SC 11088

The reaction of bovine-milk xanthine oxidase with *p*-chloromercuricbenzenesulphonic acid

Several groups of workers have commented on the inactivation of bovine-milk xanthine oxidase (xanthine:O_2 oxidoreductase, EC 1.2.3.2) by PCMB[1-3]: even in those cases where sufficient experimental data have been given, the markedly different conditions used makes a comparison of the results extremely difficult. One controversial point concerns the sensitivity of xanthine oxidase towards PCMB. It is probable that the existing disparity can be explained by the greatly increased lability of the enzyme towards some reagents (including the mercurials) in the presence of the substrate. The purpose of this communication is to present the results of some experiments regarding the reaction of xanthine oxidase with another organic mercury compound, *viz.*, PCMS.

Utilizing the spectrophotometric technique of BOYER[4], FRIDOVICH AND HANDLER[2] (and also BERGEL AND BRAY[3]) observed that PCMB reacts with the enzyme in a diphasic manner indicating that either two distinct groups in the enzyme are involved, or two similar groups with differing reactivity. The data presented in Fig. 1. show that PCMS behaves in a similar fashion. Although BERGEL AND BRAY[3]

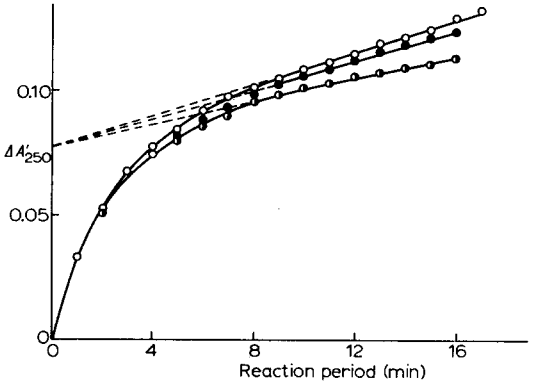

Fig. 1. Effect of inhibitors on the reaction of xanthine oxidase with PCMS. To 2.5-ml aliquots of enzyme were added 100-μl amounts of water (○—○), semicarbazide (●—●) or chalcone (◑—◑), and the reactions started by the addition of 100-μl aliquots of a solution of PCMS in water. Final concentrations: enzyme 3.6 μM (calculated from the 450-mμ absorption[5]), PCMS 190 μM, semicarbazide 2.2 mM, chalcone 74 μM. Conditions: 50 mM pyrophosphate buffer (pH 6.1), 23.6°.

reported no direct correlation between the degree of inactivation and the extent to which the mercurial had reacted, in the one experiment here, where this was checked, the inactivation paralleled the slow phase. (In these experiments the reaction times were kept short in order to obviate changes not directly attributable to the reaction considered.) It can be seen from Fig. 1 that two inhibitors, semicarbazide and

Abbreviations: PCMB, *p*-chloromercuricbenzoate; PCMS, *p*-chloromercuricbenzenesulphonic acid.

3,3',4,4'-tetrahydroxychalcone, both affected the rate at which the enzyme and PCMS reacted, and, since the later sections of all curves extrapolate back to the same absorbancy value on the ordinate, the inhibitors may be considered to act specifically on the slow phase. Moreover, the magnitude of the protective effect is in order of the inhibitory power (chalcone > semicarbazide). It is thus reasonable to accept that the slow phase does in fact reflect the reaction of the PCMS either with the active site or with a group on whose integrity the activity is dependent.

The influence of pH on the shape of the reaction curves was determined using constant enzyme, reagent and buffer concentrations but without control of the ionic strength. The initial rate and the rate of the slow phase ('final' rate) were determined, and plotted against the pH (Fig. 2). Although it is possible that the two independent reactions may be differently influenced by the variation in the ionic strength of the buffer, the marked change in the slope of the curve for the slow reaction is probably due to the ionisation of a group in the enzyme. Both the initial and 'final' rates were markedly decreased when at one pH value (6.7) the buffer was changed from pyro-

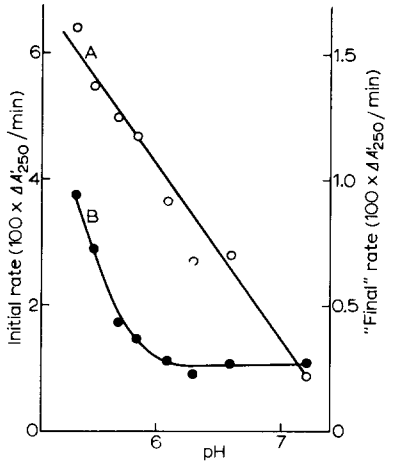

Fig. 2. Effect of pH on the reaction of xanthine oxidase with PCMS. To 1.5-ml aliquots of the pyrophosphate buffer at a suitable pH, was added 1.0 ml of unbuffered enzyme followed by 100 μl water. The reactions were started by the addition of 100-μl aliquots of a PCMS solution in water. The final pH values were checked at the end of each experiment. Curve A: initial rate of the reaction. Curve B: rate of the slow phase ("final" rate). Enzyme 3.7 μM; PCMS 192 μM; 60 mM pyrophosphate buffer; 23.5°.

phosphate to Tris. This may also be due to a difference in the ionic strength or, perhaps more likely, to an effect of the buffer ion on the reagent.

The results clearly show that a more systematic and detailed study is required before the mechanism of the reactions involved can be understood with any certainty. It would be unwise, for example, to automatically assume that the PCMS is reacting with thiol groups since the rate of both reactions increases with decreasing pH, a result contrary to that expected in view of the higher reactivity of the mercaptide ion. One might explain this observation in numerous ways, *e.g.*, by the influence of pH on the repulsive action of protective anionic groups situated close to the reactive

site. It is wiser, however, to await the results of many further experiments before accepting that bovine-milk xanthine oxidase is, in fact, a thiol-dependant enzyme.

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Uncoupling and inhibition of oxidative phosphorylation by 2-hydroxy-3-alkyl-1,4-naphthoquinones

Various 2-hydroxy-3-alkyl-1,4-naphthoquinones are potent inhibitors of electron transport in the region of the antimycin-inhibitory site of the respiratory chain[1]. The inhibition of respiration in intact mitochondria by 2-hydroxy-3-(3-methylbutyl)-1,4-naphthoquinone may be reversed by the uncoupling agent, 2,4-dinitrophenol[2], suggesting that the quinone might act on energy-conservation reactions. It is of interest to study the influence of naphthoquinones on oxidative phosphorylation under conditions where the inhibition site is bypassed. This may be done by using a system including ascorbate and catalytic amounts of TMPD which appears to supply electrons to the respiratory chain at a locus between the antimycin block and oxygen[3].

Fig. 1 is a polarographic trace of oxygen consumption by rat-liver mitochondria respiring with TMPD and ascorbate. The addition of AMP produced a characteristic stimulation of respiration and this stimulation was completely eliminated by the addition of 2-hydroxy-3-cyclohexyl-1,4-naphthoquinone. Inhibition of State-3 respiration (+ AMP) was approximately to the State-4 (no AMP) level, a property also of the inhibition of coupled respiration by oligomycin[4]. The concentration of CNQ used was sufficient to inhibit succinate oxidation completely.

Abbreviations: TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine; CNQ, 3-cyclohexyl-1,4-naphthoquinone; DNQ, 2-hydroxy-3-(3,7-dimethyloctyl)-1,4-naphthoquinone.