PARADOXICAL DETERGENT EFFECTS ON MICROSOMAL PROTEIN DISULPHIDE ISOMERASE

Robert B. FREEDMAN, Anne NEWELL and Colin M. WALKLIN
Biological Laboratory, The University of Kent, Canterbury CT2 7NJ, England

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1. Introduction

Detergents are useful probes of the interaction of membrane-bound enzymes with lipids and other membrane components; however they can also have more direct effects on enzyme activity. In this paper, data are presented on the apparently paradoxical effects of sodium deoxycholate on protein disulphide isomerase (EC 5.3.4.1), a microsomal enzyme which probably plays a role in the formation of native disulphide bonds during protein biosynthesis [1].

Knowledge of the transverse orientation of protein disulphide isomerase in endoplasmic reticulum membranes would further our understanding of its physiological role. Since very low concentrations of deoxycholate render microsomal membrane permeable to macromolecules [2], such as the protein substrate of protein disulphide isomerase, we attempted to use this technique to study transverse orientation, but we found that the rat liver microsomal enzyme was inhibited by quite low concentrations of the detergent [3]. This was subsequently found to be true also of the enzyme solubilised and partially purified from beef liver [4].

Triton X-100 is observed [5] to inhibit microsomal protein disulphide isomerase activity at conc. $< 0.01\%$, but to activate it at conc. $\geq 0.05\%$.

In view of this observation, and in view of the fact that glutathione-insulin transhydrogenase, an activity related to protein disulphide isomerase but distinct from it [1] has been purified from a deoxycholate extract of rat liver microsomes [6,7], we have studied more closely the effects of deoxycholate on beef and rat liver microsomal protein disulphide isomerase. The data indicate that two distinct effects of the detergent are involved – a direct inhibition of the enzyme in any physical state plus an activation of the enzyme when it is released from the membrane.

2. Materials and methods

Beef and rat liver microsomes were isolated as in [3,4]. Chemicals were from the same sources.

Protein disulphide isomerase was assayed as in [8] described [3]. The substrate is 'randomly' reoxidised ribonuclease and the reaction is monitored by withdrawal of aliquots as a function of time and assay of these samples for ribonuclease activity. The units of activity are those defined [3]. In studies of the effect of deoxycholate on protein disulphide isomerase activity, aliquots of 1% or 10% sodium deoxycholate were added to isomerase assay mixtures in total vol. 1 ml. Final concentrations of deoxycholate quoted are the concentrations in the assay mixture as a result of this direct addition; in some cases there is also some deoxycholate present carried through in the enzyme sample. The concentration of deoxycholate in the ribonuclease assays was always 1/300-times that in the isomerase incubation and in no case did this have any direct effect on ribonuclease activity [3].

Deoxycholate extraction of microsomal preparations was done as in [6,7]. Sodium deoxycholate, 10% in water, was added to a thick beef liver microsomal slurry in 10 mM Tris–HCl, pH 7.8, to final conc. 0.5%. Alternatively, a rat liver microsomal pellet was resuspended in the same buffer containing 0.25 M sucrose and 0.5% sodium deoxycholate. In both

cases the clarified microsomal suspension was centrifuged at 105 000 × g_{av} for 16 h at 4°C. This centrifugation gave rise to four fractions a narrow opaque layer containing little protein (0), a broad layer of solubilized material (S), a narrow viscous layer (V), immediately above the residual pellet (P). The layers were carefully removed and diluted in sucrose–TKM (0.25 M sucrose in 50 mM Tris–HCl, pH 7.5, containing 25 mM KCl and 5 mM $MgCl_2$) for assay of protein and enzyme activities. The residual pellet was rehomogenized in the same buffer.

3. Results

The protein disulphide isomerase activity of beef liver microsomes was inhibited by low concentrations of sodium deoxycholate; detergent concentrations between 0.05% and 0.1% inhibited the enzyme by more than 75%. Protein disulphide isomerase extracted from beef liver microsomes by 0.5% deoxycholate (see section 2) was also assayed in the presence of various concentrations of added deoxycholate; it too was inhibited (fig.1b) and the titration of this solubilized material closely resembled that for the intact microsomes. The enzyme in intact beef liver microsomes and in deoxycholate extract of beef liver microsomes therefore resembles those in intact rat liver microsomes [3] and in a fully solubilized and partially purified preparation from beef liver [4] in their sensitivity to inhibition by this detergent.

But the resemblance in shape of figs.1a,b conceals a significant difference; considerably less protein was taken for the assay of the deoxycholate extract so

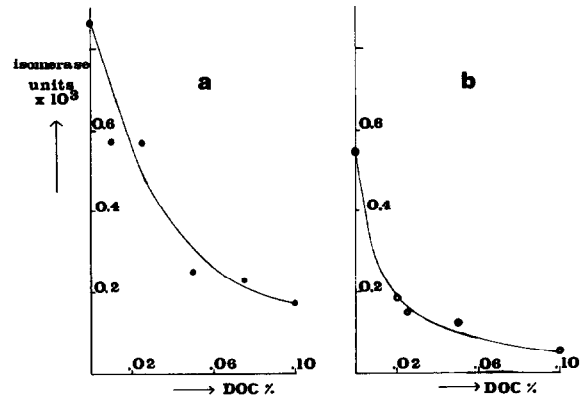

Fig.1. Inhibition of protein disulphide isomerase by added sodium deoxycholate. (a) Isomerase activity of beef liver microsomes (2.14 mg protein) in the presence of various amounts of added sodium deoxycholate. (b) Activity of deoxycholate-solubilised fraction (S) from beef liver microsomes (0.35 mg protein).

that the specific activity of this preparation in the absence of added deoxycholate (but in the presence of 0.03% deoxycholate carried through with the extract) was 4-times greater than that of the untreated microsomes. Despite the direct inhibitory effect of the detergent, the enzyme is detectable at high activity in fractions solubilized by that detergent.

Table 1 shows that this effect is observed consistently for both rat and beef liver microsomes. Samples of microsomes were extracted with 0.5% sodium deoxycholate (see section 2) and the four fractions obtained were assayed for protein and for protein

Table 1
Recovery of isomerase after deoxycholate extraction of beef and rat liver microsomes

Source	Beef		Beef		Rat	
Fraction	Protein (mg)	Isomerase (units × 10^3)	Protein (mg)	Isomerase (units × 10^3)	Protein (mg)	Isomerase (units × 10^3)
Microsomes	214	84	365	99	121	44
O	10	14	n.d.	1	1	1
S	13	21	9	18	14	107
V	10	302	5	74	42	103
P	n.d.	419	208	677	43	117

n.d., not determined

disulphide isomerase activity. In all cases net activation of isomerase was noted, and the total isomerase activity recovered in the four fractions was 7–10-fold that observed in the original microsomes. Furthermore, the activity was particularly concentrated in the solubilized fractions S and V, where the specific activities were up to 50-fold greater than in the original microsomes.

4. Discussion

Taken together [3,4] the findings of fig.1 demonstrate that inhibition by sodium deoxycholate is an intrinsic property of protein disulphide isomerase, it is not a function of the enzymes membrane-bound state or its association with other structures. Comparable inhibition is observed for both rat and beef liver microsomes, for a deoxycholate-solubilized fraction from beef liver microsomes and for a soluble and partially-purified enzyme preparation from beef liver.

Despite this inhibition, it is clear that high concentrations of the detergent release considerable quantities of the enzyme from microsomal membranes and that this solubilized enzyme is of high specific activity when assayed in the absence of added deoxycholate. The activation of microsomal protein disulphide isomerase when solubilized by the detergent is comparable to that observed when the enzyme is solubilized following acetone extraction of beef liver microsomes [4] or when it is solubilized by repeated washing of rat liver microsomes [8,9].

In fact it appears that solubilization of protein disulphide isomerase from microsomes is always accompanied by activation.

In that context, it becomes clear that the paradoxical action of sodium deoxycholate on protein disulphide isomerase is, in fact, two distinct effects:

(i) The enzyme in any physical state, is inhibited by the detergent.

(ii) The detergent can release the enzyme from microsomal membranes, and for this enzyme, solubilization is generally accompanied by significant activation.

It is pertinent to ask if other detergents might show the same combination of effects. Protein disulphide isomerase from rat liver microsomes, including the effects of Triton X-100, has been studied [5]. While most of the physical and immunological data suggest that a single form of the enzyme is present in rat liver microsomes, it was concluded [5] that distinct forms are present on the cytoplasmic and luminal faces of the vesicles. Their crucial evidence is that low concentrations of the detergent inhibit microsomal protein disulphide isomerase activity, while concentrations greater than 0.05% activate the enzyme. This is interpreted as an inhibition of a cytoplasmic-surface form of the enzyme followed by unmasking of a structurally-latent, luminal-surface form. In view of our findings with sodium deoxycholate, it is possible that these findings with Triton X-100 could be interpreted in terms of two distinct effects of the detergent, rather than two distinct forms of the enzyme.

Our results are also relevant to the use of deoxycholate solubilization in the purification of protein disulphide isomerase and other thiol-protein disulphide oxidoreductases. Glutathione-insulin transhydrogenase has been purified from deoxycholate-solubilized rat liver microsomes [6,7]; the preparation also had some protein disulphide isomerase activity. It is now clear that these activities are not alternative activities of a single enzyme species ([1] and ref. therein). However we have repeated the procedure [6,7] assaying both the transhydrogenase and isomerase activities. We find that the transhydrogenase is solubilized by 0.05% deoxycholate with a significant increase in specific activity as noted [7]. However, the activation of glutathione-insulin transhydrogenase is not so spectacular as that shown in table 1 for protein disulphide isomerase, and the specific activity of solubilized glutathione-insulin transhydrogenase is, at most, twice that of the original microsomes, whereas for isomerase it is 10–50-fold. Thus the starting material for the purification of glutathione-insulin transhydrogenase from detergent-solubilized microsomes is significantly more purified in protein disulphide isomerase than in glutathione-insulin transhydrogenase. This may be related to the fact that the transhydrogenase is significantly the more labile enzyme [4].

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