ACTIVATION BY AMP OF THE NADH OXIDASE OF MYCOBACTERIUM TUBERCULOSIS*

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Received August 4, 1964

Recent studies (reviewed by Monod, et al., 1963) have demonstrated that the role of certain compounds, often the adenine nucleotides, in the activation of enzymes may be of primary importance as metabolic regulators. In this allosteric action the allosteric effector has a catalytic role in that it functions at low concentrations, in a highly specific manner, and is not consumed in the reaction. We have recently found that AMP is an allosteric effector of the particulate NADH oxidase of M. tuberculosis. The oxidation of NADH is followed either spectrophotometrically at 340 mμ or electrometrically with the GME "Oxygraph"; the effect of AMP on the latter system is shown in Fig. 1.

NADH oxidation is not coupled to phosphorylation in this particulate system. Neither P_i nor Mg^{++} are required for the stimulation of the NADH oxidase by AMP.

The activation by AMP of the NADH oxidase is a function of the AMP concentration (Fig. 2), is half maximal at 0.2 mM AMP, and is highly specific for AMP (Table 1). ADP shows no activation of the washed NADH oxidase particles but does activate NADH oxidation by

* Supported in part by funds from grant number AI-02416-06 from the USPHS. The technical assistance of Mrs. Emma Lee Armstrong is gratefully acknowledged.

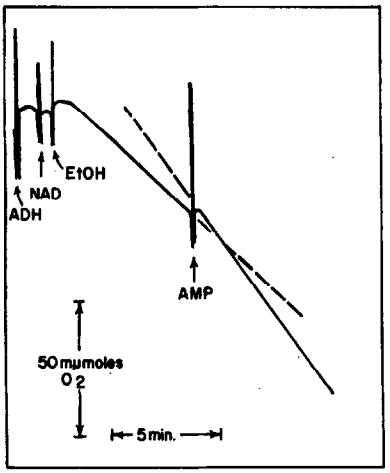

Fig. 1. Effect of AMP on the oxidation of NADH. The polarographic cell contained 200 μmoles of tris-acetate buffer of pH 7.5, 10 μmoles of $MgCl_2$ and 0.67 mg of NADH oxidase particle (Segel and Goldman, 1963) protein in a final volume of 2.0 ml. Additions were made as follows: 0.10 mg of ADH protein, 2 μmoles of NAD, 100 μmoles of ethyl alcohol and 2 μmoles of AMP. The reaction was carried out at 30°.

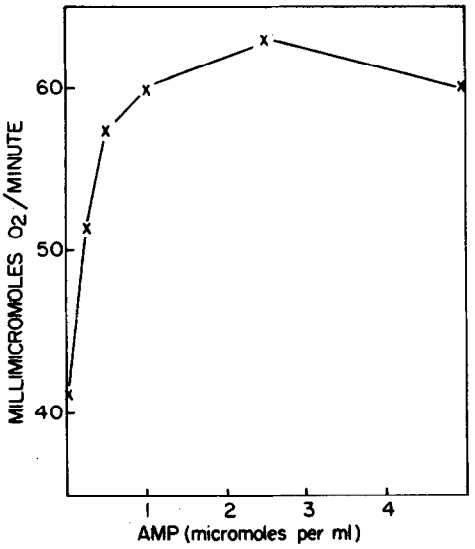

Fig. 2. Effect of the concentration of AMP on the oxidation of NADH. The conditions were the same as described for Fig. 1 except that 2.67 mg of NADH oxidase protein was used. In the several experiments the rate of O_2 consumption ranged from 40.7 to 42.9 mμmoles per minute before AMP addition.

TABLE 1

Specificity of the nucleotide activation of the NADH oxidase

Nucleotide	% of AMP activation (AMP = 100%)
AMP	100
dAMP	30
2'-AMP, 3'-AMP, ADP, ATP, CMP, UMP, TMP, IMP, 3', 5'-cyclic AMP	0

The reaction mixture contained 1 μmole of the nucleotide to be tested, 0.15 μmole of NADH, 100 μmoles of tris-acetate buffer of pH 7.5 in a final volume of 0.99 ml. All components had been equilibrated at 30°. The mixture was held at 30° for 3 minutes at which time 10 μliters of the NADH oxidase preparation were added. The change in absorbance at 340 mμ was followed.

whole extracts of M. tuberculosis. This action is probably due to the formation of AMP from ADP by a very active soluble adenylate kinase present in the crude cell-free extract.

The data presented in Table 2 lead to the suggestion that AMP exerts its allosteric effect on the dehydrogenase rather than the hemoprotein segment of the oxidase chain. The particulate NADH

TABLE 2

AMP stimulation of the NADH-menadione reductase

Additions to reaction mixture	Δ A 340 mμ per min
None	0.028
AMP (1.0 μmole)	0.033
Menadione (0.30 μmole)	0.075
AMP plus menadione	0.125

The reaction mixture consisted of 100 μmoles of tris-acetate buffer of pH 8.5, 0.15 μmole of NADH and additions as shown in a final volume of 1.00 ml. After temperature equilibration (30°) the reaction was started by the addition of 150 μg of NADH oxidase protein.

oxidase slowly loses activity on storage (-17°) or on freezing and thawing. In these "aged" particles an NADH-menadione reductase activity is observed; this reductase is stimulated by AMP (Table 2). Since menadione is rapidly autooxidized at the pH of the reaction mixture AMP must act before the menadione-oxygen shunt.

NADH oxidase activity is rapidly lost at pH 8.5 and 30°; 10^{-3} M AMP prevents this inactivation (Fig. 3). The particles treated at pH 8.5 in the absence of AMP are no longer activated by AMP. These effects are similar to the protection of certain enzymes by their allosteric effectors (Changeaux, 1961; Whiteley and Hayaishi, 1964) and to the desensitization of the enzyme to its allosteric effector (Monod et al., 1963).

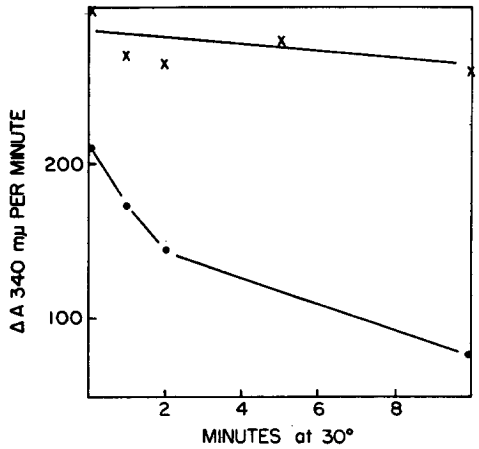

Fig. 3. Protection by AMP against pH inactivation of the NADH oxidase particles. Each cuvette (● —— ●) contained 100 μmoles of tris-acetate buffer of pH 8.5 and 150 μg of NADH oxidase protein in a final volume of 1.00 ml. In a parallel series of reactions (x —— x) 10^{-3} M AMP was also present. After the times indicated 0.15 μmole of NADH was added and the rate of NADH oxidation was followed. The preincubations and NADH oxidations were carried out at 30°.

Activation of the NADH oxidase by AMP is also noted on dilution of NADH oxidase particles preincubated with 10^{-3} M AMP at 0° (Table 3). AMP has no further activating effect on these particles. Preincubation

TABLE 3

Effect of preincubation on the AMP activation of the NADH oxidase particles

Preparation and treatment	Δ A 340 mμ per min	
	No added AMP	Plus 10^{-3} M AMP
Particles, 0°, 10 min*	0.148	0.192
Same, plus AMP**	0.187	0.192
Particles, 30°, 10 min, pH 8.5, then 0°, 10 min, pH 7.5	0.094	0.097
Same plus AMP (after the 30° treatment)	0.097	0.097

* Protein concentration was 4 mg per ml during the preincubation; 80 μg of protein were used in the NADH oxidase assay system.
** 2×10^{-3} M final concentration, during preincubation. Final concentration after dilution was 4×10^{-5} M.

of the particles at 30° and pH 8.5 before the addition of AMP abolishes this effect (Table 3).

AMP is not metabolized during NADH oxidation by an AMP-activated NADH oxidase preparation. 8-^{14}C-AMP was used as the activator in these reactions. Nucleotides were recovered following acid deproteinization of the reaction mixture. After paper chromatography of the nucleotides radioactivity was found only in the spot corresponding to authentic AMP which was also the spot occupied by the ^{14}C-nucleotide of the no-enzyme control.

The specificity of the AMP activation of the NADH oxidase, the protection by AMP against NADH oxidase inactivation, the activation by AMP on preincubation of the NADH oxidase and the absence of metabolic alteration of AMP during NADH oxidation are all taken as evidence that AMP functions as an allosteric effector of NADH oxidation in M. tuberculosis.

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