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Short Communications

Xanthine Oxidase Type D (Dehydrogenase) in the Intestine and other Organs of the Rat

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It has been reported from this laboratory (Della Corte & Stirpe, 1972) that the xanthine oxidase activity of rat liver extracts appeared as a dehydrogenase (type D) and could be converted into an oxidase (type O) irreversibly by proteolysis, or reversibly by treatment with reagents for thiol groups. The activity of the enzyme of other rat organs appeared as an oxidase, and in all cases but intestine could be converted into a dehydrogenase by incubation at 37°C with dithioerythritol before assay (Table 1).

Joyce & Duke (1971) reported that the xanthine oxidase from mouse duodenum had a different electrophoretic mobility from that of the liver enzyme. However, no differences in mobility were observed if the intestinal enzyme was prepared in the presence of soya-bean trypsin inhibitor, thus suggesting that 'the difference between tissue forms may be due to an artifact of preparation', i.e. to conversion of one form into another one by the intestinal proteolytic enzymes.

The present experiments were performed to verify this possibility and to ascertain whether the different forms corresponded to the type D and the type O xanthine oxidase.

Tissue extracts were prepared with or without trypsin inhibitor and 2-mercaptoethanol and were assayed for xanthine oxidase and/or dehydrogenase activity as described previously (Della Corte & Stirpe, 1972). The results are summarized in Table 2. When the intestine was washed with and homogenized in buffer containing the soya-bean trypsin inhibitor at a concentration similar to that used by Joyce & Duke (1971) (8mg/ml; personal communication from Dr. E. J. Duke), the xanthine oxidase appeared as type O, but could be partially converted into type D by preincubation with dithioerythritol. The intestinal enzyme was still of the type O, but could be converted completely into the type D (i.e. it was entirely of the reversible type O) if the trypsin inhibitor in addition to being added to the buffer was also given to the animal by stomach tube before it was killed. The xanthine oxidase appeared as type D if the buffer used for homogenization and dialysis contained 2-mercaptoethanol in addition to the trypsin inhibitor. The xanthine oxidase of lung and spleen appeared

Table 1. *Effect of dithioerythritol on the xanthine oxidase activity of various organs*

Organs were homogenized with 5vol. of 0.1M-tris–HCl buffer, pH8.1, and the 100000*g* supernatant was prepared and assayed for enzyme activity as described by Della Corte & Stirpe (1972). Assay mixtures contained, in a final volume of 3ml: 0.1M-tris–HCl buffer, pH8.1, 60μM-xanthine, 0.67mM-NAD^+ (when present) and 0.2ml of supernatant. One unit of enzyme activity corresponds to the formation of 1μmol of product/min.

	Xanthine oxidase activity (munits/100mg of tissue)					
	Untreated supernatant			Supernatant kept for 30min at 37°C with 10mM-dithioerythritol		
Acceptor ...	O_2	O_2+NAD^+		O_2	O_2+NAD^+	
Tissue	Uric acid formed	Uric acid formed	NADH formed	Uric acid formed	Uric acid formed	NADH formed
Heart	6	6	0	0	6	4
Liver	8	34	27	5	35	32
Small intestine	42	45	0	32	32	0
Spleen	24	24	1	2	18	16
Pancreas	11	11	0	3	7	10
Lung	15	14	0	1	11	10
Kidney	10	10	0	1	8	7

Table 2. *Xanthine oxidase and xanthine dehydrogenase activity of various organs*

Organs were homogenized with 5vol. of 0.1M-tris–HCl buffer, pH8.1, and the 100000*g* supernatant was prepared and assayed for enzyme activity as described by Della Corte & Stirpe (1972). Assay mixtures contained, in a final volume of 3ml: 0.1M-tris–HCl buffer, pH8.1, 60μM-xanthine, 0.67mM-NAD^+ (when present) and 0.2ml of supernatant. One unit of enzyme activity corresponds to the formation of 1μmol of product/min. Trypsin inhibitor was type II-S from Sigma Chemical Co. (St. Louis, Mo., U.S.A.): the amount given by stomach tube was 25mg in 5ml, 15min before death.

		Xanthine oxidase activity (munits/100mg of tissue)		
		Acceptor ... O_2 (air)	O_2+NAD^+	
Source of enzyme and treatment	Trypsin inhibitor in homogenizing buffer (5mg/ml)	Uric acid formed	Uric acid formed	NADH formed
Homogenate in tris–HCl buffer				
Intestine	–	24	24	0
Intestine, supernatant kept for 20min at 37°C with 10mM-dithioerythritol	–	19	18	0.4
Intestine	+	35	34	2
Intestine, supernatant kept for 20min at 37°C with 10mM-dithioerythritol	+	19	28	8
Intestine, trypsin inhibitor given by stomach tube	+	30	30	0
Intestine, trypsin inhibitor given by stomach tube, supernatant kept for 20min at 37°C with 10mM-dithioerythritol	+	3	19	18
Homogenate in tris–HCl buffer containing 10mM-2-mercaptoethanol				
Intestine, trypsin inhibitor given by stomach tube	+	8	18	12
Intestine, trypsin inhibitor given by stomach tube, supernatant kept for 20min at 37°C with 10mM-dithioerythritol	+	4	20	16
Lung	–	4	9	6
Lung, supernatant kept for 20min at 37°C with 10mM-dithioerythritol	–	2	9	8
Spleen	–	6	17	12
Spleen, supernatant kept for 20min at 37°C with 10mM-dithioerythritol	–	2	14	13

also largely as type D if these organs were homogenized in the presence of 2-mercaptoethanol. If the intestinal supernatant prepared in the presence of trypsin inhibitor but without 2-mercaptoethanol (and therefore containing the xanthine oxidase of the reversible O type) was dialysed overnight against buffer containing 2-mercaptoethanol, the xanthine oxidase activity remained of the reversible O type. This indicates that the simple addition of the thiol to the buffer does not convert the enzyme from type O into type D. Therefore it seems that the extrahepatic xanthine oxidase prepared in the presence of 2-

mercaptoethanol is type D because the thiol prevents its conversion into type O, which otherwise occurs during the preparation of the tissue extracts.

These results indicate that the 'native' xanthine oxidase of all rat organs is probably of the type D. The liver enzyme remains a dehydrogenase throughout the preparation of the extract, whereas the enzyme from other organs is converted into an oxidase presumably as a result of the oxidation of thiol group(s). The intestinal enzyme is further converted into the irreversible type O by the action of the intestinal proteolytic enzymes.

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Della Corte, E. & Stirpe, F. (1972) *Biochem. J.* **126**, 739
Joyce, P. & Duke, E. J. (1971) *Biochem. J.* **125**, 111P