Alpha-class Glutathione Transferases from Pig: A Comparative Study

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**Abstract**

Glutathione transferases (GSTs, EC 2.5.1.18) possess multiple functions and have potential applications in biotechnology. This thesis contributes to knowledge about glutathione transferases from *Sus scrofa* (pig). The study is needed for better understanding of biochemical processes in this species and is desirable for drug development, for food industry research and in medicine.

A primary role of GSTs is detoxication of electrophilic compounds. Our study presents porcine GST A1-1 as a detoxication enzyme expressed in many tissues, in particular adipose tissue, liver and pituitary gland. Based on comparison of activity and expression profiles, this enzyme can be expected to function in vivo similarly to human GST A2-2 (Paper II).

In addition to its protective function, human GST A3-3 is an efficient steroid isomerase and contributes to the biosynthesis of steroid hormones in vivo. We characterized a porcine enzyme, pGST A2-2, displaying high steroid-isomerase activity and resembling hGST A3-3 in other properties as well. High levels of pGST A2-2 expression were found in ovary, testis and liver. The properties of porcine enzyme strengthen the notion that particular GSTs play an important role in steroidogenesis (Paper I).

Combination of time-dependent and enzyme concentration-dependent losses of activity as well as the choice of the organic solvent for substrates were found to cause irreproducibility of activity measurements of GSTs. Enzyme adsorption to surfaces was found to be the main explanation of high variability of activity values of porcine GST A2-2 and human Alpha-class GSTs reported in the literature. Several approaches to improved functional comparison of highly active GSTs were proposed (Paper III).

*Keywords:* glutathione transferase, substrate selectivity, steroidogenesis, Sus scrofa, functional comparison, irreproducibility, reproducible assays

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urn:nbn:se:uu:diva-144119 (http://urn.kb.se/resolve?urn=nbn:se:uu:diva-144119)
If you are not part of the solution, you are part of the precipitate.

Henry J. Tillman

There is no God, no destiny but only you, your dream and your choice.

To my family
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


Reprints of the papers were made with permission of the publishers.

Paper not included in the thesis:

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Abbreviations

5AD  5-androstene-3,17-dione
5PD  5-pregnene-3,20-dione
cDNA  complementary deoxyribonucleic acid
CDNB  1-chloro-2,4-dinitrobenzene
CHP  cumene hydroperoxide
DNA  deoxyribonucleic acid
GSH  glutathione
GST  glutathione transferase
hGST  human glutathione transferase
mRNA  messenger ribonucleic acid
NAD⁺  nicotinamide adenine dinucleotide
NADPH  nicotinamide adenine dinucleotide phosphate, reduced
Non  trans-2-nonenal
PCR  polymerase chain reaction
PEITC  phenethyl isothiocyanate
pGST  porcine glutathione transferase
RNA  ribonucleic acid
Introduction

The present work concerns a group of enzymes, glutathione transferase, that are involved in several cell processes. The classical function of these enzymes is defense. Glutathione transferases catalyze reactions between the ubiquitous tri-peptide glutathione and numerous reactive compounds coming every day with food, water and air as well as some compounds produced in our body in process of normal metabolism. By doing so, glutathione transferases deprive dangerous chemicals of chemical reactivity and, thereby, protect proteins, genome and lipids from adverse modifications. Some glutathione transferases interact with signal proteins, thereby regulating the intensity of signal transduction in the cell. The role of glutathione transferases in biosynthesis of steroid hormones has begun to emerge in recent years. The introduction is aimed at presenting the state of art in three scientific fields brought together in this thesis: glutathione and glutathione transferases, biosynthesis of steroid hormones and rationale for studies of *Sus scrofa* (pig) as a model organism. Some historical facts are also included in order to illustrate how the glutathione transferases area has been developing.

Glutathione

Glutathione serves to glutathione transferases as cofactor and the invariant substrate. Apart from that, glutathione is an important cell component by itself.

*Discovery and structure.* The history of glutathione spans over 100 years [1]. In 1888, the name *philothion* was coined by Joseph de Rey-Pailhade for an extracted from yeast substance characterized by its ability to form hydrogen sulfide from sulfur. Philothion was also found in many animal tissues such as beef muscle and liver, sheep brain, lamb small intestine, fish muscle, fresh egg white, and in small amounts in tendons, adipose tissue and blood. In 1921, philothion was rediscovered by Frederick Gowland Hopkins studying muscular contraction. Philothion was renamed to *glutathione* by Hopkins after he had concluded that it is a di-peptide containing glutamate and cysteine. The important biological function of philothion/glutathione was recognized immediately, but the ultimate structure of glutathione was
Glutathione is a tri-peptide of unusual chemical structure (Figure 1), where cysteine and the C-terminal glycine are coupled via standard peptide bond, while the N-terminal glutamic acid forms a peptide bond to cysteine via the carboxyl group of the side chain instead of the alpha-carboxyl group.

Occurrence. Glutathione seems to occur in all aerobic organisms, in all the cell types, bile and urine at mM concentration and in blood plasma at µM concentration [4-6]. In human cells, intracellular glutathione concentration varies from approximately 0.3 mM (erythrocytes) to 10 mM (hepatocytes) [6].

Chemical properties. The thiol group of the glutathione molecule carries the properties of ordinary thiols. A thiol is an electron donor and readily acts as a reductant. Glutathione reduces disulfides, peroxides, nitrate esters, metal ions like copper(II), free carbon radicals, iodine, diazenes etc [4, 6]. Glutathione disulfide is a product of all oxidative reactions. Due to polarizability, the sulfur atom is a good nucleophile and reacts with electrophilic carbon in a variety of compounds. Glutathione also chelates metal ions both physiological (zinc and calcium) and toxic (mercury, lead, cadmium, tin, tellurium, platinum) [7].

Functions. Cytotoxic electrophiles come with food and air. They are also synthesized in the cell as main or side products of natural metabolism [6, 8, 9]. Glutathione competes with DNA, RNA, proteins and other essential components of the cell for reactive electrophiles and has an advantage of doing it fast being harbored by special enzymes - glutathione transferases. Cytotoxic electrophiles are often hydrophobic, while glutathione is easily soluble in aqueous solutions. Glutathione conjugates with hydrophobic compounds become water-soluble, are pumped through the cell membrane [10], metabolized to mercapturic acids and excreted [11, 12]. Not surprisingly, the protective role of glutathione extends to cytotoxic drugs and is associated with resistance to many anticancer agents [13]. Drugs such as chlorambucil (Leukeran®), melphalan (Alkeran®), mechlorethamine (Mustargen™), ethacrynic acid (Edecrin®) have been recognized as substrates of glutathione transferases. Others, like cisplatin (Platinol®), are
not substrates of glutathione transferases. Instead, cancer cell resistance to cisplatin has been shown to be dependent on the glutathione status [13-16].

Glutathione status is defined as total amount and a ratio of glutathione to glutathione disulfide in the cell [4]. Glutathione is continuously oxidized to glutathione disulfide but the level of the latter is balanced below 5 % [4] due to the action of intracellular glutathione reductase at the expense of NADPH [17]. So, glutathione and glutathione disulfide work as a redox buffer by providing a very large pool of reducing equivalents [18]. Due to reactive oxygen species production [19], cells may undergo oxidative stress [20]. Glutathione is the main scavenger of oxygen reactive species in mitochondria [21]. Recent findings also suggest the role of nuclear glutathione in cell proliferation via redox status and regulation of chromatin structure [22]. In addition, various essential cellular processes (cell signaling, glycolysis, calcium homeostasis, protein folding, redox balance, mitosis etc) are subjects to regulation by glutathionylation of the proteins involved [23].

When under certain circumstances (starvation, intensive drug treatments, chronic alcohol abuse etc) glutathione is depleted, cells get damaged [24]. Glutathione depletion has been shown to induce or potentiate apoptosis [25].

**Glutathione transferases**

*Discovery and occurrence.* Enzymes catalyzing conjugation reactions of glutathione to various xenobiotics were discovered in 1961 by two groups [26, 27]. The enzymes were named glutathione transferases (abbreviated as GSTs) and classified as EC 2.5.1.18 by the Enzyme Commission. Just like glutathione, glutathione transferases seem to occur in all living organisms and all tissues in various amounts. Eukaryotes appear to possess multiple forms of glutathione transferases with different substrate specificities. In rats, for example, at least thirteen GST isoforms were characterized [28]. Originally, glutathione transferases were isolated and studied because of the conjugation reactions they catalyzed. In recent years, relatively easy access to genomic data facilitated identification of new enzymes and the original view on glutathione transferases as solely detoxication enzymes has been markedly changed.

*Classification and nomenclature.* Glutathione transferases are combined into superfamily and divided into classes based on sequence similarity. For convenience, classes are often grouped into families based on their localization in the cell.

Classes of cytosolic glutathione transferases (also known as soluble or canonical) are designated with Greek letters (Alpha, Mu, Pi etc) and abbreviated with capital Roman letters (A, M, P etc) [29, 30]. In mammalia, seven classes of soluble glutathione transferases have been identified: Alpha,
Mu, Pi, Omega, Sigma, Theta, and Zeta, of which the first three are specific to mammalia. The mean amino acid sequence identity within a class is usually at least 40%, while the mean interclass identity in mammalia is no more than 30%.

The less multifarious family of microsomal glutathione transferases (membrane-associated proteins involved in eicosanoid and glutathione metabolism or MAPEG) resides in the subcellular membranes. In bacteria, plasmid-encoded bacterial fosfomycin-resistant glutathione transferases compose the smallest family [31]. The nomenclature is constantly being refined due to new types of glutathione transferases discovered.

Structure. Soluble glutathione transferases are dimers of two identical subunits [32] or subunits belonging to the same class [33]. Heterodimers between Mu and Pi subunits have also been reported [34]. Each subunit consists of two domains. The thioredoxin domain provides the binding site for glutathione, while the C-terminal domain is mainly responsible for binding of the variable substrate [32].

Microsomal glutathione transferases are homotrimers [35]. The only one known to form tetramer is a GST from Plasmodium falciparum (one of the parasite species causing malaria). The tetramer is catalytically inactive, but it dissociates to catalytically active dimers in the presence of glutathione [36].

Characteristic properties. All glutathione transferases have some features in common. The vast majority of glutathione transferases catalyze conjugation of glutathione to hydrophobic compounds bearing electrophilic centers [37]. Since most of the substrates are harmful for the cell [32], glutathione transferases are often referred to as detoxication enzymes. Some substrates are oxidation products of Phase I metabolizing enzymes (cytochromes, monooxygenases). Therefore, glutathione transferases are also referred to as Phase II enzymes [38]. Glutathione transferases are promiscuous, that is each particular subunit is capable of recognizing multiple substrates. Glutathione transferase isoforms have overlapping substrate selectivity profiles, that is, a particular compound may be a substrate for several subunits both from the same class and different classes. Glutathione transferases may also be called moonlighting enzymes, because the same isoform may have non-catalytic functions beside the catalytic ones. Finally, expression of many glutathione transferases is inducible by xenobiotics [28], and Alpha, Pi, Mu and Theta classes have been found to be polymorphic [39, 40].

Mechanisms and functions of glutathione transferases

Alpha-class GSTs, a subject of the present study, catalyze addition, substitution and isomerization reactions. These enzymes also function as ligandins and are involved in protein-protein interactions.
Glutathione activation. How do glutathione transferases catalyze reactions with glutathione? The enzyme enhances nucleophilicity of the thiol group by lowering the pK\textsubscript{a}(-SH) from 9 in solution down to 6.5 when bound in the active site. Besides, both the thiolate and the second hydrophobic substrate are desolvated in the active site. Other parts of the glutathione molecule presumably serve to position the thiol in the active site [37]. Calculations predict that Alpha- and Pi-class GSTs have similar glutathione activation mechanism, while that of Mu-class GST differs [41].

Addition and substitution reactions. Glutathione transferases catalyze nucleophilic addition and substitution reactions with multiple structurally diverse substrates [28]: aryl and alkyl halides, α,β-unsaturated carboxyls, esters and organic nitrate esters, organic thiocyanates, epoxides, bulky substrates like aflatoxin and benzopyrene, small substrates like diiodoethane and acrolein, or long-chain substrates like 9-hydroperoxylinooleic acid. In all cases, the mechanism is based on nucleophilic attack of thiolate followed by either detaching of the leaving group (substitution reactions) or by protonation of the disengaged electron pair (addition reactions). Glutathione transferases also catalyze peroxide reduction [42], in which two glutathione molecules are consumed per peroxide molecule. The first reaction step is enzymatic and the second reaction step is non-enzymatic.

Isomerization reactions. In isomerization reactions, glutathione is not expended. Cis-trans isomerization of maleylacetoacetate to fumarylacetoacetate is a part of catabolism of phenylalanine and tyrosine catalyzed by human GST Zeta [43]. The reaction proceeds via a covalent intermediate of glutathione with maleylacetoacetate (Figure 2) [44].

Human GST P1-1, M1-1 and A1-1 catalyze the isomerization of 13-cis-retinoic acid to all-trans-retinoic acid. The reaction could be expected to proceed via the same mechanism as maleylacetoacetate isomerization; however, it seems to be glutathione-independent [45]. Glutathione-conjugate intermediates may also be formed via S-O linkage. This mechanism is used

![Figure 2. Mechanism of isomerization of maleylacetoacetate to fumarylacetoacetate proposed in [44].](image-url)
by human GST S1-1 (as well as rat Alpha, Mu and Pi classes [46]), efficient prostaglandin D synthase [47], for endoperoxide ring opening [48].

Mammalian Alpha-class GSTs catalyze double-bond migration in steroids also using glutathione as a cofactor [49]. The latest mechanism of this reaction proposed for human GST A3-3 (most efficient steroid isomerase in mammalia [50]) suggests glutathione as acid-base catalyst in concerted proton transfer from C4 to C6 (Figure 3) [51]. (For comparison, bacterial ketosteroid isomerases employ aspartate residue for proton abstraction/donation and stabilize intermediate dienolate in the oxoanion hole of the active site [52]). A similar mechanism is used by insect Epsilon-class GST in conversion of dichlorodiphenyltrichloroethane (the insecticide DDT) to dichlorodiphenyldichloroethylene [53]. Glutathione has been proposed to abstract proton from the electrophilic carbon, which results in chloride elimination.

GSTs as ligandins. Soluble glutathione transferases of the Alpha, Mu and Pi classes have non-catalytic function as ligandins [54-57]. They non-specifically bind a range of “ligands” – steroids and their metabolites, heme, hemin, bilirubin, several dyes, various drugs and carcinogens. Since all these compounds are hydrophobic, it has been speculated that their binding to soluble glutathione transferases would facilitate transport of these compounds in the cell. Later experimental studies have shown that GST-mediated transport of bilirubin from the plasma membrane to the endoplasmic reticulum membrane occurs via a series of diffusional jumps between membranes. At that, GSTs seem to be intermediate stations that actually slow down the process of bilirubin transfer [58].

GSTs in protein-protein interactions. Murine GST Pi has been shown to interrupt signal transduction by specific inhibition of the phosphorylation ability of Jun N-terminal kinase [59]. As reported, the enzyme binds as monomer, which is the only evidence for functioning of monomeric GSTs in vivo. Interactions with stress-activated kinases have also been reported for human GST A1-1 [60, 61] and mouse GST M1-1 [62]. Another example of GST-dependent protein-protein interaction is provided by Sigma-class GST from octopus [63]. This enzyme builds a base for the liquid-crystal structure.
of the eye-lens. It has been demonstrated that polymer-like GST chains consist of protomers interacting non-covalently. Whether protomers are monomers or dimers has yet to be determined.

Steroid hormones

Steroid hormones play crucial roles in homeostasis and development in humans. Mineralocorticoids (e.g. aldosterone) maintain the balance of salt and water in the body. Glucocorticoids (e.g. cortisol) regulate the metabolism of proteins, carbohydrates and lipids. Estrogens (e.g. estradiol) and androgens (e.g. testosterone) primarily influence the development, maturation, and function of reproductive system in females and males, respectively. Progestogens (e.g. progesterone) are important for maintaining pregnancy and as precursors of all other groups of steroid hormones [64].

Synthesis of steroid hormones takes place mainly in adrenal gland, testis, ovary, placenta and mammary gland. Steroid hormones are fat-soluble, pass easily through cell membranes and bind to ligand-dependent intracellular transcription factors thereby initiating target gene expression [64]. Steroid hormones also induce non-genomic responses by triggering release of intracellular calcium and activation of protein kinases [65, 66].

The source of all steroid hormones is cholesterol. Much of the cholesterol is synthesized de novo in liver and carried to other body sites by lipoproteins present in the blood plasma [67]. The biosynthesis of steroid hormones starts from the cleavage of the cholesterol side chain and proceeds through a series of reactions catalyzed by two major classes of proteins: the NADPH-dependent cytochromes P450 and NAD+-dependent hydroxysteroid dehydrogenases [68, 69]. Cytochromes and dehydrogenases involved in steroid biosynthesis are membrane-bound enzymes associated with the mitochondrial membranes or the endoplasmic reticulum. Reactions take place both in mitochondria and endoplasmic reticulum and obviously involve transport of intermediate steroid forms between the two organelles.

Double-bond isomerization. 3β-Hydroxysteroid dehydrogenase is the main enzyme that catalyzes oxidation of 3β-hydroxyl group in steroid hormones. The same enzyme catalyzes the following $\Delta^5 \rightarrow \Delta^4$ isomerization of a double bond [70]. Both reactions are indispensable steps in production of all types of steroid hormones. 3β-Hydroxysteroid dehydrogenase has long been thought the only enzyme at this point of biosynthesis.

However, evidence has been accumulated indicating that the isomerization reaction might be catalyzed by another enzyme. In 1955, it was shown that 3β-hydroxyl group oxidation and isomerization are catalyzed by separate enzymes in Pseudomonas testosteroni [71]. The next step was a publication of 1976 describing that steroid-isomerase from rat is glutathione-dependent [72]. Next year, these steroid isomerases from human and rat liver
were recognized as glutathione transferases [73]. By 2001, all human Alpha-class GSTs were demonstrated to catalyze steroid isomerization [49, 50]. Their catalytic efficiencies are at least 600 fold lower than that of bacterial ketosteroid isomerase. However, as compared to human 3β-hydroxysteroid dehydrogenase, hGST A1-1 and A3-3 have 33 and 333 fold higher catalytic efficiencies. Since Alpha-class GSTs are cytosolic enzymes while all known steroidogenic enzymes are membrane-bound, it was unclear whether such efficiency is significant in vivo. In 2008, human GST A3-3 was finally shown to complement 3β-hydroxysteroid dehydrogenase isomerase activity in human cell lines [74]. As reported, 74% of the steroid-isomerase activity with 5-androstene-3,17-dione is glutathione-dependent in the human adrenocortical cell line H295R, and 10-30% of the isomerization leading to progesterone in the placental choriocarcinoma cell line JEG3 is catalyzed by hGST A3-3. On one side, it is quite logical that a more active enzyme contributes to steroid production. On the other side, it is not clear why and by what means steroids migrate from intracellular membranes to cytosolic Alpha-class GSTs and back. Besides, none of similiar efficient GSTs have been found in other mammalian or eutherian species.

Bovine GST A1-1 expression has been found to be up-regulated in granulosa cells responsible for follicular growth and dominance [75], to be tissue- and cell-specific, associated with steroidogenically active cells, and hormonally regulated in the ovarian follicle [76]. Bovine GST A1-1 shares 84% amino acid sequence identity with hGST A3-3 and almost identical residues identified as critical for isomerase activity of human GST A3-3 [77]. Nevertheless, the specific activity of bovine GST A1-1 in steroid-isomerization reaction is 0.4% and $k_{cat}$ is 0.3% of those values for hGST A3-3, while $K_M$ is 28% of that for hGST A3-3 [77]. Theoretically, bovine GST A1-1 may contribute to steroidogenesis in bovine ovary on the condition that its expression is sufficiently high. In reality, whether GSTs contribute to steroidogenesis in other eutherian/mammalian species beside humans has remained uncovered. We therefore decided to study glutathione transferases of Alpha-class from pig, a species with exceptional physiological similarity to humans. Besides, pigs have found many applications in our life and additional knowledge about them would be valuable.

Pigs as objects of research

Porcine glutathione transferases, in particular their relation to steroidogenesis, are a subject of investigation in the present study for several reasons. The pig (Sus scrofa) has been intensively studied as an alternative to rodents, primates and other model organisms. Pigs and humans are very similar at the genetic level. The porcine genome consists of nineteen chromosome pairs and the genome size is $2.7 \times 10^9$ base pairs [78] which is
87% the human genome size [79]. There is extensive homology between human and porcine genomes; however, correspondence of chromosomes is not one-to-one but rather patchy [80]. The pig genome sequencing project is not finished yet and further refinement is anticipated.

Pigs are testing animals. Testing of new drugs always requires preclinical trials, where main pharmacokinetic, pharmacodynamic properties and toxicity can be assessed. It is of importance to know whether pigs and humans have reasonably similar drug metabolizing ability. Porcine cytochromes P450, Phase I metabolizing enzymes, have been characterized and compared to the human cytochromes in order to make interspecies extrapolation possible [81]. Besides, activity modulation of some metabolizing enzymes can be useful in drug treatments. Glutathione transferases are the main enzymes of Phase II drug metabolism. While human glutathione transferases have been extensively characterized, porcine glutathione transferases are yet to be studied.

Pigs in foodstuff industry. Pigs remain one of the most important food sources. Both a producer and a consumer are interested in high quality pork at minimum cost. Many efforts have been put into breeding of sows with high reproduction efficiency and improved fertility traits [82]. The genetic approach, involving search for genetic markers associated with large litter size and high level of pre-weaning viability, requires a better understanding of the porcine reproductive system and how it is affected by different factors [82]. Other methods focus on development of reproductive technologies [83]. Steroid production is known to influence pork meat quality [84]. Fat content (lean pork is desirable) and boar taint (undesirable) are both related to steroidogenesis. Also, porcine reproductive ability drops under prolonged stress and the mechanism behind has been proposed to be cortisol-mediated [85]. Gene expression in pigs has been investigated using high throughput methods in order to expand pig-human comparative maps, to understand porcine gene expression and to identify genes differentially expressed in specific tissues or cell types [86]. Alpha-class glutathione transferases have been found among genes differentially expressed during pig ovarian follicular differentiation [87]. Pi-class glutathione transferase has been identified among genes up-regulated in ovaries and ovarian follicles of pigs selected for increased ovulation rate [88]. It has also been shown that follicle-stimulating hormone and testosterone enhance the Alpha-class GST mRNA and protein levels in porcine Sertoli cells responsible for support, protection and nutrition of developing spermatozoa [89]; while tumor necrosis factor alpha acts as inhibitor of Alpha-class GST expression in Sertoli cells [90]. How glutathione transferases contribute to the aforementioned processes is yet to be understood.

Pigs in medicine. The anatomy and physiology of pigs are very similar to those of humans. Therefore, pigs and, in particular, miniature pigs are used as mammalian models for human biology [91, 92]. Pig is an important model
in studies of heart physiology, reproductive function, skin physiology, brain, tissue engineering etc [91]. Pigs and humans have similar progression of metabolic (obesity, heart diseases) and infectious (numerous organisms cause infection across species) diseases. Pigs suffer from hypertension, hypercholesterolemia, dyslipidemia, insulin resistance and atherosclerosis. Pigs, along with rodents and dogs, are models for food allergy [92, 93]. The neonatal swine model mimics the physical (emesis, lethargy, diarrhea, respiratory distress) and immunological characteristics of food allergy in human beings.

Porcine tissues and cells have been used in xenotransplantation [94]. Heart valves from pigs last 10-15 years of work in the human heart. Porcine tissues (skin, intestine) are used to repair hernias, ulcers, for wound care, plastic surgery and weight-loss surgery. Entire organ xenotransplantation involves more obstacles and, at present, it is only used for model studies [94]. A promising tool to overcome rejection is gene therapy technology applied to pigs [95]. It must be pointed out that primates would genetically be more suitable for transplantations, however, pigs are more available and their use in biomedical experimentation has become routine practice since they are slaughtered for food.

Summary

Glutathione transferases are multifunctional enzymes present in virtually all living organisms. The vast majority of the enzymes catalyze glutathione conjugation to a variety of reactive electrophiles that an organism receives with food, water and air. Deleterious products of metabolism, such as lipid peroxides, are also neutralized by conjugation to glutathione.

Biosynthesis of all steroid hormones proceeds through an indispensable step – migration of $\Delta^5$-double bond. Human GSTs A3-3 and A1-1 catalyze this reaction more efficiently than the classical dehydrogenase. The contribution of hGST A3-3 to steroid hormone production has been demonstrated \textit{in vivo}. Although Alpha-class GSTs from all organisms possess steroid-isomerase activity to some extent, no other organism with significant steroid-isomerase contribution by Alpha-class GSTs has been identified. Therefore, the significance and commonness of glutathione transferases in steroid biosynthesis remains uncertain. Among many mammalian species that could be chosen as a subject for our study, pigs appear most attractive due to a variety of applications humans have already found for these animals in every-day life. The study of porcine glutathione transferases is a contribution to our knowledge of glutathione transferases variety, and is desirable for drug development, for food industry research and in medicine.
Present investigation

Aims

The present work was aimed to investigate the variety of Alpha-class GSTs from the pig, to explore whether there were porcine GSTs that could contribute to steroidogenesis, to characterize the enzymes, and to compare porcine and human Alpha-class GSTs.

Identification of research objects (Papers I and II)

No porcine Alpha-class GSTs (ascribed to Alpha-class based on sequence similarity), were characterized when the present study was initiated. The UniProt knowledgebase of protein sequence and function contains information about three cytosolic GSTs: Alpha 1-1, Omega and Pi (Swiss-Protein accession numbers P51781, Q9N1F5 and P80031, respectively), three putative Mu-class subunits (TrEMBL accession numbers Q000H9, Q000H8 and Q29583) and three cytosolic GST subunits not yet classified (Q29057, Q29188, Q29581). Amino acid sequences of five GST subunits share >30% sequence similarity to human GST A3-3 (Swiss-Prot accession number Q16772). Pi-class GST (P80031) has earlier been characterized [96]. The putative GST subunits Q29188 and Q29581 consist of less than 120 amino acid residues, lack C-terminal domain and are, therefore, unlikely to have any catalytic activity. Finally, GST A1-1 (P51781) and one of the putative GSTs that we named A2-2 (Q29057) contain all elements of functional glutathione transferases and showed 83 and 84% sequence identity to human GST A3-3, respectively. The corresponding cDNA sequences have been identified among genes that change expression levels during ovarian follicular differentiation in pigs [87]. Hence, two Alpha-class GSTs were chosen for cloning and characterization in the present study.

We have attempted to find more Alpha-class GSTs in the porcine genome. Four or five Alpha-class GST genes, with six exons each (like in humans), could be recognized on chromosome 7. Unfortunately, the porcine genome assembly (NCBI build 1.1, based on a single Duroc sow) is not yet completed and does not allow unambiguous mapping and annotation of even those Alpha-class GSTs for which cDNAs have been identified. The presence of four-five GST sequences in the porcine genome does not
necessarily imply that four-five GST proteins are expressed *in vivo*. In the human genome, five Alpha-class GSTs have been identified on chromosome 6 [97]. It has been demonstrated that all the genes have genomic elements obligatory for translation and code for proteins of equal length, 222 amino acid residues including the initial methionine. Yet *in vivo*, human GST A5-5 has not been detected at the mRNA or the protein level in human tissues in contrast to human GST A1-1, A2-2, A3-3 and A4-4.

At the protein level, one [98], three [99], four [100] or five [101] Alpha-class GSTs have been isolated from porcine liver in earlier studies. Four or five Alpha-class GSTs have also been detected in porcine ovary [102]. However, characterization of the proteins that would allow unambiguous identification of the corresponding amino acid sequences is lacking. Further investigations, such as proteomic analyses, would be desirable for definitive identification of the expression pattern of GSTs in porcine tissues.

Cloning and purification (Papers I and II)

Porcine Alpha-class GSTs were cloned from ovary and testis of the domesticated Large White pig intercrossed with European Wild Boar. A sequence of cloned porcine GST A2-2 cDNA was identical to the corresponding sequence published in the database. However, the sequence of pGST A1-1 we cloned differed from the published sequence at three positions (*Figure 4*). The first difference is a silent mutation in residue Asn73. The second one leads to a conservative replacement of Tyr174 in the published sequence by Phe174. The third difference is the most conspicuous. Due to a missing nucleotide in our sequence, as compared to the published one, ten C-terminal amino acid residues were changed. The deduced amino acid sequence of our clone showed higher similarities with pGST A2-2 and

![Figure 4](image)

*Figure 4.* Aligned amino acid sequences of pGST A1-1 and A1-1*. Active site residues involved in glutathione and the second substrate binding are marked with triangles and gray color, respectively. The residues are deduced from comparison of human Alpha-class GSTs with structures solved.
In order to understand where the differences come from, the original cDNA published in the database was re-sequenced and no discrepancies were found. Experimental artifacts could also be excluded for pGST A1-1 that we have cloned due to repeated sequencing of the cDNA isolated from two different tissues and two different animals. Genome studies revealed that the published pGST A1-1 sequence (hereafter referred to as pGST A1-1*) is recognized for first two differences, while only the pGST A1-1 cDNA we cloned is recognized at the third position. Thus, it is not clear whether pGST A1-1* variant has arisen due to breed difference, an allelic variant, or is a cloning artifact in the previous study.

Porcine GST A1-1 and A2-2 cDNA inserted into expression vectors were biosynthesized in bacteria. A purification method had to be developed because of crystallization trials planned. The widely used glutathione-affinity chromatography yielded only 11.2 mg of pGST A2-2 from 3 liters of bacterial culture. The bulk of overexpressed enzyme was found in the flow-through. Cation-exchange chromatography on CM-Sepharose yielded up to 32 mg pGST A2-2 from 1 liter of bacterial structure. The best results were achieved by a method including fractional precipitation with ammonium sulfate, cation-exchange on SP-Sepharose and affinity chromatography on S-hexylglutathione-Sepharose. This purification procedure yielded 150 mg of high-purity pGST A2-2 from 3 liters of bacterial culture. For purification of pGST A1-1, a combination of fractional ammonium sulfate precipitation and S-hexylglutathione affinity chromatography was used. The yield was 200 mg of at least 95% purity protein from 1.5 liters of bacterial culture.

Activity profiles (Papers I, II and III)

Six substrates were chosen for functional characterization of the enzymes (Figure 5). Addition reactions are represented by phenethyl isothiocyanate (PEITC) and trans-2-nonenal (Non). PEITC is a natural compound present in many edible cruciferous vegetables such as cabbage, broccoli, Brussel sprouts, rucola, cauliflower, collard, kohlrabi, swede etc. This compound has been shown to inhibit the growth of breast [103] and ovarian [104] cancer cells in humans at nutritionally relevant concentrations. Nonenal is also a natural compound occurring in our body. It is an oxidation product of unsaturated fatty acids present, for example, in the skin surface [105]. Substitution reactions are represented by 1-chloro-2,4-dinitrobenzene (CDNB). CDNB is used in organic synthesis (e.g. as a precursor for Sanger’s reagent) and is an artificial substrate for many glutathione transferases. The CDNB assay is convenient and often used as a standard for quantification of glutathione transferases. Cumene hydroperoxide (CHP) is an intermediate in
industrial production of phenol and acetone. It is an artificial substrate for glutathione transferases, however, peroxides are common in the cell due to constant oxidative stress [19, 20]. By definition, CDNB, CHP and PEITC may be referred to as xenobiotics. Isomerization reactions are represented by two steroid compounds: 5-androstene-3,17-dione (5AD) and 5-pregnene-3,20-dione (5PD). In vivo, 5-androstene-3,17-dione is a product of oxidation of dehydroepiandrosterone and 17α-hydroxyprogesterone and is a precursor of estrogens, testosterone and dihydrotestosterone. 5-Pregnene-3,20-dione is a product of pregnenolone oxidation and is an intermediate for all groups of steroid hormones.

Porcine GST A1-1 demonstrated highest specific activity with CDNB. With all other substrates tested, pGST A1-1 activity was ≤7% of that with CDNB. Activity with Non was the lowest (0.06%).

Porcine GST A2-2 demonstrated highest specific activity with 5AD. The activity with CDNB and PEITC was 25% of that with 5AD, and activity with other substrates did not exceed 4% of that with 5AD. Experimental aspects described and discussed in Paper III pose limitations on the amount of GST that can be used in the assay. Below 20 nM enzyme in the reaction mixture, enzyme activity is underestimated due to enzyme adsorption to the container walls. Considering that pGST A2-2 activity with 5AD was measured using 2-3 nM enzyme without blocking but in the presence of glycerol, the true activity can be estimated to at least 2 fold of the measured one. Thus, the enzyme is even more selective towards 5AD compared to other substrates tested. Activity with other substrates in this case would not exceed 13% of that with 5AD. In other words, specific activities of pGST A1-1 and A2-2 with their most active substrates exceed activities with other tested substrates at least 7 fold.

Steady-state parameters were also determined. The highest specificity constant $k_{cat}/K_M$ of porcine GST A1-1 was in the reaction with CDNB. CHP and PEITC were moderately good substrates with the $k_{cat}/K_M$ values of about 20% of that with CDNB. Steroids and Non were the least reactive substrates with the catalytic efficiencies below 5% of that with CDNB. Thus, pGST
A1-1 catalytic efficiency profile characterizes the enzyme as directed to detoxication of xenobiotics.

For porcine GST A2-2, the highest specificity constants were in the reactions with steroids. The $k_{cat}/K_M$ values for 5PD and PEITC were about 10% of that with 5AD. Other substrates have catalytic efficiency values below 3% of that with 5AD. Thus, pGST A2-2 catalytic efficiency profile characterizes the enzyme as directed to steroid isomerization.

Functional analogies (Papers I and II)

Functional analogies between porcine and human Alpha-class GSTs were established taking into account not only kinetic characteristics of the enzymes but also the distribution of the enzymes in various tissues. Indeed, it does not matter how active or selective an enzyme is if it is not biosynthesized in the part of the body where the presence of this activity would make sense. In contrast, low enzyme activity may be compensated by the presence of the enzyme in large amounts or by a certain balance in concentrations of the substrates.

Comparison of catalytic efficiency profiles of porcine and human Alpha-class GSTs reveals that pGST A1-1 is similar to hGST A2-2 (Figure 6A). Porcine GST A1-1 is characterized by the low $k_{cat}/K_M$ values for the steroid substrates, which distinguishes the enzyme from hGST A1-1 and A3-3. The enzyme also lacks preference to Non in contrast to hGST A4-4.

The highest levels of the pGST A1-1 mRNA were detected in adipose tissue, liver, and pituitary gland. Lower levels of the mRNA were found in adrenal gland, kidney, lung, prostate, skin, gonads. No pGST A1-1 mRNA was detected in brain tissue. The expression pattern of pGST A1-1 was most similar to those of hGST A1-1 and A2-2 [97], suggesting that pGST A1-1 in the pig in a similar manner is mainly involved in detoxication.

Prominent $k_{cat}/K_M$ values for the steroid substrates position pGST A2-2 between hGST A1-1 and hGST A3-3 (Figure 6B). Preferences of the enzyme among less favorable substrates point to closer similarity of pGST A2-2 to hGST A3-3 (Figure 6B, inserts). Human GST A2-2 and A4-4 have very low activity with 5AD compared to other substrates. Human GST A5-5 has been poorly characterized so far and its expression in vivo has not yet been detected [97, 106].

The highest levels of the pGST A2-2 mRNA were detected in gonads and liver, while the expression in kidney, skin and pituitary gland was at significantly lower levels. Comparison of the expression profiles revealed that the pGST A2-2 expression pattern shares features of hGST A1-1 and A3-3 expression profiles. In contrast to hGST A3-3, pGST A2-2 expression was detected in liver and no splice variants were observed. Nevertheless,
tissue expression of pGST A2-2 appears to be in agreement with steroid biosynthesis sites in pigs.

Inhibition studies were performed using tributyltin chloride, a ubiquitous contaminant whose detrimental effects on growth and steroid hormone production have been shown in mice [107], rats [108], cattle [109] and pigs [110]. The type and strength of inhibition were similar for pGST A2-2 and hGST A3-3 as tested with two different substrates: 5AD and CDNB, which strengthens the notion of functional similarity between these two enzymes.

It should finally be mentioned that one-to-one correspondence between human and porcine GSTs does not necessarily exist. Instead, the pool of porcine GSTs could collectively perform the same functions as the pool of human GSTs.

Structure-function relationships (Paper I and II)

Structure-function relationships may be established due to the paradigm that the primary structure of an enzyme defines its secondary, tertiary, quaternary structure and structure, in turn, defines all the possible functions the enzyme may have. Many efforts have been made to make such relationships predictive, which would accelerate proteome characterization and greatly benefit biotechnological studies.
### Figure 7. Structure-function relationship for Alpha-class GSTs characterized so far.

Most reactive substrates for each group of enzymes are highlighted. aThe $k_{\text{cat}}/K_M$ values were estimated based on available specific activity. bThe $k_{\text{cat}}/K_M$ values were estimated based on assumption that they do not exceed 80% of those with 5-androstene-3,17-dione. cThe $k_{\text{cat}}/K_M$ value could not be estimated and is absent.

<table>
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<th>HYDROPEROXIDE</th>
<th>PHENETHYL</th>
<th>ISOThIOCYANATE</th>
<th>5-ANDROSTEN-3,17-DIONE</th>
<th>5-PREGNENE</th>
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Porcine GST A1-1 and A2-2 share 98% amino acid sequence identity. The catalytic properties of the enzymes differ much more than could be expected from such a value if overall sequence identity would be a good predictor for substrate selectivity or absolute activity values.

More definite conclusions could be drawn from comparison of amino acid residues composing the active site. The active site in glutathione transferases is conventionally divided in two parts: the H-site including residues lining the cavity for binding of various hydrophobic substrates and the G-site including residues positioning glutathione. Out of seventeen residues forming the H-site in Alpha-class GSTs, ten residues were found to differ in the compared enzymes (Figure 7). Major differences in substrate preference might be explained by the residues of the H-site differing between the enzymes compared.

By mutational analysis, five H-site residues have been identified as necessary for high steroid-isomerase activity of hGST A3-3 [111, 112]. Those residues 10, 12, 111, 208 and 216 are highlighted in bold in Figure 7. Considering other Alpha-class enzymes sequences, the relationship between the residues identified and the steroid-isomerase activity becomes less clear. Porcine GST A1-1 and human GST A1-1 have only one unfavorable residue in the H-site, Met208, however their catalytic efficiencies with 5AD differ 28 fold.
Another relationship has been found in the present study. It seems that a pair of residues 208+212 must be bulky for smaller substrates like CDNB or CHP and small for large substrates like steroids. The pair of Ser10+Ile12 in hGST A2-2 occupies a similar volume as the pair of Phe10+Gly12 in other GSTs. For steroid substrates, this difference is essential, so hGST A2-2 activity with 5AD is much lower than that of hGST A1-1. Smaller substrates, binding of which is permitted by the 208+212 pair, seem to tolerate such replacement, so activity with CDNB is dominant over activities with other substrates for both pGST A1-1 and hGST A2-2. In view of such reasoning, the putative pGST A1-1* can be expected to have higher activity with smaller substrates and lower activity with steroid substrates.

Assay specifics for very active GSTs (Paper III)

During kinetic studies of pGST A2-2, it was noticed that the enzyme gradually loses activity while diluted to 150 nM. This extent of dilution was required to fulfill linearity condition for measuring activity within the initial rate range for at least 30 sec of the reaction. The same phenomenon was observed for hGST A3-3 that required even higher dilution. High variability of values for Alpha-class activities was found in the literature for the reaction with 5AD catalyzed by hGST A1-1, A3-3 and pGST A2-2; for the reaction with CDNB catalyzed by hGST A1-1 and A4-4; and for the reaction with nonenal catalyzed by hGST A4-4 [reviewed in Discussion of Paper III]. Occasional activity loss has also been noted for hGST A2-2 mutants, hGST A4-4, and hGST Mu enzymes [personal communications in our laboratory].

Accurate assays are very important in investigations of glutathione transferases. Activity comparisons are routinely made between different GSTs as well as between different substrates of the same GST. Enzyme instability may cause errors in estimation of GST activities (if a significant amount of enzyme is inactivated by the time the first measurements take place). Inaccurate values of activity may also lead to errors in GST quantification (optimization of purification, cell extracts, markers in diagnostics etc). Apparent steady-state kinetic parameters may be influenced by the direction of measuring: from low to high (underestimation of V_{max}) or from high to low (overestimation of V_{max}) enzyme concentrations. Erroneous conclusions about cooperativity might be made as well. During inhibition studies, enzyme instability may interfere with the determination of strength and type of inhibition. Thus, GST instability is of importance for fundamental enzyme characterization as well as for drug design and lead optimization processes where GST activities are relevant.

GST adsorption. We therefore analyzed the observed activity loss for pGST A2-2, hGST A3-3 and hGST P1-1, for comparison. For these enzymes, activity decreased when enzyme concentration in stock solutions
was below 1 µM. Irreproducible kinetic measurements could be observed in two manifestations: as time-dependent activity loss and as concentration-dependent specific activity. Time-dependent activity loss could to some extent be prevented by addition of glycerol, while glutathione addition did not have any effect. Glycerol also partially prevented concentration-dependent activity loss at low enzyme concentrations. At first, we assumed that decrease of activity at nanomolar GST concentrations could reflect the appearance of inactive or very weakly active monomeric subunits in solution. However, some discrepancies between the experimental data and a dimer dissociation model questioned such interpretation. Additional experiments demonstrated that adsorption of enzyme to the walls of plastic containers and tubes used to store diluted enzymes explained the major part of activity loss. Blocking of plastic surfaces with bovine serum albumin abolished activity decrease effects at >10 nM enzyme concentration in stock solution (corresponds to >0.2 nM in the reaction mixture).

**GST dissociation.** Monomeric subunits of Alpha- and Pi-class GSTs seem to be either inactive or very weakly active [113, 114, 115]. Moreover, the very existence of a folded Pi-class GST monomer has been questioned [116]. Dissociation constants for Alpha- and Pi-class GSTs have not been yet determined due to difficulties of working with enzymes at low concentrations, however, it is clear that $K_d$ values for Alpha- and Pi-class GSTs are below 1 nM and 7.5 nM, respectively [113]. Therefore, activity decrease observed below 0.2 nM enzyme concentration in the reaction mixture apparently reflects dimer dissociation process. Most naturally occurring GSTs do not possess such activity that would require subnanomolar enzyme concentrations in reaction mixture. However, in view of continued optimization of GSTs for increased activities, dimer-monomer equilibrium should be kept in mind as a possible source of underestimation of enzymatic reaction rate.

**GST inhibition.** Another factor contributing to variations of activity values is inhibition of GSTs by organic solvents used to dissolve hydrophobic substrates. As follows from the present study, Alpha-class GSTs are inhibited by ethanol to a larger extent than by methanol. So, methanol is a better choice than ethanol at least because it can be used at lower concentrations. Earlier studies on GST inhibition by organic solvents have also led to the recommendation to check the effect of solvents for any combination of substrate and enzyme [117, 118].

**Suggested improvements.** For functional comparisons of GSTs, where high enzyme dilutions have to be used in experiments and blocking is too problematic, it would be advisable to use glycerol, to approximate or, at least, indicate the enzyme concentration used in the assay. Dimer dissociation effects can be expected below 0.2 nM for Alpha-class GSTs, which should also be taken into account for enzymes requiring high dilution. Inhibition of GSTs by organic solvents should also be taken into account.
Conclusions and future perspectives

In the present work, a comparative study of porcine Alpha-class GSTs has been initiated. Two enzymes were cloned, purified and characterized. Based on analysis of expression profiles, kinetic properties and inhibition studies, porcine GST A1-1 and A2-2 were suggested to be functional analogues of human GST A2-2 and A3-3, respectively. Porcine GST A1-1 seems to function as a detoxication enzyme, while pGST A2-2 may contribute to steroidogenesis \textit{in vivo}. Beside human GST A3-3, porcine GST A2-2 is the only known eutherian glutathione transferase possessing high steroid-isomerase activity.

Further investigations are necessary to understand the relationships between two major systems of the organism: pyridine nucleotides-dependent steroid hormone synthesis and glutathione-dependent protective system. \textit{In vivo} contribution of porcine GST A2-2 to steroidogenesis is yet to be determined. Since estimation of expression levels of GSTs in the present study was semi-quantitative, quantitative real-time PCR would be desirable to assess actual levels of mRNA of Alpha-class GSTs as well as 3β-hydroxysteroid dehydrogenases from pig. Kinetic characterization of the latter has not been accomplished yet. Also, since more than two Alpha-class GSTs are encoded in the porcine genome, characterization of additional enzymes may be anticipated. Little is known about kinetic properties and expression patterns of GSTs from other classes. Finally, investigation of porcine enzymes will contribute to our knowledge about pigs in general.

The present study also revealed that activities of highly active GSTs can be underestimated in routine kinetic measurements, which affects functional comparisons. Such factors as adsorption to container walls, inhibition by some organic solvents, and dissociation of the enzyme should be taken into account. Certain analysis improvements were suggested. Besides, more accurate kinetic measurements at very low concentrations of human GST A3-3 open the possibility to use the enzyme as a tool to study activity and stability of Alpha-class monomers.
Alfa-klass glutationtransferaser från gris: en jämförande studie


Artikel I

Steroidhormoner upprätthåller saltbalansen i kroppen, reglerar ämnesomsättningen och påverkar utveckling, mognad och funktion av det reproduktiva systemet samt är viktiga för att upprätthålla graviditet. I grisar är hormonproduktionen även känd för att ha inflytande på fläskköttets kvalitet. Via en rad av reaktioner är alla steroidhormoner biosyntetiserade

I detta arbete har vi identifierat, klonat, renat fram och karaktäriserat en ny isoform av glutationtransferas från gris, gris GST A2-2, vilken har hög steroidisomerasaktivitet. Förekomsten av enzymet i olika vävnader i gris samt dess katalytiska egenskaper med olika substrat och en hämmare har jämförts med humana glutationtransferaser. Utifrån detta kan vi säga att gris GST A2-2 kan ha samma funktion i gris som humant GST A3-3 har i människa, vilket betyder att GST A2-2 troligtvis bidrar till biosyntesen av steroidhormoner.

**Artikel II**


**Artikel III**
Den aktuella studien avslöjade även att aktiviteten av högaktiva glutationtransferaser är underskattade i rutinmässiga kinetikmätningar. Detta påverkar den funktionella jämförelsen mellan olika isoformer. Faktorer såsom adsorption till behållares väggar, hämning av vissa organiska lösningsmedel och dissociation av enzymet bör tas i beaktande. Utifrån detta har vissa förbättringar av analyser föreslagits.
Slutsats
Vi har undersökt olika alfa-klass glutationtransferaser från gris samt karakteriserat två nya enzymer och genom jämförelse av humana alfa-klass glutationtransferaser föreslagit deras fysiologiska funktion. Vi har även föreslagit förbättringar för kinetikmätningar med högaktiva glutationtransferaser.

Översatt från engelska av Cecilia Blikstad och korrekturläst av Olof Moden.
Просто о сложном

Трансферазы глютатиона свиньи: сравнительное исследование класса Альфа.

Данная диссертация посвящена группе ферментов, которые важны для нормального функционирования многих организмов, в том числе организма человека. Они называются трансферазы глютатиона, что буквально означает «осуществляющие перенос глютатиона».

Каждый день с пищей, водой и воздухом мы поглощаем вещества, способные химически модифицировать клеточные белки, жиры и ДНК. Это не только искусственные добавки, но и обычные натуральные компоненты растительных и животных продуктов. Кроме того, в клетках синтезируются некоторые соединения, которые становятся «вредными» только когда их синтезируется слишком много. В организме есть защитная система, которая препятствует появлению мутаций, поддерживая функционирование клетки и организма в целом.

Глютатион – это маленькая молекула, которая присутствует во всех клетках нашего организма в довольно большом количестве. Глютатион конкурирует с белками, ДНК и жирами в реакциях с вредными веществами и, тем самым, не допускает повреждения компонентов клетки. Трансферазы глютатиона сильно ускоряют реакции глютатиона с реакционно способными соединениями (называемыми в этом случае субстратами). Защитная функция трансфераз глютатиона долгое время считалась единственной. Но вдруг выяснилось, что одна из трансфераз глютатиона человека катализирует важную стадию биосинтеза стероидных гормонов.

Изучение трансфераз глютатиона свиньи необходимо по ряду причин. Во-первых, на этих животных тестируются фармакологические свойства и токсичность лекарственных препаратов. Поскольку принцип действия многих лекарств основан на их токсичности для клеток, они тоже являются потенциальными субстратами для трансфераз глютатиона. Сравнение свойств ферментов человека и свиньи позволит предсказывать поведение лекарств без их тестирования на животных, что удешевит производство и сделает его более гуманным.

Во-вторых, свинина остается одним из основных продуктов питания. Производители заинтересованы в уменьшении своих расходов и считают важным изучать репродуктивную функцию свиней. Для
потребителей же имеет значение мясо хорошего качества, с пониженным содержанием жира и без привкуса характерного для мяса хряков. Стероидные гормоны регулируют функционирование репродуктивной системы и метаболизм жиров, а также являются причиной появления нежелательного привкуса у свинины. Возможность участия трансферазы глутатиона в биосинтезе стероидных гормонов свиней — один из предметов исследования данной работы.

В-третьих, анатомия и физиология свиней и людей очень похожа. Свиньи и люди страдают от одних и тех же заболеваний таких как ожирение, болезни сердца, гипертония, гиперхолестеринемия, дислипидемия, невосприимчивость к инсулину, атеросклероз, инфекционные заболевания. Поэтому свиней часто используют как модель организма человека при изучении многих заболеваний и пищевой аллергии, а также для изучения нормального функционирования сердца, кожи, репродуктивной системы, мозга и других органов. Трансферазы глутатиона участвуют еще и в передаче сигналов в клетке. Нарушение этого процесса является причиной некоторых заболеваний. Также хотелось бы упомянуть, что ткани свиней используются в трансплантологии для замены сердечного клапана, заживления грыж, язв, ран, операций пластической хирургии.

В данной диссертации мы установили, что свиньи и люди обладают схожим набором трансфераз глутатиона класса Альфа. Мы смогли выделить и изучить два таких фермента. Выяснилось, что один из них, а именно изоформа А2-2, скорее всего играет в организме свиней ту же роль, что и обнаруженная в тканях человека изоформа А3-3, то есть участвует в биосинтезе стероидных гормонов. Второй изученный нами фермент, изоформа А1-1, обладает свойствами характерными для защитной функции и более всего напоминает изоформу А2-2 человека. Такие выводы нам позволили сделать сравнение каталитических свойств, характера ингибирования и присутствия данных ферментов в одних и тех же тканях свиньи и человека.

Кроме того было показано, что на точность и воспроизводимость результатов при работе с очень каталитически активными трансферазами глутатиона влияют степень разбавления ферментов и присутствие органических растворителей. При большом разбавлении анализ осложняется адсорбией фермента пластиковыми поверхностями и диссоциацией. Мы предполагаем, что наш наблюдения объясняют наличие слишком расходящихся измерений в литературе, предлагаем ряд способов для преодоления затруднений и надеемся, что это улучшит качество измерений в будущем.

По результатам данной диссертации опубликованы три статьи в международных рецензируемых журналах.
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Finally,
Chroooomepiiiik! Where are you, my red animal? Miss you much.
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