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Occurrence of the disulfide interchange enzyme in the liver microsomes of various animal species

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Chimica biologica. — *Occurrence of the disulfide interchange enzyme in the liver microsomes of various animal species* (*). Nota di GUIDO MOLEA, DOLORES D'AVERSA e FRANCESCO DE LORENZO, presentata (**) dal Corrisp. F. CEDRANGOLO.

RIASSUNTO. — È stato in precedenza dimostrato che un enzima, purificato dai microsomi di fegato di bue e presente in tutti i tessuti bovini, catalizza la riattivazione della forma ridotta ed inattiva della ribonucleasi pancreatica bovina e di altre proteine pure ridotte o contenenti ponti disolfuro incorretti.

In questa Nota sono presentati i risultati della riattivazione enzimatica della ribonucleasi pancreatica bovina ridotta, in presenza dei microsomi di fegato di varie specie animali come: cane, coniglio, pecora, anitra, bue e cavallo, ed anche in presenza dei microsomi delle uova di riccio di mare fecondate. La presenza dell'enzima microsomiale in queste diverse specie animali contribuisce a definire il ruolo di questa attività enzimatica, come processo generale deputato alla corretta formazione dei ponti disolfuro nelle proteine.

It was shown that rat and beef liver microsomes [1, 2] as well as microsomes of other bovine tissues tested [3], and pig, pigeon and chicken pancreas tissue[4] contain an enzyme that catalyzes the reactivation of the inactive fully reduced or of randomly cross-linked forms of bovine pancreatic ribonuclease, lysozyme [2], soy bean trypsin inhibitor [5] and pepsinogen [6]. The enzyme isolated from beef liver microsomes was purified and characterized [7, 8] and it was shown to catalyze sulphydryl-disulfide interchange in proteins [9].

The enzyme may be assayed by measuring the reactivation of fully reduced proteins in the presence of an added oxidizing agent [1, 2], or the reactivation of the inactive forms of ribonuclease and soy bean trypsin inhibitor (in which incorrect disulfide bonds have been introduced) in the presence of low levels of a reducing agent [9].

Since the disulfide interchange enzyme catalyzes the reactivation of several animal and plant proteins and since the enzyme is present in all tissues tested and is localized at the site of protein synthesis, it is suggested that the enzyme may catalyze, *in vivo*, the correct pairing of half-cystine residues in newly synthesized polypeptide chains.

We have investigated the enzymatic reactivation of fully reduced bovine pancreatic ribonuclease in the presence of liver microsomes of various animal species and in the presence of microsomes of fertilized sea urchin eggs (*Paracentrotus Lividus*).

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EXPERIMENTAL PROCEDURE.

The livers were removed as soon as possible after slaughter of the animals and rapidly processed to prepare microsomes as previously described [1]. The sea urchin eggs (*Paracentrotus Lividus*) were fertilized in sea water medium; two hours after fertilization, the eggs were homogenized and the microsomes prepared. Bovine pancreatic ribonuclease (Type II–A, Sigma) was fully reduced with β–mercaptoethanol in 8 M urea, and the reduced protein was separated from the reagents by gel filtration as previously described [10]. Titration of aliquots of the effluent with *p*–mercuribenzoate as described by Boyer [11] showed the presence of eight half-cystine residues per mole of ribonuclease. Stock solutions of reduced ribonuclease in 0.1 M acetic acid were kept for not longer than 1 day at 0° to minimize the possibility of spontaneous reactivation. The supernatant fraction of beef liver microsomes was used as the oxidizing system [1]. The concentration of proteins in the microsomal suspensions were determined by the method of Lowry et al. [12]. Assays for reactivation of reduced ribonuclease were performed in duplicate; aliquots were removed from the incubation mixture and assayed for ribonuclease activity by measurement of the rate of digestion of yeast RNA (Type II, Sigma) at pH 5.0 [13]. Blank determinations were carried out to permit correction for endogenous ribonuclease activity in the microsomal preparation.

RESULTS AND DISCUSSION.

In a previous communication [3] we showed the presence of the disulfide interchange enzyme in the microsomes of many bovine tissues. The highest amounts of the enzyme were observed in the microsomes of those tissues which secrete large quantities of protein containing disulfide bonds i.e. γ–globulins, serum albumin, thyroglobulin.

In Table I are summarized the results of the specific activities of the disulfide interchange enzyme from liver microsomes of various animal species and from microsomes of fertilized sea urchin eggs. It can be seen that the disulfide interchange enzyme was present in the livers of all animal species tested, the highest levels being found in the dog and rabbit liver microsomes. It has to be noted that, as earlier described [2, 3], inhibition was observed when levels of microsomal proteins higher than 1 mg/ml were used for the reactivation process. It is noteworthy that the level of the disulfide interchange enzyme is higher in the liver microsomes of horse foetus (where a higher rate of protein synthesis occurs) than in horse liver microsomes. Of interest is the finding of the disulfide interchange enzyme in the microsomes of fertilized sea urchin eggs; since this animal species far precedes the mammals evolutionary origin, it gives a good evidence of the constant philogenetic presence and of the general distribution of this enzymatic reaction.

TABLE I.

Comparison of specific activities of the disulfide interchange enzyme from liver microsomes of various animal species and from microsomes of sea urchin eggs.

All incubations were carried out in a Dubnoff shaking bath at 37° for 15 min. Reactivation mixtures contained (total volume) Tris–HCl 1×10^{-1} M, pH 7·4,25 μg of reduced ribonuclease, 25 μl of the dialyzable beef liver supernatant fraction, and washed microsomes.

Liver microsomes	Specific Activity (*)	Liver microsomes	Specific Activity (*)	Microsomes	Specific Activity (*)
Dog	177	Beef	73	Fertilized sea urchin eggs .	20
Rabbit	122	Pig	67		
Sheep	96	Horse foetus .	54		
Drake	88	Horse	34		

(*) The enzyme activity is calculated as the percentage of reactivation of the reduced RNAase in respect to the activity of an equal amount of native RNAase. Specific activities are obtained by dividing these figures for the protein level of the microsomal suspension used. These levels were always below 1mg/ml; in this range an essential linear relationship was found between ribonuclease reactivation and enzyme concentration.

The localization of the enzyme within the microsomal fraction of many tissues [3], its general occurrence in the different animal species, and its wide spectrum of substrate specificity, suggest that the enzyme, *in vivo*, plays an important role in the terminal stages of biosynthesis of proteins containing S–S bonds.

Studies are now in progress in our laboratory to investigate the distribution of this enzyme in a large number of marine organisms of different phyla.

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