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Properties of the xanthine oxidase from human liver

The xanthine oxidase (xanthine:O_2 oxidoreductase, EC 1.2.3.2) of mammalian tissues has been considered to be an oxidase due to its capacity to use O_2 as electron acceptor. However, in some tissues, including human liver[1,2], its activity is stimulated by methylene blue; this was interpreted as an indication of the existence of a dehydrogenase, as well as of an oxidase[2].

It was reported that the oxidation of xanthine by freshly prepared rat-liver supernatant occurred mainly through a dehydrogenation reaction requiring an electron acceptor such as NAD^+ or methylene blue. The reaction became an oxidase-type reaction, *i.e.* O_2 was used as acceptor, if the supernatant was kept at −20° for some hours[3] or if it was preincubated either with proteolytic enzymes[4] or in the presence of any subcellular fraction or in anaerobiosis[5]. The presence of xanthine accelerated the effect of proteolytic enzymes or of anaerobiosis. It was postulated that these changes might be consequent to conversion of the enzyme from dehydrogenase (named type D) to oxidase (named type O)[5].

TABLE I

CONVERSION OF HUMAN-LIVER XANTHINE OXIDASE FROM TYPE D TO TYPE O

The enzyme assays were performed at 25° in a mixture containing, in 1 ml: 100 μmoles of Tris–HCl buffer (pH 8.1), 60 nmoles of xanthine, 0.67 μmole of NAD^+ or 0.33 μmole of methylene blue (when present) and 0.05 ml of liver supernatant. The supernatant was preincubated in the presence of trypsin (100 μg/ml) and/or of xanthine (1 mM), before dialysis.

Case No.	*Xanthine oxidase activity (nmoles uric acid formed per min per 100 mg protein)*					
	Assayed immediately			*24 h at −20°*		
	O_2	NAD^+	*Methylene blue*	O_2	NAD^+	*Methylene blue*
1	23	134	132	133	116	228
2	25	129	—	78*	81*	—
3	21	134	211	139	127	—
4	15	127	216	105	117	—
5	24	165	245	180	183	—
6	18	113	198	91	94	—

	5 min at 37° with trypsin		*5 min at 37° with trypsin and xanthine*		*5 min at 37° in anaerobiosis with xanthine*	
	O_2	NAD^+	O_2	NAD^+	O_2	NAD^+
2	—	—	—	—	73	73
3	55	136	79	79	89	73
4	38	48	53	48	66	73
5	55	158	114	114	114	114
6	—	—	—	—	51	49

* 14 h instead of 24.

The present study was undertaken to investigate the characteristics of the xanthine oxidase of human liver.

Samples of human liver weighing about 1 g were obtained from surgical biopsies performed under pentothal/NO_2–O_2 anaesthesia from patients (4 women and 2 men, age 40–72 years) operated on for cholecystitis (No. 1, 2, 3 and 5) or for peptic ulcer (No. 4 and 6). The samples were immediately put in an ice-cold 0.1 M Tris–HCl buffer (pH 8.1) and were homogenized in the same buffer (1 g of liver *plus* 5 ml of buffer) within 30 min of excision. The homogenate was centrifuged at 600–800 $\times g$ for 20 min and then at 100 000 $\times g$ for 1 h, and the resulting supernatant was dialyzed for 3 h against a continuous flow of 300 vol. of 0.1 M Tris buffer (pH 8.1). Enzyme activity was assayed by the method of ROWE AND WYNGAARDEN[6] with or without NAD^+ or methylene blue, as reported previously[3], except that the volume of the reaction mixture was reduced to one third and that the assay was performed in 1-ml cells. Protein was determined using the method of GORNALL *et al.*[7].

The xanthine oxidase activity of human-liver supernatant had the same general characteristics as those of rat liver, although the specific activity of human supernatant was lower. The xanthine oxidase activity of the freshly prepared supernatant was very weak with O_2 as acceptor; the activity was about 7-fold higher with NAD^+ (which was reduced to NADH during the reaction) and about 10-fold higher with methylene blue (Table I). Storage at $-20°$ for 24 h brought about changes similar to those observed in rat-liver supernatant[3], *i.e.* the rate of uric acid formation with O_2 reached the rate observed with NAD^+ as acceptor. The activity in the presence of methylene blue appeared unchanged in the single assay allowed by the available material.

The possibility of changes induced by proteolysis or by anaerobiosis was investigated initially by preincubating the supernatant for 60 min, since this length of time ensured complete conversion of the enzyme activity of rat-liver supernatant[5]. The first results showed that after preincubation the xanthine oxidase activity was very low either with O_2 or with NAD^+, as compared with the activity of the non-preincubated supernatant, thus indicating that the human enzyme was inactivated at 37°. Consequently the length of the preincubation was reduced to 5 min; although some loss of activity was still observed under these conditions, it was ascertained that the human xanthine oxidase was partially converted from type D to type O after trypsinization in the absence of xanthine and that the conversion was complete after trypsinization in the presence of xanthine or after preincubation in anaerobiosis in the presence of xanthine. As it was observed with rat-liver supernatant[3–5], the reduction of NAD^+ was diminished or abolished after the human xanthine oxidase had been converted to type O, regardless of how this was obtained.

The inactivation of xanthine oxidase at 37° was investigated further. When the preincubation was performed in air, 50% of the oxidase activity was lost in 30 min, and the inactivation was even more marked for the dehydrogenase activity (Fig. 1). The preincubation in anaerobiosis in the presence of xanthine brought about a very rapid loss of the dehydrogenase activity during the first few minutes. The inactivation became much slower after the conversion of the enzyme from dehydrogenase to oxidase (Fig. 2), thus indicating that the latter form is more stable than the dehydrogenase.

These results indicate that most of the human-liver xanthine oxidase in its native state is a dehydrogenase, for which NAD^+ is probably the physiological acceptor,

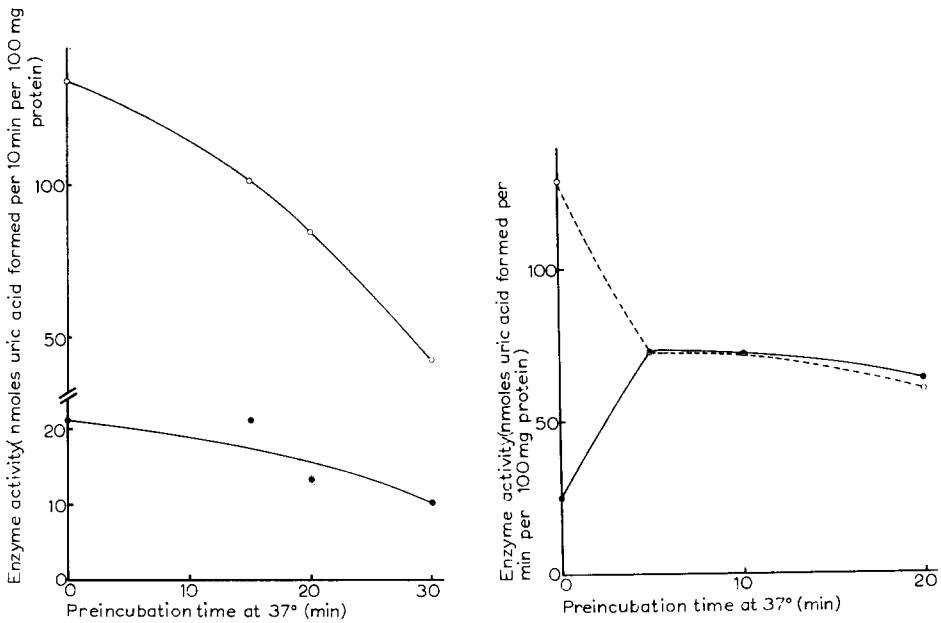

Fig. 1. The effect of preincubation on the xanthine oxidase activity. Liver supernatant was preincubated in air and then assayed with O_2 (●—●) or NAD^+ (○—○).

Fig. 2. The effect of preincubation in anaerobiosis in the presence of xanthine on the xanthine oxidase activity. Liver supernatant was preincubated at 37° under N_2 in the presence of 1 mM xanthine and then was dialyzed and assayed with O_2 (●—●) or with NAD^+ (○— — —○).

and becomes an oxidase after the treatments described above. It has been observed with rat liver that at least the changes caused by preincubation in anaerobiosis are reversible[5]; consequently, it is possible that the interconversion dehydrogenase–oxidase may have a role in the physiological regulation of xanthine oxidase activity.

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