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Received March 16th, 1970

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BBA 61212

The regulation of rat liver xanthine oxidase: Conversion of type D (dehydrogenase) into type O (oxidase) by a thermolabile factor, and reversibility by dithioerythritol

It has been reported from this laboratory that most of the "xanthine oxidase" (EC 1.2.3.2) activity of rat liver is detectable as an NAD^+-dependent dehydrogenase (called type D) in freshly prepared supernatant. The activity is converted into an oxidase (called type O) by various treatments of the supernatant, such as storage at $-20°$, treatment with proteolytic enzymes, preincubation under anaerobic conditions, and preincubation in air in the presence of particulate subcellular fractions[1–3].

The effect of the preincubation of the supernatant with other subcellular fractions (or of the preincubation of the whole homogenate) was tentatively interpreted as due to the action of proteolytic enzymes[3]. Evidence is now presented that the conversion of xanthine oxidase from type D into type O by subcellular particles is different from the conversion operated by proteolysis and is due to a thermolabile factor, apparently similar to an enzyme oxidizing thiol groups.

Rat liver was homogenized with 5 vol. of 0.1 M Tris–HCl buffer (pH 8.1) and was centrifuged at 600 × *g* for 20 min at 0°. The first supernatant so obtained was centrifuged again at 100 000 × *g* for 1 h to separate a particle-free supernatant and a sediment. The sediment from a 10-ml centrifuge tube was resuspended with 2 ml of buffer and was added to the particle-free supernatant obtained from the same tube. 2 ml of buffer were added to the supernatant in control experiments. After preincubation at 37° for 20 min the sediment was separated again by centrifugation, the supernatant was dialyzed and the activity of xanthine oxidase type D and type O was determined by following spectrophotometrically the formation of uric acid with

and without NAD^+, respectively[3]. The reduction of NAD^+ was also measured.

The results are summarized in Table I. Preincubation at 37° of the supernatant alone brought about a slight conversion of the dehydrogenase into the oxidase, a process probably facilitated by the presence of endogenous xanthine[3]. The conversion of type D into type O was complete (as defined by STIRPE AND DELLA CORTE[3], footnote to p. 3856) if the supernatant was preincubated with the sediment, whereas the presence of boiled sediment was without effect. The changes caused by preincubation with the sediment were completely reversed if the supernatant was incubated again at 37° in the presence of 10 mM dithioerythritol. Preincubation of the supernatant with trypsin in the presence of 1 mM xanthine brought about an apparently similar conversion of the xanthine oxidase activity which, however, could not be reversed by dithioerythritol. Thus rat liver xanthine oxidase undergoes two types of conversion, an irreversible one upon treatment with proteolytic enzymes, and a second one which is reversed by dithioerythritol. The reversibility of the latter conversion upon addition of a thiol compound suggests the possible involvement of a modification of one or more thiol group(s) in the conversion of xanthine oxidase. This is confirmed by the fact that dithioerythritol reverses the conversion following other

TABLE I

EFFECT OF DITHIOERYTHRITOL ON THE CONVERSION OF XANTHINE OXIDASE ACTIVITY OF RAT LIVER SUPERNATANT

The enzyme activity was assayed at 25° in a mixture containing in a final volume of 3 ml: 0.1 M Tris–HCl buffer (pH 8.1), 60 μM xanthine, 0.7 mM NAD^+ (when present) and supernatant (0.2 ml when O_2 was the acceptor or 0.15 ml when NAD^+ was the acceptor)

Treatment	*Xanthine oxidase activity*		
	(nmoles uric acid formed per min per 100 mg protein)		*(nmoles NAD^+ reduced per min per 100 mg protein)*
Acceptor:	O_2	NAD^+ and O_2	NAD^+
Expt. 9			
Supernatant kept at 0° (control)	53	294	246
Supernatant at 37° for 20 min	102	271	153
Supernatant at 0° with sediment	66	258	200
Supernatant at 37° for 20 min with sediment	346	354	0
Supernatant at 37° for 20 min with boiled sediment	106	271	160
Supernatant at 37° for 20 min with sediment, then for 20 min with 10 mM dithioerythritol	35	248	220
Expt. 2			
Supernatant, untreated	93	507	391
Supernatant at 37° for 90 min with trypsin (100 μg/ml) and 1 mM xanthine	480	500	9
Supernatant at 37° for 90 min with trypsin (100 μg/ml), 1 mM xanthine and 10 mM dithioerythritol	490	510	49
Supernatant at 37° for 90 min with trypsin (100 μg/ml) and 1 mM xanthine, then for 30 min with 10 mM dithioerythritol	440	495	32

treatments (storage at −20°, *etc.*) and that reagents oxidizing or binding with thiol groups convert xanthine oxidase from type D into type O (E. DELLA CORTE AND F. STIRPE, unpublished experiments).

These results indicate also the presence in the sedimentable fraction of rat liver of a factor causing the reversible conversion of xanthine oxidase activity from type D into type O. This factor seems to be present in all subcellular fractions, since any fraction is capable of converting xanthine oxidase[3]. Attempts to solubilize the factor from microsomes with 0.7% deoxycholate failed, since the converting activity of microsomes was lost on addition of deoxycholate, thus suggesting that integrity of subcellular membranes may be required to allow the factor to work. Finally, the thermolability of the factor suggests that it may be an enzyme, or an enzyme-containing system present in the sedimentable fraction, possibly similar to the microsomal enzyme catalyzing disulfide interchange in proteins[4].

We think that this information on the role of thiols in the interconversion of xanthine oxidase type D and O will be useful for achieving the purification of liver xanthine oxidase in its D form.

The work was aided by a grant from Consiglio Nazionale delle Ricerche, Rome.

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Received April 16th, 1970