ACTIVATION BY Hg^{2+} OF ACETOACETYL-CoA REDUCTASE IN EXTRACTS OF *RHODOPSEUDOMONAS SPHEROIDES* AND *RHODOMICROBIUM VANNIELII*

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1. Introduction

In examining the activities of enzymes concerned in poly-β-hydroxybutyrate formation in Athiorhodaceae an activation of acetoacetyl-CoA reductase (EC 1.1.1.36, D-3-hydroxyacetyl-CoA: NAD(P) oxidoreductase) by Hg^{2+} was observed. Some properties of this unusual activation are examined in cell-free extracts of several bacterial species and in a partially purified preparation of acetoacetyl-CoA reductase from *Rhodopseudomonas spheroides*.

2. Methods

The non-sulphur, purple bacteria, *Rhodopseudomonas spheroides* (N.C.I.B. No. 8253) and *Rhodomicrobium vannielii*, were grown photosynthetically as described earlier [1] on a malate-glutamate medium [2]. Other bacteria were grown on nutrient broth at 30° in an Orbital Incubated Shaker. Organisms were harvested in late log phase of growth, washed once with 10 mM sodium phosphate, pH 7.5, and resuspended in a small volume of the same buffer. After ultrasonic treatment at 0° for three minutes unbroken cells, debris and some particulate material were removed by centrifugation at 40,000 *g* for 2 hr at 0°. The supernatant fraction from this used for enzyme assays and protein concentration was determined by the procedure of Layne [3].

Acetoacetyl-CoA reductase was assayed by the measurement of ΔE_{340} due to the oxidation of NADH in the presence of the artifical substrate *S*-acetoacetyl-*N*-acetylcysteamine, this being preferable to the natural substrate acetoacetyl-CoA [4]. Assays were carried out using a Unicam SP700 recording spectrophotometer, at room temperature. Cuvettes contained, in μmole, sodium pyrophosphate-HCl buffer, pH 7.5, 200; NADH, 0.5; *S*-acetoacetyl-*N*-acetylcysteamine, 10 and bacterial extract 0.5-3.0 mg in a total volume of 3 ml. Specific activity of enzyme activity was expressed as μmole NADH oxidised/min/mg protein.

S-Acetoacetyl-*N*-acetylcysteamine was prepared by slow addition of diketone (3.94 g) in ether to an ice cool solution of *N*-acetylcysteamine (5.7 g) in ether. After standing at room temperature for 6 hr the oily mixture was chilled to -10° and the crystalline product collected by filtration. The *N*-acetylcysteamine was prepared by reaction under reflux of thioacetic acid with ethylenimine [5].

3. Results and Discussion

The optimum pH of acetoacetyl-CoA reductase, in extracts of *R. vannielii*, was 7.4 and the effect of addition of metal ions to the standard assay mixture is shown in table 1. Relatively little effect was observed except by Hg^{2+} and, to a lesser extent, by Cd^{2+}. The increase in activity was seven fold in extracts of *R. vannielii* and three fold in extracts of *R. spheroides* (fig. 1). Dialysis of extracts against sodium pyrophosphate buffer increased slightly the activation by Hg^{2+} and this stimulation by Hg^{2+} was not removed by the addition of EDTA (mM) to the assay system (fig. 1). *p*-Chloromercuribenzoate (0.2 mM) produced a similar activation to that of Hg^{2+} in extracts from three other species of Athiorhodaceae (*Rhodopseudomonas*

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Table 1
Effect of metal ion on the acetoacetyl-CoA reductase activity in *Rhodopseudomonas vannielii.*

Addition	Concentration (mM)	Alteration in acetoacetyl-CoA reductase activity, relative to control
None	–	100
Hg^{2+}	0.10	200
Hg^{2+}	0.20	700
Hg^{2+}	0.30	350
Mn^{2+}	0.05	150
Mn^{2+}	0.20	100
Cd^{2+}	0.30	200
Na^{2}	0.20	100
AsO_4	0.50	80
Pb^{2+}	0.50	80

Enzymic activity was measured as described in Methods, except as indicated in the table; metal ions added last and results are expressed relative to a control.

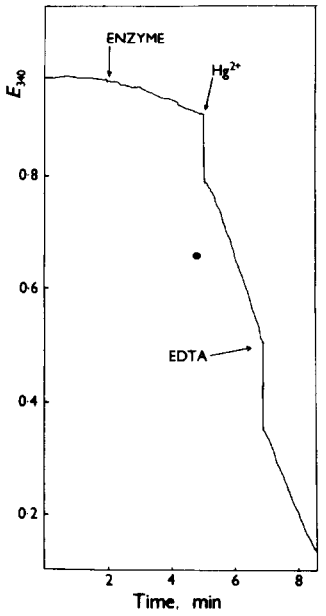

Fig. 1. Activation of acetoacetyl-CoA reductase from *Rhodomicrobium vannielii* by the addition of 0.2 mM Hg^{2+}, and the lack of reversal by EDTA (mM).

capsulata, Rhodopseudomonas gelatinosa, Rhodopseudomonas palustris) or from four species of aerobic heterotrphic bacteria was found.

The complete reaction mixture was necessary, prior to the addition of Hg^{2+}, for activation to occur. When Hg^{2+} was preincubated with the enzyme, in the absence of substrate some degree (approx. 10%) of inhibition occurred. Gel filtration on Sephadex G-200 produced a fraction purified 15 fold from *R. spheroides*, which exhibited the same activation by Hg^{2+} as obtained in the intact cell free extract. Variation in substrate concentration in the presence and absence of Hg^{2+} produced significantly different results. The inclusion of Hg^{2+} in the reaction mixture caused the substrate concentration-enzymic rate curve to become more sigmoidal (fig. 2).

The non-enzymic interaction of Hg^{2+} with reduced pyridine nucleotides, and the consequent cautions necessary in interpreting the effect of Hg^{2+} on enzyme assays, has been recently reported [6]. It was suggested that Hg^{2+} forms a weak bond with NADH or NADPH, but that in the presence of a chelating agent, or protein, no such association occurred. The activation of malate dehydrogenase from pig heart mitochondria by Hg^{2+} has been described by Kuramitsu [7] which exhibits similarities to the activation by Hg^{2+} of acetoacetyl-CoA reductase reported here. In both cases it is necessary for the substrate to be present during activation of the enzyme by Hg^{2+}; it would appear that a conformational change occurs, caused perhaps by the breakage of SH-group linkages,

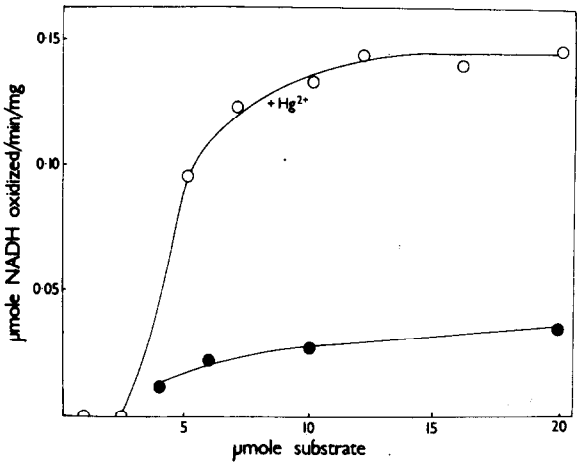

Fig. 2. Acetoacetyl-CoA reductase in extracts of *Rhodopseudomonas spheroides* with substrate (*S*-acetoacetyl-*N*-acetylcysteamine) concentration in the presence and absence of Hg^{2+} (0.2 mM).

that results in activation only when the catalytic site is occupied. Whilst clearly this is not a physiological mode of activation within the bacterial cell it may be that Hg^{2+} mimics the effect of a natural activator.

Acknowledgements

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