Microsomal UDP-Glucuronyltransferase in Rat Liver: Oxidative Activation

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Abstract: Activation of microsomal UDP-glucuronyltransferase (UDPGT) activity by treatment of hepatic microsomes with either detergents or Fe^{3+}/ascorbate pro-oxidant system has been reported; however, definite mechanisms underlying these effects have not been clarified. In this work, we characterize Fe^{3+}/ascorbate-induced activation of UDPGT activity prior to solubilization with Triton X-100 and after the oxidation process provoked the solubilization of the enzyme. We observed a time-dependent increase in UDPGT activity up to 20 min. incubation of the microsomes with Fe^{3+}/ascorbate (3-times); after 20 min. incubation, however, we observed a time-dependent decrease in this activity to basal levels after 4 hr incubation. Treatment of microsomes with 0.1% Triton X-100 (5 min.) lead to a similar increase in UDPGT activity; higher detergent concentrations produced a dose-dependent decrease in this activity to basal levels with 1% Triton X-100. Interestingly, UDPGT activity was susceptible to activation only when associated to microsomal membranes and the loss of activation correlated with the solubilization of this activity. UDPGT activation by either Fe^{3+}/ascorbate or Triton X-100 was correlated with an increase in *p*-nitrophenol apparent K_m and V_{max} values. This activation was prevented or reversed by the reducing agents glutathione, cysteine or dithiothreitol when it was induced by the Fe^{3+}/ascorbate. Furthermore, the latter provoked a significant decrease in microsomal thiol content, effect not observed after treatment with Triton X-100. Our results suggest that the main mechanism responsible for Fe^{3+}/ascorbate-induced UDPGT activation is likely to be the promotion of protein sulfhydryl oxidation; this mechanism appears to be different from detergent-induced UDPGT activation.

UDP-glucuronyltransferase isoenzymes (UDPGT, EC 2.4.1.17) comprise a superfamily of integral microsomal glycoproteins with their catalytic domain located in the lumen of the endoplasmic reticulum. These enzymes catalyze the conjugation of glucuronic acid with different substrates, including many structurally different compounds, such as phenols, carboxylic acids, aliphatic and aromatic alcohols, certain aromatic amines, and physiological molecules, including bile acids, sex hormones, and serotonin (Sánchez & Tephly 1973; Kasper & Henton 1980; Martin & Black 1994).

Since the UDPGT catalytic domain is oriented to the endoplasmic reticulum lumen, there is a lipid physical constraint for the access of UDP-glucuronic acid (UDPGA) to the enzyme active site. In fact, a number of methods and conditions that perturb the lipid phase of the microsomal membrane have been tested, all of them leading to an increase in UDPGT activity. These methods include sonication and treatment with phospholipases, bilirubin, organic solvents, detergents and lipid peroxidation-inducing agents (Bentley *et al.* 1979; Dannenberg *et al.* 1990; Fulceri

et al. 1994). A compartmentation-based hypothesis has been widely accepted and developed in order to explain the activation phenomenon. The destruction of the vesicular structure of microsomes and the passage of UDPGA to the enzyme's active site through holes in the microsomal membranes result in an increase in UDPGT activity, supporting a complex model of compartmentation. However, inhibition of UDPGT activity by treatment of microsomes with elevated concentrations of detergents or for extensive lengths of time has also been reported (Sánchez & Tephly 1973; De Groot *et al.* 1985; Lear *et al.* 1991). This conflicting evidence suggests different mechanisms underlying the activation of UDPGT.

As stated above, all treatments used to study UDPGT activation lead to conformational changes on the protein and/or lipid environment. However, treatment of microsomes with oxidant agents, such as the Fe^{3+}/ascorbate pro-oxidative system, induce additional oxidative effects on endoplasmic reticulum proteins, including UDPGT that have not been addressed. It is important to note that UDPGT has 3 cysteine residues in its amino acidic chain (Gorski & Kasper 1977; Zakim & Dannenberg 1992); thus, it is reasonable to suggest that these redox-sensitive groups may be involved in the UDGPT catalytically-active conformation. In this work, we studied the oxidative effect of Fe^{3+}/ascorbate system on rat liver microsomal UDPGT activity. We studied how different conditions affected UDPGT

activity, such as changes in the lipid integrity of the microsomal membrane, potential solubilization of UDPGT and sulfhydryl oxidation of microsomal proteins. In order to discriminate these changes, we treated hepatic microsomes with Triton X-100, which will induce changes in the lipid integrity of microsomal membrane, or Fe^{3+}/ascorbate, which will additionally lead to protein oxidation.

We found that both Fe^{3+}/ascorbate and Triton X-100 treatments of rat liver microsomes led to an increase in UDPGT activity only when assays were performed in conditions at which all of the enzyme activity was still bound to the membrane. In the other hand, when treatments induced UDPGT activity solubilization, the activation effect was lost. Only the oxidative, and not the detergent-induced UDPGT activation was prevented and reversed by reducing agents. Overall, our data support two different mechanisms for the UDPGT activation: a) changes in lipid environment of the enzyme, and b) conformational changes of the enzyme produced by oxidative stress. Physiological and pathological implications of these findings are discussed.

Materials and Methods

Chemicals. Cysteine, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), 2,4-dithiothreitol (DTT), glutathione (GSH), p-nitrophenol (PNP), UDP-glucuronic acid (ammonium salt) (UDPGA) and Triton X-100 were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of analytical grade.

Animals. Adult male Sprague Dawley rats (200–250 g), derived from a stock maintained at the University of Chile, were used. They were allowed free access to pelleted food, maintained with controlled temperature (22°) and photoperiod (lights on from 07:00 a.m. to 7 p.m. hr). All animal procedures were performed using protocols approved by the Institutional Ethical Committee of the Faculty of Chemical and Pharmaceutical Sciences, University of Chile.

Microsomal preparation. Animals were fasted for 15 hr with water *ad libitum* and sacrificed by decapitation. Livers were perfused *in situ* with 4 volumes of 25 ml 0.9 % W/V NaCl, excised, and placed on ice. All homogenization and fractionation procedures were performed at 4° and all centrifugations were performed using either a Suprafuge 22 Heraeus centrifuge or an XL-90 Beckmann ultracentrifuge. Liver tissue (9–11 g wet weight), devoid of connective and vascular tissue, was homogenized with five volumes of 0.154 M KCl, with eight strokes in a Dounce Wheaton B homogenizer. Homogenates were centrifuged at 9,000 × g for 15 min., and sediments were discarded. Supernatants were then centrifuged at 105,000 × g for 60 min. Sediments (microsomes, enriched in endoplasmic reticulum) were stored at −80° until use. Protein determinations were performed according to Lowry *et al.* (1951).

Treatment of microsomes. Rat liver microsomal preparations (1 mg/ml) were treated either with 450 μM Fe^{3+}/1.0 mM ascorbate for different periods of time (2–240 min.), at 37°, or with different concentrations (0.1–1.0% V/V) of Triton X-100 for 5 min. at 20°.

UDPGT activity. p-Nitrophenol conjugation was studied essentially according to Letelier *et al.* (1985). Activity was assayed determining the remaining p-nitrophenol after 15 min. incubation at the following conditions: 0.5 mM p-nitrophenol; 2 mM UDPGA, 100 mM Tris HCl, pH 8.5, 4 mM $MgCl_2$ and 2 mg/ml microsomal protein. Control samples were performed in absence of UDPGA. Reactions were stopped adding trichloroacetic acid (5% final concentration);

samples were then centrifuged at 10,000 × g for 10 min. in a Suprafuge 22 Heraeus centrifuge and NaOH was added to the mixture in order to achieve a 0.5 M final concentration. Remaining p-nitrophenol was determined at 410 nm using control samples of known p-nitrophenol initial concentration as standards. Reaction rates were determined at conditions where product formation were linearly-dependent to time and protein concentration.

Determination of UDPGT solubilization. Following each treatment of microsomes, samples were centrifuged at 105,000 × g for 60 min. in a XL-90 Beckmann ultracentrifuge. Pellets (P-105) were used to estimate UDPGT remaining activity in membranes. Supernatants (S-105) were used to assess solubilization of UDPGT activity. UDPGT activities were assayed as above.

Lipid peroxidation assay. The extent of lipid peroxidation following Fe^{3+}/ascorbate preincubation was estimated by determining TBARS, essentially according to Díaz-Araya *et al.* (1998).

Microsomal thiol content. Microsomal thiols were titrated with DTNB before and after a Fe^{3+}/ascorbate preincubation, as described by Jiménez *et al.* (1997). Thiol concentration was estimated by the equimolar apparition of 5-thio-2-nitrobenzoic-acid ($\varepsilon_{410}=13,600$ M^{-1} cm^{-1}).

Statistical analysis. Data groups (means±S.E.M.) were compared using Student's t-test for paired observations. Statistical significances were considered at $P<0.05$.

Results

Fe^{3+}/ascorbate and Triton X-100 effects on microsomal UDPGT activity.

Preincubation of microsomes from 2 to 20 min. with Fe^{3+}/ascorbate (fig. 1A) displayed a time-dependent significant increase in microsomal UDPGT activity. While maximum activity (3-times increase) was observed at 20 min., longer preincubation periods led to a progressive loss of the activation, reaching a basal activity value at 4 hr preincubation.

Similarly, UDPGT activation was detected after preincubation of microsomes with Triton X-100 (fig. 1B). Triton X-100 preincubation (0.1–0.5%) led to an increase in microsomal UDPGT activity in a dose-dependent manner, with a maximum activity (3.8-times increase) observed at 0.5%. Higher concentrations of this detergent produced a loss of UDPGT activation in a dose-dependent manner, reaching basal activity level with 1% detergent concentration.

Solubilization of microsomal UDPGT activity induced by Fe^{3+}/ascorbate or Triton X-100. In order to assess the solubilization of microsomal UDPGT activity induced by changes in the enzyme's lipid environment induced by both systems, microsomal suspensions were further centrifuged after each treatment; UDPGT activity was assayed in soluble (S-105) and particulate fractions (P-105) after each treatment. As summarized in table 1 (selected data), both treatments showed specific conditions at which all of the UDPGT activity remained associated to the P-105 fraction (20 min. Fe^{3+}/ascorbate and 0.1% Triton X-100 preincubations). Longer periods of preincubation with Fe^{3+}/ascorbate (30 to 240 min.) or higher concentrations of Triton X-
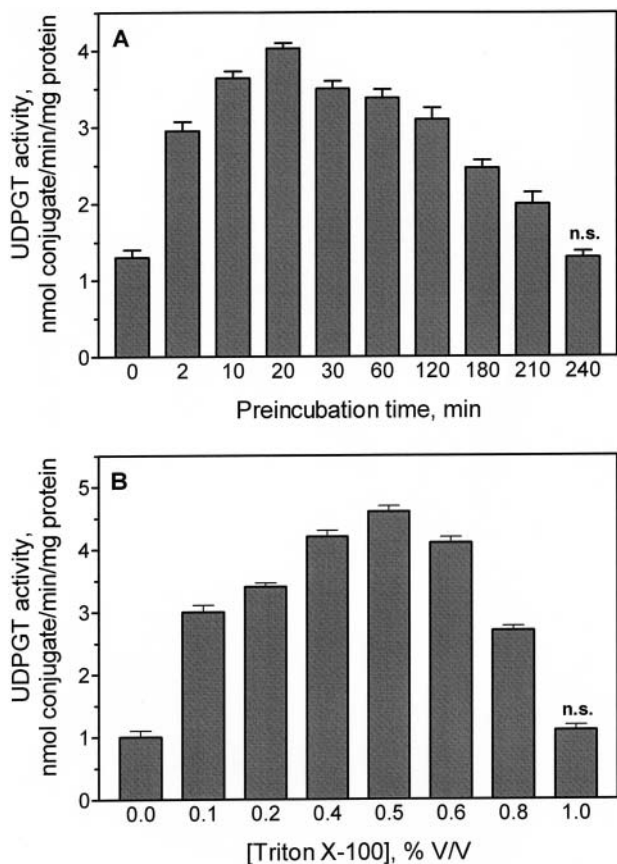
Fig. 1. Fe^{3+}/ascorbate or Triton X-100 effects on UDPGT activity. Microsomes (1 mg/ml) were preincubated for different periods of time with 450 μM Fe^{3+}/1 mM ascorbate (**A**), or with different Triton X-100 concentrations (**B**), as detailed in Materials and Methods; following preincubations, UDPGT activity was measured as described in Materials and Methods. Activity values are expressed as nmol of conjugate/min/mg of microsomal protein (mean±S.E.M. of at least 4 independent experiments). n.s. stands for not significant compared to control microsomal suspensions (P>0.05).

Table 1.

Solubilization of microsomal UDPGT activity after preincubation of rat liver microsomes with fe^{3+}/ascorbate or Triton X-100.

Condition	Microsomal activity	P-105 associated activity	S-105 associated activity
Control	1.26±0.06	1.27±0.05	N.D.
Fe^{3+}/ascorbate preincubation			
20 min.	3.50±0.16	3.60±0.16	N.D.
120 min.	2.99±0.13	0.84±0.03	2.14±0.10
240 min.	1.30±0.10	0.88±0.05	0.29±0.08
Triton X-100 preincubation			
0.1% V/V	3.10±0.15	3.07±0.14	N.D.
0.5% V/V	4.20±0.19	0.87±0.04	3.40±0.15
1.0% V/V	1.10±0.08	N.D.	1.02±0.11

Microsomal activity refers to the total UDPGT activity assayed in the preparations, without further centrifugation. P-105 and S-105 correspond to pellet and supernatant of rat liver microsomes (1 mg/ml) respectively, obtained by centrifugation following preincubation with Fe^{3+}/ascorbate or Triton X-100 under conditions detailed in Materials and Methods. UDPGT activity values are expressed as nmol of conjugate/min./mg of microsomal protein (mean±S.E.M. of at least 4 independent experiments). All values are statistically different from their respective controls (P<0.05). N.D. stands for not detectable activity.

100 (0.2 to 1.0%) led to a progressive solubilization of the UDPGT activity into the S-105 fraction.

Oxidative effects induced by Fe^{3+}/ascorbate on microsomes.

Microsomal lipid peroxidation. Fe^{3+}/ascorbate produced a time-dependent linear increase in microsomal lipid peroxidation (fig. 2A). Addition of thiol reducing agents, such as GSH, cysteine and DTT significantly prevented lipid peroxidation induced by this pro-oxidant system (fig. 2B).

Microsomal thiol content. Microsomal preincubation with Fe^{3+}/ascorbate led to a rapid decrease in thiol content (2 min.). This decrease remained constant up to the maximum preincubation period assayed (20 min., fig. 3). Triton X-100 preincubation (0.1%) did not modify microsomal thiol content (fig. 3).

Activation of UDPGT induced by Fe^{3+}/ascorbate. Effects of sulfhydryl reducing agents. As shown in fig. 4, addition of

GSH, cysteine and DTT both protected (fig. 4A) and reversed (fig. 4B) the Fe^{3+}/ascorbate-induced activation effect. Furthermore, DTT prevented (fig. 5A) and reversed (fig. 5B) the activation of UDPGT induced by Fe^{3+}/ascorbate in a concentration-dependent manner.

UDPGT kinetic parameters in the presence of Fe^{3+}/ascorbate and Triton X-100. To evaluate possible changes in apparent kinetic parameters of UDPGT activity induced by oxidation and/or solubilization, we estimated the apparent kinetic constants under selected conditions (summarized in table 2): i) microsomes without treatment, ii) microsomes preincubated for 20 min. with Fe^{3+}/ascorbate, iii) microsomes preincubated with 0.1% Triton X-100 for 5 min., iv) soluble fraction obtained after preincubation of microsomes for 240 min. with Fe^{3+}/ascorbate and v) microsomes treated with 0.5% Triton X-100 for 5 min. Under conditions ii and iii, all UDPGT activity remained associated to the microsomal membranes. Preincubation of microsomes with either Fe^{3+}/ascorbate for 20 min. and 0.1% Triton X-100 for 5 min. increased apparent K_m (6–7-times), V_{max} (8–9-times) and catalytic efficiency (1.3-times) compared to control values.

Finally, apparent K_m and V_{max} of solubilized UDPGT were higher than their control values. Four hr of Fe^{3+}/ascorbate preincubation led to a 27- and 3.2-times increase in apparent K_m and apparent V_{max} of UDPGT activity, respectively. In contrast, 5 min. preincubation of microsomes with 0.5% Triton X-100 led to a 3.1 and 5.3-times in apparent K_m and apparent V_{max}, respectively. Interestingly, while preincubation of microsomes with Fe^{3+}/ascorbate (4 hr) led to a decrease in solubilized UDPGT catalytic effici-
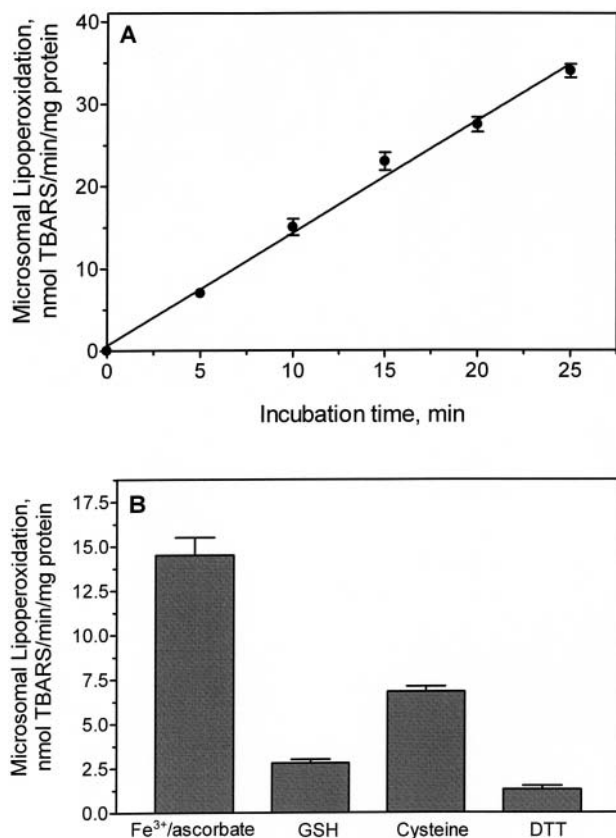
Fig. 2. Microsomal lipid peroxidation induced by Fe^{3+}/ascorbate. **A)** Microsomes (1 mg/ml) were incubated with 450 μM Fe^{3+}/1 mM ascorbate for different periods of time (up to 20 min.) and then microsomal lipid peroxidation was determined as described in Materials and Methods. **B)** Microsomes (1 mg/mL) were preincubated with or without GSH, cysteine or DTT (2 mM final concentrations) for 15 min. before a Fe^{3+}/ascorbate treatment for 20 min. Afterwards, lipid peroxidation was assayed as described in Materials and Methods. Data correspond to the mean±S.E.M. of at least 4 independent experiments.
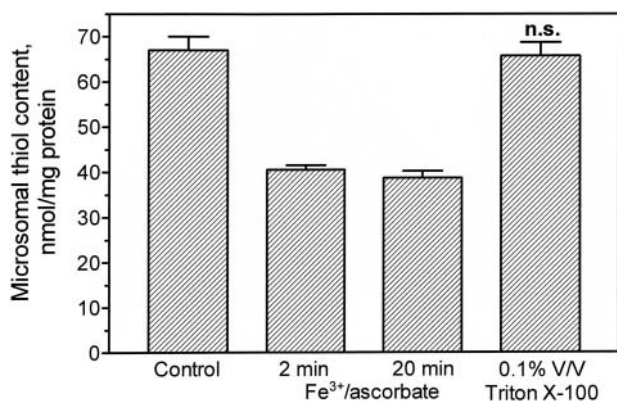
Fig. 4. Effect of reducing agents on the UDPGT activation induced by Fe^{3+}/ascorbate. Microsomes (1 mg/ml) were incubated with or without GSH, cysteine or DTT (2 mM final concentrations) for 15 min. either before (**A**) or after (**B**) incubation with 450 μM Fe^{3+}/1 mM ascorbate for 10 min. UDPGT activity was measured as described in Materials and Methods. Data correspond to mean±S.E.M. of at least 4 independent experiments. Dotted lines indicate control values for UDPGT activity (with no treatment). n.s. stands for not significant.

ency (about 8-times), 0.5% Triton X-100 preincubation led to an increase in this catalytic efficiency (1.7-times).

Discussion

Molecular mechanisms underlying UDPGT activation induced by oxidant or detergent agents has not been clarified; thus, the aim of this study was to discriminate physical from chemical changes involved in UDPGT activation. It has been reported that the treatment of microsomes with either detergents or the Fe^{3+}/ascorbate pro-oxidative system reduces the physical constraint imposed by microsomal membranes for the access of UDPGA to the active site of UDPGT. Effects of these treatments are time- and concentration-dependent (Sánchez *et al.* 1981; De Groot *et al.* 1985; Fulceri *et al.* 1994).

It has been proposed that an environmental physical change would explain the activation of UDPGT observed in the presence of these agents. However, there is also evidence of inhibition of the UDPGT activity when the microsomes are treated with both high concentrations or pro-

Fig. 3. Effect of Fe^{3+}/ascorbate or Triton X-100 on microsomal thiol content. Microsomes (1 mg/ml) were incubated with and without 450 μM Fe^{3+}/1 mM ascorbate for 20 min. or with 0.1% V/V Triton X-100 for 5 min. before titration of microsomal thiol content, as detailed in Materials and Methods (mean±S.E.M. of at least 4 independent experiments). n.s. stands for not significant.
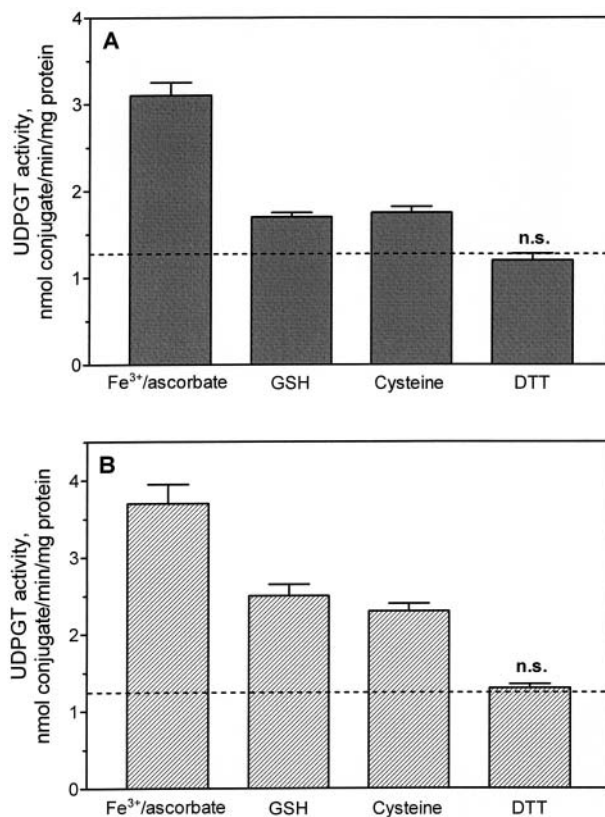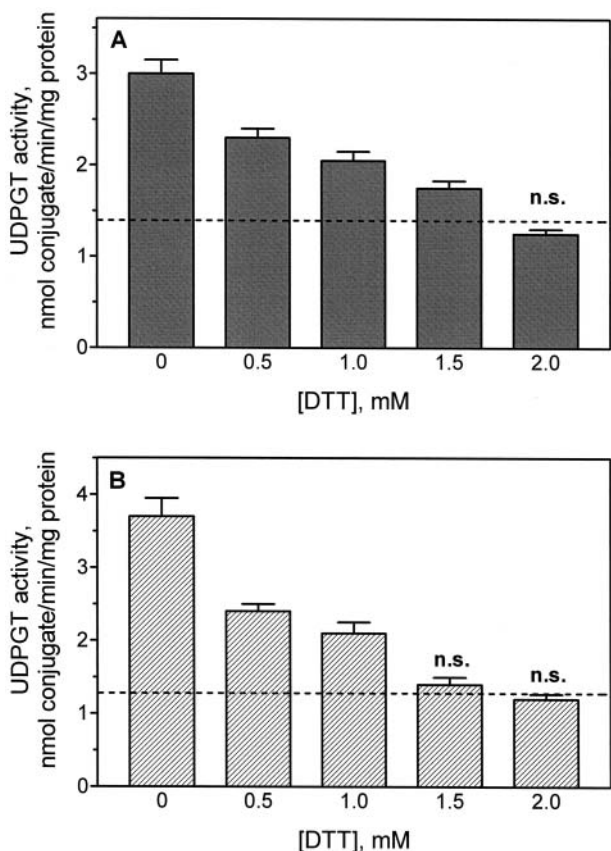
Fig. 5. Effect of DTT on UDPGT activation induced by Fe^{3+}/ascorbate. Microsomes (1 mg/ml) were incubated with different DTT concentrations for 15 min. either before (**A**) or after (**B**) incubation with 450 μM Fe^{3+}/1 mM ascorbate for 10 min. UDPGT activity was assayed as described in Materials and Methods. Data correspond to mean±S.E.M. of at least 4 independent experiments. Dotted lines indicate control values for UDPGT activity (with no treatment). n.s. stands for not significant.

Table 2.

Microsomal UDPGT kinetic constants following treatment of rat liver microsomes with Fe^{3+}/ascorbate or Triton X-100.

Condition	$K_{m\ app}$ PNP, (mM)	$V_{max\ app}$,	Catalytic efficiency
Control	0.56±0.02	2.78±0.13	4.96
Membrane-associated activity following:			
20 min. Fe^{3+}/ascorbate	3.98±0.12	26.16±0.92	6.57
0.1% V/V Triton X-100	3.28±0.16	21.49±0.86	6.55
Solubilized activity following:			
240 min. Fe^{3+}/ascorbate	15.03±1.3	8.94±0.93	0.59
0.5% V/V Triton X-100	1.76±0.06	14.87±0.67	8.45

Rat liver microsomes (1 mg/ml) were preincubated with Fe^{3+}/ascorbate or Triton X-100 as described in Materials and Methods. Samples were further centrifuged to obtain a membrane-associated fraction (P-105) and a soluble fraction (S-105), as detailed in Materials and Methods. Kinetic constants were estimated from Lineweaver-Burk plots (not shown). $V_{max\ app}$ values are expressed as nmoles of conjugate/min/mg of microsomal protein (mean±S.E.M. of at least 4 independent experiments). Values are statistically different from their respective controls ($P < 0.05$).

longed periods of incubation with detergents (Sánchez *et al.* 1973; De Groot *et al.* 1985). UDPGT catalyzes a reversible reaction, either catalyzing the conjugation of its substrate with UPDGA or the reverse reaction, in which UDP is the co-factor; thus, the actual enzymatic activity is the resultant of these two reactions. Zakim & Vessey (1975) studied changes in the affinity constants for UPDGA and UDP induced by treatment of UDPGT with either phospholipase A or Triton X-100; they found that Triton X-100 under certain conditions increased the activity for the reverse reaction and inhibited the forward reaction. These findings could account for the total loss of UDPGT activation observed when microsomes were treated with 1% Triton X-100. It is also possible that higher detergent concentrations would interfere with intramolecular lipophilic interactions, leading to a decrease in UDPGT activity. In addition, we observed that treatment of microsomes with the Fe^{3+}/ascorbate pro-oxidant system induces different physicochemical changes, such as: lipid peroxidation, decrease in microsomal thiol content and/or microsomal protein solubilization, including that of UDPGT. In contrast, Triton X-100

treatment is likely to promote only a solubilization effect (fig. 2A and 3, and table 1).

Noteworthy, maximum oxidative activation of UDPGT activity was reached at 20 min. of Fe^{3+}/ascorbate preincubation, a condition at which all of the activity remained associated to the microsomal membrane (P-105), even though there is a significant lipid peroxidation phenomenon in this condition (fig. 1A and 2A, table 1). Our oxidative activation results are in agreement to those observed by Bentley *et al.* (1979), which also report a biphasic effect of the Fe^{3+}/ascorbate system on UDPGT activity. Nevertheless, this oxidative activation is most likely to be explained by a protein-oxidative mechanism, and not only by the shortening of phospholipid hydrocarbon chains, a membrane perturbation mechanism proposed by these authors. Our data further corroborated this redox-dependent activation mechanism, i.e. prevention and reversion of this activation by thiol reducing agents (fig. 4). Moreover, DTT was able to completely prevent and reverse this activation effect in a concentration-dependent manner (fig. 5). Treatment with either reducing agents did not lead to any changes in Triton X-100-induced UDPGT activation (not shown), suggesting that Triton X-100 did not induce any oxidative effects on UDPGT.

Fe^{3+}/ascorbate pro-oxidative system and Triton X-100 alter the membrane fluidity through different mechanisms, the first modifies the lipid structure and the later solubilizes the membrane lipids. These changes are not reversible. However, we found that the oxidative activation of UDPGT, a chemical mechanism, was totally reversed by DTT, a thiol reducing agent and greatly by endogenous reducing agents (GSH, cysteine) (fig. 4A). Triton X-100 activation was not affected by these reducing agents (data not shown). These results may indicate that the main mechanism involved in the activation of UDPGT is likely to be the oxidation of cysteinyl residues.

The amino acid composition of the UDPGT isoenzymes (approximately 531 amino acids) displays a relatively low sulfur content accounted for 3 cysteine and 14 methionine residues per mole of transferase (Gorski & Kasper 1977; Zakim & Dannenberg 1992). Therefore, it is possible that redox-sensitive cysteine residues are involved in the regulation of UDPGT activity. This suggestion is further supported since UDPGT activity appears to display reactivity towards sulfhydryl alkylating agents, such as *p*-hydroxymercuri-benzoate, *p*-chloromercuriphenyl-sulfonate, and mersalyl acid; apparently, these agents act in a concentration-dependent biphasic manner and differently towards different UDPGT isoforms (Isselbacher *et al.* 1962; Sánchez *et al.* 1981). Ikushiro *et al.* (2002) reported UDPGT activation induced by the non-physiological reducing agent, DTT, suggesting that protein reduction and not oxidation is the mechanism underlying UDPGT activation. The experimental conditions used by these authors, however, are different from those used in this study: UDPGT activity was significantly activated by DTT in concentrations higher than 5 mM in the absence of oxidants. In contrast, in our study we demonstrated reversion of the Fe^{3+}/ascorbate effects by DTT with 2 mM being the highest concentration. It is possible that higher DTT concentrations may lead to the reduction of additional microsomal elements, which in turn activate UDPGT. Further experiments are needed to address these postulates.

If cysteine residues are involved in catalytic activity of UDPGT, redox changes on these amino acid residues might alter apparent kinetic parameters of the enzyme. After 20 min. treatment with Fe^{3+}/ascorbate, both apparent K_m for p-nitrophenol and apparent V_{max} increased 7 and 10-times, respectively; although the apparent affinity for p-nitrophenol decreased, the increase of catalytic efficiency (1.3 times, table 2) was probably due to an increase in the apparent K_m for UDPGA. Treatment of microsomes with 0.1% Triton X-100 produced a similar increase in these kinetic constants (table 2). Drastic experimental conditions using both agents lead to the solubilization of UDPGT and induced clearly distinguishable changes in these kinetic constants. Changes on the solubilized enzyme induced by the treatment of microsomes with Fe^{3+}/ascorbate were far more drastic than those observed with Triton X-100 treatment. Consequently, UDPGT catalytic efficiency was the lowest at this oxidative condition, approximately 8- lower than its control (table 2).

It is widely accepted that enzymatic reactions in a cellular environment are not likely to proceed in the same way under *in vitro* conditions, thus the V_{max} measured *in vitro* (equilibrium kinetics) does not represent actual *in vivo* conditions (flow kinetics). Consequently, it is of great importance to work with enzymes bound to microsomes, conditions that are more in agreement *in vivo* conditions. Our findings on maximum activity when all of the protein was still bound to the membrane fractions and not solubilized further explain the activation of UDPGT under physiological conditions. UDPGT is located next to the cytochrome P450 oxidative system in the endoplasmic reticulum, this oxidative activation mechanism may be physiologically relevant for xenobiotic biotransformation processes since: i) metabolites generated by the cytochrome P450 system are substrates of UDPGT, and ii) cytochrome P450 system is an acknowledged source of pro-oxidant agents. For instance, it is known that nitrofurantoin and nifurtimox are drugs that induce oxidative stress by nitro-reduction, a reaction catalyzed by the cytochrome P450 reductase, a member of the cytochrome P450 system. We have also demonstrated that these lipophilic drugs, in oxidative conditions, are capable of inducing the activation of UDPGT (Letelier *et al.,* 2004).

In summary, treatment of microsomes with either Fe^{3+}/ascorbate or detergent induce activation of UDPGT activity by different mechanisms: the former involves mainly oxidative processes on proteins and lipid membrane components, while the latter only changes in the lipid conformation of the microsomal membrane. The oxidative activation phenomenon may account for a physiological oxidative crosstalk between phase I and II drug metabolizing enzymes in order to enhance detoxication of lipophilic substances. This is an interesting field of research and we are currently testing this hypothesis.

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