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THE INFLUENCE OF LIPID PEROXIDATION PRODUCTS (MALONDIALDEHYDE, 4-HYDROXYNONENAL) ON XANTHINE OXIDOREDUCTASE PREPARED FROM RAT LIVER

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Abstract—Depending on metabolic conditions, xanthine oxidoreductase acts as either a dehydrogenase (XDH) or an oxidase (XOD). The metabolism of hypoxanthine and xanthine by the oxidase is associated with the production of reactive oxygen radicals. Reaction of reactive oxygen radicals with polyunsaturated fatty acids (lipid peroxidation) leads to the formation of malondialdehyde (MDA) and 4-hydroxynonenal (HNE), known to modify proteins by reaction with NH_2- and SH-groups. Therefore, these aldehydes could influence both the activity of xanthine oxidoreductase and the XOD/XDH ratio. We found that incubation of xanthine oxidoreductase with MDA leads to an initial increase in XDH activity and to a continuous decrease in XOD activity, whereby the total activity decreases. This was in contrast to the effects of HNE which did not alter the XDH activity; XOD was however activated. This demonstrates that the lipid peroxidation products MDA and HNE are able to modify xanthine oxidoreductase similarly to a feed-back mechanism.

Xanthine oxidoreductase metabolizes hypoxanthine and xanthine to uric acid. Under physiological conditions, this enzyme acts as a dehydrogenase (XDH†). Different metabolic states (hypoxia, ischemia) lead to the conversion of the dehydrogenase form of xanthine oxidoreductase to an oxidase form (XOD) [1, 2], which relates the metabolism of oxypurines with the generation of reactive oxygen radicals. Those are able to react with proteins [3, 4], carbohydrates [5, 6], nucleic acids [7] and polyunsaturated fatty acids [8]. The reaction with polyunsaturated fatty acids leads to lipid peroxidation. During lipid peroxidation lipid hydroperoxides decompose to short-chain hydrocarbons and very active aldehydes [malondialdehyde (MDA), 4-hydroxynonenal (HNE)] [9, 10]. It is known that these aldehydes are able to modulate protein functions by reacting with SH- and NH_2-groups [11, 12]. Since SH-group modulation is included in the regulation and conversion of xanthine oxidoreductase [13], it seems possible that these aldehydes could influence the activity of the dehydrogenase and oxidase forms of xanthine oxidoreductase. Therefore we investigated the influence of MDA and HNE on xanthine oxidoreductase prepared from rat liver.

MATERIALS AND METHODS

Xanthine was purchased from Ferak (Berlin, Germany), hypoxanthine from Chemapol (Praha, Czechoslovakia), milk XOD from Serva (Heidelberg, Germany) and MDA–bis(diethylacetal) from Merck-Schuchardt (Hohenbrunn bei München, Germany). HNE was a generous gift from Prof. H. Esterbauer, Institute of Biochemistry, University of Graz, Austria.

Enzyme preparation. According to Kaminski and Jezewska [14], rat liver tissue was homogenized in 4 vol. 0.15 M sucrose, 0.10 M Tris–HCl buffer at pH 7.4 with an Ultra Turrax (4 × 30 sec). The homogenate was centrifuged for 10 min at 27,000 *g* and 4° in a Beckman JA 21 centrifuge, and for a further 60 min at 160,000 *g* in a Beckman L8 centrifuge at 4°. The resulting supernatant was precipitated within the range of 1.4–2.4 M ammonium sulfate and centrifuged for 10 min at 27,000 *g*. The pellet was dissolved in 0.25 M sucrose (about 1 mL for the pellet from 1 g tissue wet wt). The total activity was 0.125 ± 0.027 U/mL (N = 4; 9.0 mg protein/mL) consisting of 0.102 ± 0.018 U/mL XOD (81.8 ± 3.7%) and 0.024 ± 0.01 U/mL XDH (18.3 ± 4.0%).

These enzyme preparations were incubated with MDA and HNE at different concentrations and incubation times as indicated in the figure legends. The blank was prepared under the same conditions without the addition of aldehydes.

Aldehyde preparation. MDA solution was prepared according to Esterbauer *et al.* [15] and Haberland *et al.* [16]. MDA-bis(diethylacetal) solution (10 mM) was incubated in 1% H_2SO_4 for 1 hr at 25°. After hydrolysis the pH was adjusted to 6.5 by 10 N NaOH. The concentration of MDA was calculated by measurement of absorption at 245 nm (ε = 13.75 × 10^3 L/mol/cm).

HNE solution was prepared according to Jürgens

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† Abbreviations: MDA, malondialdehyde; HNE, 4-hydroxynonenal; XDH, xanthine dehydrogenase; XOD, xanthine oxidase.

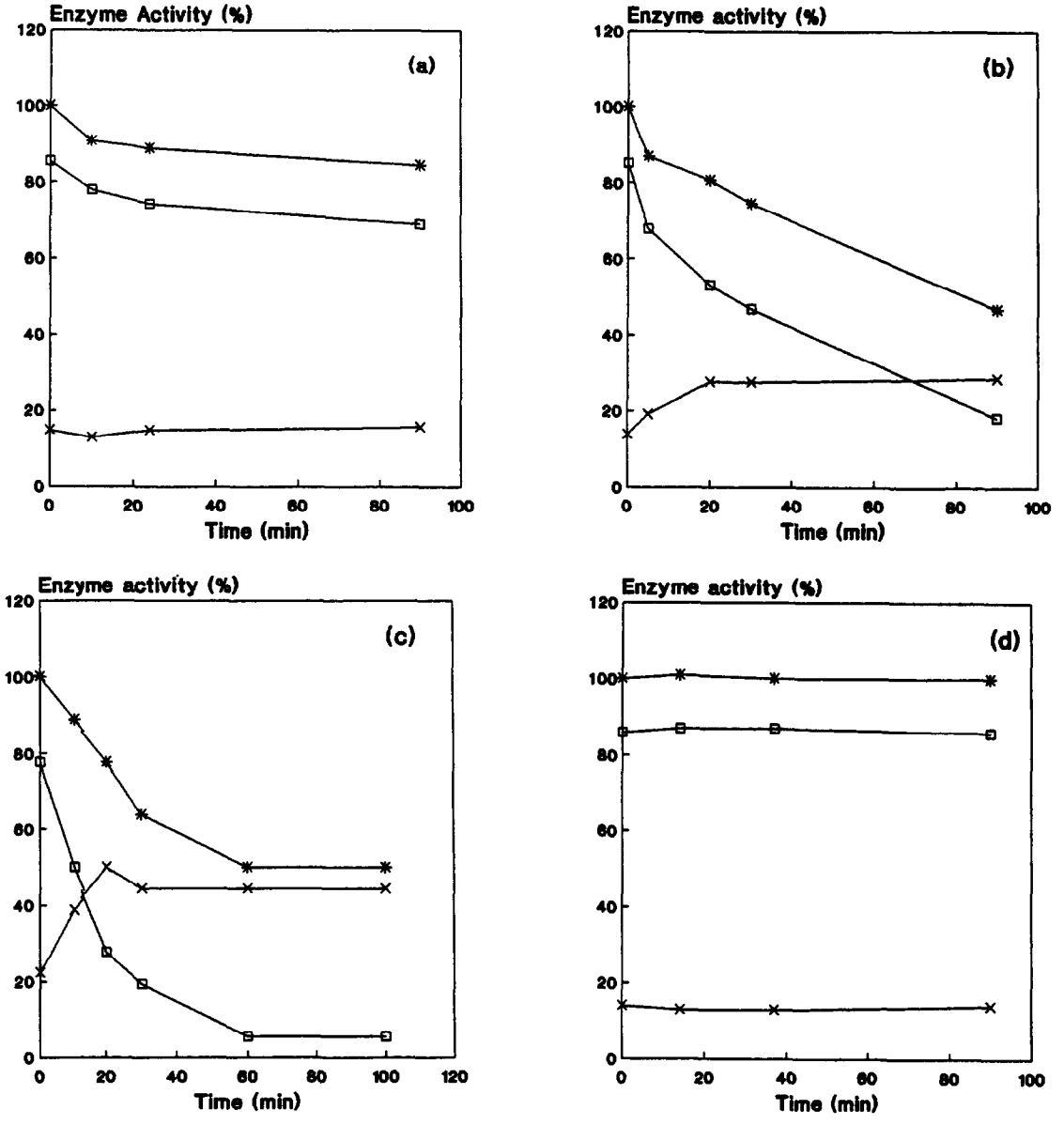

Fig. 1. Influence of different concentrations of MDA on rat liver xanthine oxidoreductase. Xanthine oxidoreductase (0.1 U/mL) was incubated with (a) 0.1 mM MDA; (b) 1.0 mM MDA; (c) 5.0 mM MDA; (d) without MDA. XOD + XDH activity (*), XOD activity (□), XDH activity (×).

et al. [17]. The concentration of HNE was calculated by measurement of absorption at 225 nm (ε = 13.75 × 10^3 L/mol/cm).

XDH and XOD measurement. The estimation of XDH and XOD activity was carried out according to the method described by Kaminski and Jezewska [14]. The activity of XOD was determined by measurement of uric acid generation at 302 nm, the activity of XDH by formation of NADH and 340 nm. The reaction mixture contained the sample, 0.05 mM xanthine without or with 0.147 mM NAD for XDH estimation, in 50 mM Tris–HCl buffer at pH 8.0 in a final volume of 2.5 mL.

RESULTS

During incubation of the xanthine oxidoreductase preparation with different concentrations of MDA or HNE the activity of xanthine oxidoreductase was monitored. A decrease in XOD activity occurred, dependent on MDA concentration (0.1, 1.0, 5.0 mM MDA: Fig. 1a–d). MDA at 0.1 mM led to a small decrease in the total activity after a long incubation time (90 min). This was the result of a diminution of the XOD activity; XDH was not affected (Fig. 1a). Incubation with 1.0 mM MDA led to an inhibition of the total activity of xanthine oxido-

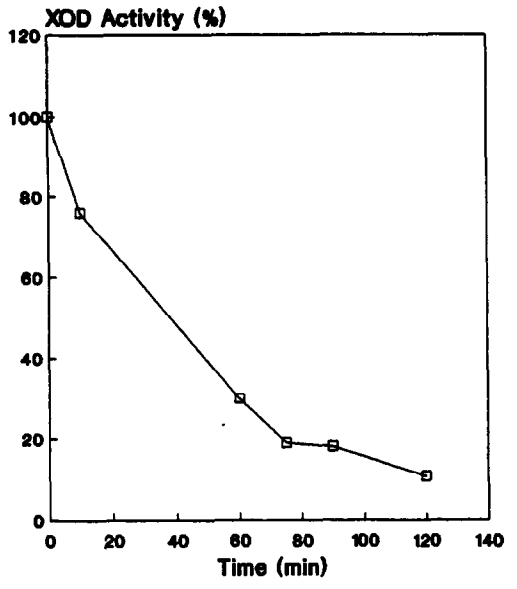

Fig. 2. Inactivation of commercial milk XOD (Serva, 0.10 U/mL). XOD was incubated with MDA in a final concentration of 1.0 mM.

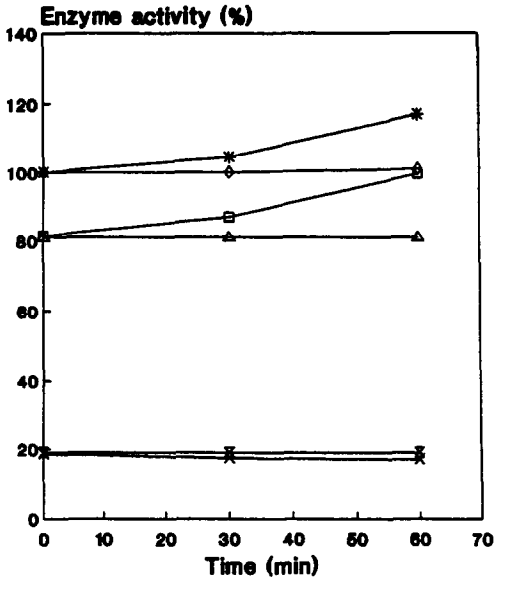

Fig. 3. Influence of 0.01 mM HNE on rat liver xanthine oxidoreductase. XOD + XDH activity (*), XOD activity (□), XDH activity (×). The initial concentration of xanthine oxidoreductase was 0.11 U/mL. XOD + XDH/ctr (◇), XOD/ctr (△) and XDH/ctr (×) are the control experiments without HNE.

reductase, caused by a reduced XOD activity. In contrast to this decrease in XOD activity, the XDH activity was enhanced by about 30% up to 30 min after the start of incubation (Fig. 1b). MDA at 5.0 mM led to a greater decrease in total activity, caused by extensive XOD inhibition. Sixty minutes after the start of incubation almost no XOD activity was detectable. The increase in XDH activity stopped after 20 min and reached a maximum of 200% of the initial activity (Fig. 1c). In the absence of MDA (but with the addition of the same concentration of 1% H_2SO_4 at pH 6.5) no effects were observed either on the XDH/XOD ratio or on total activity (Fig. 1d). The influence of MDA on commercial milk XOD was tested also. Thirty minutes after the start of incubation the XOD activity was inhibited by approximately 50% (Fig. 2).

HNE led to a continuous activation of XOD, whereas XDH was not significantly affected. In the range of 0.001–0.14 mM HNE the activation of XOD by HNE was almost entirely independent of HNE concentration. An experiment using 0.01 mM HNE is presented (Fig. 3).

Commercial milk XOD (Serva, 0.1 U/mL) was not affected by HNE.

DISCUSSION

SH-groups are involved in the conversion and regulation of xanthine oxidoreductase [13]. The oxidase form of the enzyme is regarded as being one of the most important sources of oxygen radicals in ischemia and reperfusion. Oxygen radicals are able to induce lipid peroxidation leading to the formation of many different metabolites including aldehydes, e.g. MDA and HNE [10]. It is generally accepted that such aldehydes react with the SH- and NH_2-groups of proteins [17], where both inactivation and activation could be demonstrated [18, 19]. Whereas HNE is more reactive with SH-groups in comparison with NH_2-groups, MDA reacts preferentially with NH_2-groups to form Schiff bases [11, 12]. Therefore, it seemed possible that MDA and HNE would react also with the SH- and NH_2-groups of xanthine oxidoreductase. In this way, the aldehydes could modify xanthine oxidoreductase activity. In our experiments MDA effected a continuous decrease in the oxidase form of xanthine oxidoreductase. This decrease in XOD activity was the cause of the diminished total activity of xanthine oxidoreductase, observed during incubation with MDA. An enzyme inhibition by MDA was also demonstrated by Leclerc *et al.* [20], who investigated the influence of MDA on Ca^{2+}, Mg^{2+}-ATPase in hemoglobin S erythrocyte membranes. Other enzymes such as superoxide dismutase and glutathione peroxidase were also inactivated by aldehydes (MDA, HNE), but the sensitivity of the antioxidative enzymes towards the aldehydes was different [21].

The xanthine dehydrogenase form increased in the first period of incubation with MDA. Longer incubation times and higher concentrations did not lead to further changes in XDH activity, although XDH contains NH_2- and SH-groups [13]. We also found an enhanced enzyme activity after treatment with MDA during incubation of the sarcolemma-localized β-receptor–adenylyl cyclase system.* Con-

* Unpublished data.

cerning the β-receptor–adenylyl cyclase system, comparable results were obtained by Paradisi *et al.* [22].

We were unable to decide whether the increase in XDH activity is a result of reconversion of XOD or direct activation by MDA. The effects of MDA on XOD could also be demonstrated using commercial milk XOD. In contrast, HNE induced different effects on commercial milk XOD and prepared liver XOD. Commercial XOD was not influenced by HNE, whereas the XOD activity of the liver enzyme preparation increased time-dependently. Since HNE did not change the XDH activity, a conversion of XDH to XOD during incubation of the liver enzymes with HNE could be excluded as a cause of the increase in activity. Our experiments demonstrate that xanthine oxido-reductase activity can be influenced by products formed during lipid peroxidation in a similar way to a feed-back mechanism. To determine whether the inhibition of XOD by the lipid peroxidation-generated MDA could be regarded as a defence mechanism, diminishing oxygen radical formation by XOD, requires further experimentation.

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