POSSIBLE UTILIZATION OF SOME PHASE II ENZYMES BIOTRANSFORMATION FOR XENOBIOTIC DETERMINATIONS

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Abstract

Exposure of human or animal to xenobiotics induces complex cellular responses (metabolic changes leading to adverse outcomes) results in perturbation of many events.

The presence of xenobiotic substances (foreign molecules) in a living system can easily disrupt their balance by inhibiting, enhancing or interacting with one or more of the components or chemical reactions on which its situation depends.

The present paper presented two phase II enzymes biotransformation UDP-glucuronosyltransferases and glutathione-S-transferases and their possible utilization in vitro, for detection of some xenobiotics

Key words: biotransformation; enzymes, xenobiotics, mycotoxins, bioactivation, toxicity

1. Introduction

All living organisms are chemically dynamic systems, functioning as living entities as a result of interdependent chemical reactions. The interdependent chemical reactions in continuous high flow are maintained in a state of balance. [21]

- lipid peroxidation;
- oxidative stress;
- inflammation;
- genotoxicity;
- cytotoxicity.

Animals have evolved different enzyme systems for converting (biotransformation) xenobiotics into water soluble metabolites (hydrophilic metabolites) that can be eliminated via bile and urine.

The biotransformation (biochemical processes) is the sum of the processes by which a xenobiotic is subject to chemical change in living organisms.

The biotransformation reactions occur in liver, lungs, kidney and intestines, the differrences in enzyme activities may provide protecttion against certain xenobiotics, but can increase the toxicity of others [18]

Biotransformation is not strictly related to detoxification, because in a number of cases the metabolites are more toxic than the parent pollutants, and in that case, the term of bioactivation or toxification is used. Metabolites may have comparable or greater toxicity in organism than the parent pollutant since during the biotransformation process functional groups (hydroxyl, amine, carboxylic) are inserted into the xenobiotic by oxidation, reduction or hydrolysis reactions (phase I biotransformation).

Generally speaking, the addition of the reactive functional groups increases the chemical reactivity of the pollutant that reacts with a highly polar or ionic species (conjunction) to form a product that has much higher water solubility (phase II biotransformation) and can be excreted [3]

General pathways of xenobiotics metabolism phase I and phase II biotransformation are presented in figure 1.

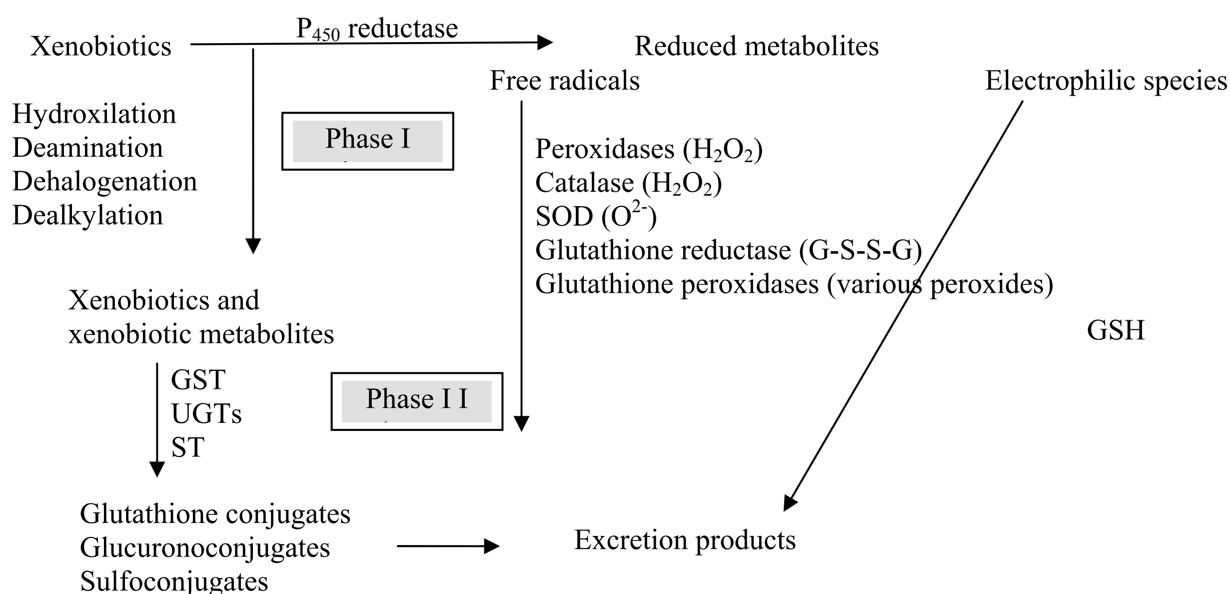

Fig. 1 General pathways of xenobiotics metabolism

Phase I and II biotransformation reactions are important to understand metabolism of endogenous molecules as well of exogenous molecules (xenobiotics).

The benefit of these mechanisms is an increase in the protective activities towards the xenobiotic to which the cell is exposed [17].

Phase I metabolism does not result in a large change in molecular weight or water solubility of the xenobiotics, but from oxidation reactions, new functional groups are produced, which facilitate interaction with phase II enzyme. Phase II metabolism (conjugation) induce increase in molecular weight and water solubility of xenobiotics or their metabolites. Phase I reactions are mediated by the cytochrome P_{450} and other enzymes: monooxygenases, peroxidases, dehydrogenases, hydrolases, amine oxidases, xantine oxidases [22].

Cytochrome P_{450} is a generic name for a superfamily of hemoproteins with an enzymatic function, catalyzing mainly a monooxigenase reaction. Among the 18 families of cytochrome P_{450} genes, 3 encode for proteins specialized in the metabolism of endogenous and exogenous compounds, which usually are the main components of a multiprotein complex for electron transfer, and exhibit broad substrate specificity [5].

In contrast with phase I enzymes biotransformation, phase II conjugating enzymes have received less attention.

2. Possible utilization of some phase II enzymes biotransformation for xenobiotic determinations

Phase II metabolism is general regarded as a detoxification pathway and inhibition of these enzymes can lead to increased toxicity of xenobiotics or their metabolites.

More of xenobiotics or their reactive electrophilic metabolites formed in phase I metabolism by cytochrome P_{450} enzymes and other enzymes, are general detoxificated by phase II metabolism in presence of conjugated enzymes.

The conjugation activities are catalyzed by UDP-glucuronosyltransferases (UGTs), which utilize as co-substrate UDP-glucuronic acid, sulfotransferases (ST) which utilize as co-substrate 3'-phosphoadenosine-5'-phosphosulphate, and glutathione-S-transferases (GSTs), which utilize as co-substrate glutathione [7, 11].

Glutathione-S-transferase and UDP-glucuronosyltransferase are major important Phase II enzymes used to conjugate electrophilic substances with glutathione and glucuronic acid [8].

The effectiveness of GSTs is dependent on the supply of GSH, which is dependent by γ-glutamylcysteine synthase and GSH synthase (the last is the rate limiting enzyme).

Because GSTs react with electrophiles, radicals and reactive oxygen species, they have a major role in the protection against oxidative stress.

In animal organisms are three family members of GSTs:

- membrane microsomal which are involved in the metabolism of endogenous compounds;
- mitochondrial;
- cytosolic which are involved in the metabolism of exogenous compounds.

The cytosolic enzymes (A, Mu, O, Pi, S, T, Z) are localized in different tissues with organ specific expression pattern and have a promiscuous substrate specificity [2, 13].

Glutathione-S-conjugates can undergo further extracellular metabolic catalyzed by γ-glutamil transferase and cysteinylglycine dipeptidase, which lead to the formation of cysteine S-conjugates. This metabolic pathway specific to thioether compounds is referred to as mercapturic acid pathway [19].

The glutathione transferases (EC.2.5.1.18) are a family of enzymes that catalyse the nucloephilic interaction of reduced glutathione with a wide range of electrophilic compounds (conjugation reaction). This reaction is a critical step in the cellular detoxication of endogenous and exogenous (xenobiotics) substrates because the conjugates are more soluble than the original compounds and are easy removed from the cell. GSTs are found in all aerobic organisms, and act as detoxifying enzymes.

The cytosolic GSTs are homodimers or heterodimers, and each monomer has two domain first hydrophilic and second hydrophobic. The cytosolic glutathionetransferases contain a glutathione binding site which bind glutathione, and a hydrophobic substrate binding site, in which the substrate binds (substrates) for reaction with glutathione [6].

GSTs are dimmers of approximately 50 kDa, and the active site of these enzymes consists of a GSH-binding site (G site) and xenobiotic binding site (H site).

Each subunit of the dimmer has its active site with tyrosine residue or serine residue, where stabilizes the thiolat anion of GSH, during of conjugation reaction.

GSTs which is found in plants is able to detoxify several classes of herbicides: triazines (atrazine), thiocarbamates, chloroacetamides and diphenylethers [16].

Substrates of glutathione conjugation can be electrophile xenobiotics as well as electrophilic phase I metabolites, or other phase II conjugates.

Glutathione conjugation can occur spontaneously or more efficient catalyzed by GSTs and glutathione conjugates are not deconjugated in the same manner as sulphate or glucuronide metabolites. The peptide bonds in the glutathione molecule of the metabolite may be hydrolyzed in two steps to form cysteinylglycine metabolite and cysteine metabolite, which by N-acetylation to form mercapturate metabolites [15].

Glutathione transferases, in addition to their enzymatic role has also linking role because binds to non-substrate lipophilic molecules (fatty acids, bile acids, hemin, bilirubin, drugs) and are involved in the storage and transport of these molecules in the aqueous phase.

Non-substrate ligands inhibit in a competitive or noncompetitive mechanism enzymatic activity of GST, which induce an inter-relationship between binding and enzymatic functions [10].

For determination of chloro-xenobiotics it can be use the ability of GST to catalyse the conjugation of GSH with chloro-xenobiotic with concomitant proton release (HCl).

The concentration of released proton is proportional to the amount of conjugated substrate and is measured potentiometric using a pH electrode (Fig. 2). The concentration of released protons reflects the progress of the xenobiotic/GSH conjugation reaction and can be measured by pH change.

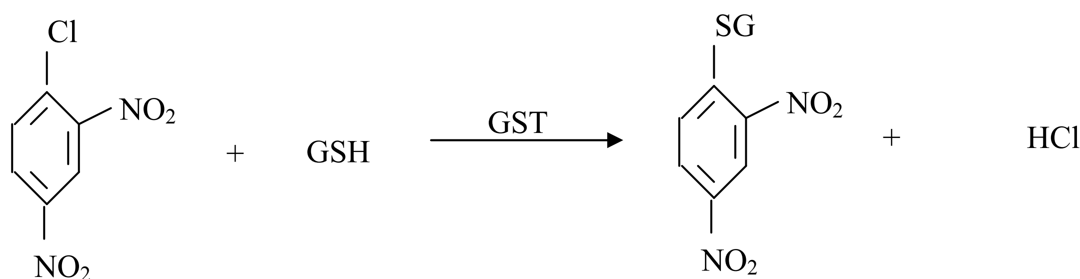

Fig. 2 Reaction of 2,4-dinitrochlorobenzene with glutathione in presence of glutathione S-transferase

For determination of chloro-herbicides can be use the ability of GST to catalyse the conjugation of GSH with these pesticides (Fig. 3).

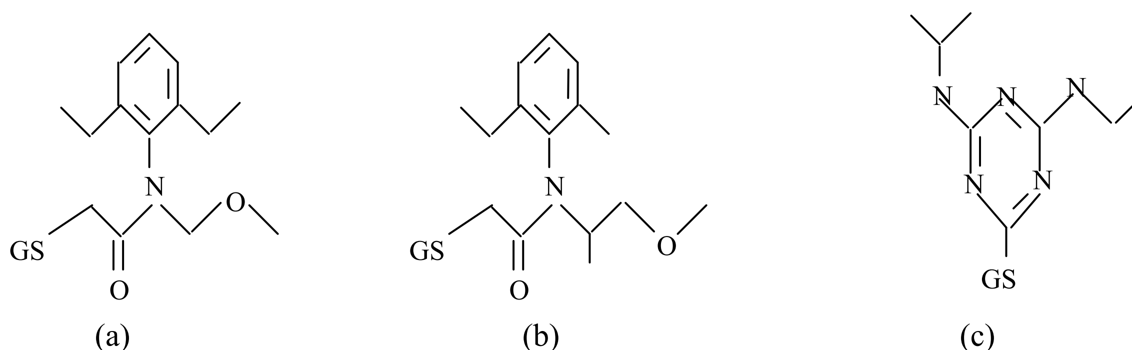

Fig. 3 Structure of herbicide–GSH conjugates (a) alachlor; (b) metalachlor; (c) atrazine

Glucuronidation is a phase II metabolism reaction (major detoxification pathway) in all vertebrates, in which glucuronic acid is conjugated to a small lipophilic endogenous or exogenous compound (drug, pollutant, dietary compound, hormone, and toxin). The process is an S_{N2} reaction where the configuration of the glucuronic acid changes from α- to β-anomer and the most products are O- and N-glucuronides.

Glucuronides have high hydrophility as an aglicone and are more easily excreted from body.

The glucuronidation is catalyzed by UDP-glucuronosyltransferases that are classified into families UGT1 and UGT2 and 3 subfamilies (UGT1A, UGT2A, UGT2B [1].

The glucuronidation reaction is catalyzed by a family of membrane-bond isoenzymes the UDP-glucuronosyl-transferases and involves the transfer of a glucuronic acid residue from UDP-glucuronic acid to substances possessing hydroxyl-, amino-, carboxyl-, or sulfhydril groups, converting them to water-soluble β-D-glucuronides [4]. The structure of β-O-glucuronide is presented in figure 4.

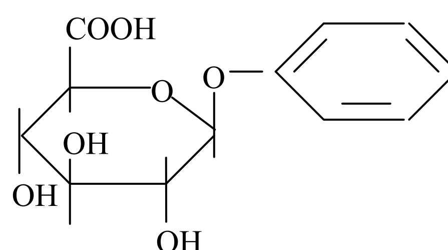

Fig. 4 Structure of β-O-glucuronide

UDP-glucuronosyltransferases are located mainly in the endoplasmatic reticulum of liver and to a lesser extent, in all other mammalian tissues.

UDP-glucuronosyltransferases play an important cytoprotective role, either by preventing the accumulation of potentially toxic xenobiotics or by avoiding their subsequent bioactivation to even more toxic reactive intermediates.

Phenol derivatives and most of micotoxins,

possess in their structures one or more hydroxyl groups and can react with UDP-glucuronic acid to forms glucuronides.

Micotoxins are a group of secondary metabolites of fungi that grow on a variety of food and feed at any stage of production.

The effects on the health of humans and animals including: carcinogenic, mutagenic, teratogenic and immunosupresive effects.

Phenols and phenol derivatives may be metabolites of normal metabolism or xenobiotics and their low solubility induces in humans the development of toxicities.

The human UGTs are bound to the internal membrane, a location which confers advantages because these enzymes have direct access to metabolites produced by phase I biotransformation reactions, and disadvantages because restricts the access of xenobiotics, cofactors and glucuronidated products to and from the active enzymatic site.

The glucuronidation reactions transfers glucuronic acid from UDP-glucuronic acid to functional groups (aromatic and aliphatic alcohols, carboxylic acids, amines, sulfhydryl) on hydrophobic xenobiotics or their metabolites to form the respective β-glucuronides (O-, C-, N-, or S-glucuronide) [9].

The factors that determine UGT substrate specificity are not well understood, but small and hydrophobic molecules are often glucuronidated by multiple UGT enzymes.

The UGT enzymes are associated in vitro and in vivo as homo or hetero-oligomrs, and oligomerization may be required for enzymatic activity, or in some cases may alter the glucuronidation reactions.

The studies on glucuronidation have demonstrating the following generalizations regarding the catalytic activity of UGT enzymes [12, 14]:

- all are capable of forming O-linked glucuronides;
- UGT1A4, UGT1A9, and UGT2B7 are responsible for the conjugation of carboxilyc acids;
- UGT1A4 and UGT1A3 are capable of forming N-glucuronides with amines;
- UGT1A1 is responsible to form the glucuronide with bilirubin;
- UGT1 and UGT2 catalyze the glucuronidation of steroids;
- UGT2B7 is responsible for the glucuronidation of opioids.

Mycotoxins are potentially hazardous to man and domestic animals and are responsible for many diseases and they can also enter the human food chain via animal products (eggs, milk, cheese, meat) as result of eating contaminated food.

The mycotoxins are chemically and structurally diverse, are synthesized by simple biosynthetic reactions from small molecules (lactates, pyruvates, acetates) and have a diverse range of toxic effects (acute or chronic): carcinogenic, mutagenic, nephrotoxic, teratogenic, hepatotoxic, neurotoxic, immunosuppresive, fertility inhibition.

Mycotoxins are stable compounds (to survive storage and processing), are difficult to remove, and the best method of control is prevention.

Ochratoxins (OTA) are dihydroisocoumarins produced by several Aspergillus and Penicillium and are natural opportunistic biodeterioration agents (Fig.5). The concentration of OTA in most common cereals and other starch rich foods does not exceed a few ppb [20].

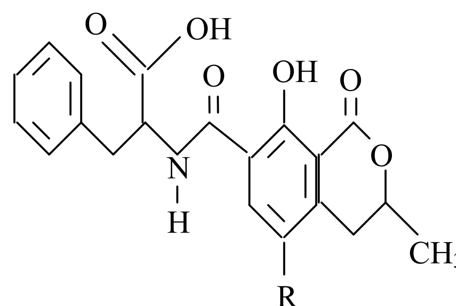

Fig. 5 Structures of ochratoxins: ochratoxin A (R: Cl); ochratoxin B (R: H)

Zwaralenone (ZEA) is produced as a secondary metabolite by a number of Fusarium species and their concentration in food can vary from a few micrograms up to 300 mg/Kg.

Two hydroxyl groups are presented in ZEA structure (Fig. 6) which can react as a substrate with UDP-glucuronic acid as a co-substrate, in presence of UGT (conjugation).

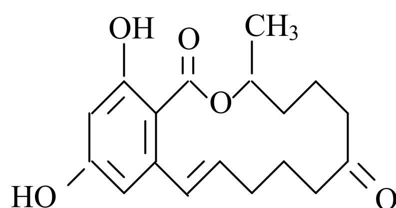

Fig.6 Chemical structure of zearalenone

Zearalenone is rapid absorbed after oral administration and two biotransformation pathways have been suggested:

- reduced to zearalenol by hydroxylation in presence of some dehydrogenases (phase I biotransformation);
- conjugation of zearalenone or its reduced metabolites (phase II biotransformation) with glucuronic acid, catalyzed by UDP-glucuroniltransferases [23].

3. Conclusion

In this paper a summary of the biotransformation process has been described, which is formed from two phases, insisting on phase II.

Two phases II enzymes biotransformation has been characterized, and presented some possible reactions which can be catalyzed *in vitro*.

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