MECHANISM OF ACTION OF 2,4-DINITROPHENOL ON THE SOLUBLE ATPASE FROM MITOCHONDRIA

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The action of 2,4-dinitrophenol on the soluble ATPase from mitochondria has been suggested to be related to its action as an uncoupler of oxidative phosphorylation [1] but there has been little definitive information about the mechanism of either action of 2,4-dinitrophenol. It has been found that the action of 2,4-dinitrophenol on the soluble ATPase is correlated with the ligand exchange rate of the metal ion used to activate the soluble ATPase; 2,4-dinitrophenol stimulates the activity of the enzyme when the metal has a slow exchange rate. It is suggested that the non-phosphorylated high energy intermediate thought to be involved in mitochondrial oxidative phosphorylation could be a high energy level interaction between a metal ion and the ATPase coupling factor.

When ATP is the substrate and with certain bivalent metal ions as activator the activity of the soluble ATPase from mitochondria is stimulated by low, but inhibited by high, concentrations of 2,4-dinitrophenol. With other metal ions as activators or when ITP is the substrate 2,4-dinitrophenol produces only inhibition of the enzyme [2-5]. The activity of the ATPase depends on the ionic radius of the activating metal ion [6] but there is no correlation of the effect of 2,4-dinitrophenol with the ionic radius of the metal activator.

The effect of 2,4-dinitrophenol, when ATP is the substrate is correlated with the rate at which the metal ion exchanges coordinated water molecules (fig. 1). Stimulation of the ATPase activity is found when the metal has a slow water exchange rate. It would appear that a ligand exchange on the metal is contributing markedly to limiting the overall rate of reaction in these cases, and 2,4-dinitrophenol either increases this rate of ligand exchange or provides an alternative path-

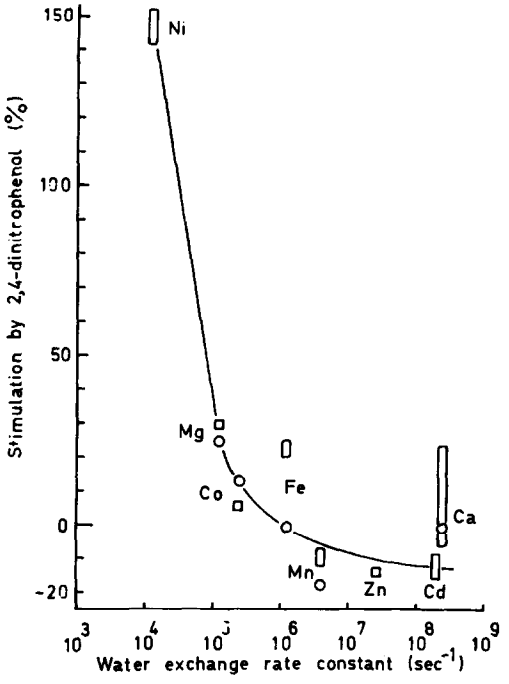

Fig. 1. Relation between the effect of 2,4-dinitrophenol on the soluble ATPase from mitochondria and the water exchange rate of the bivalent metal ion used as activator. Water exchange rate constants from Eigen [7,8]. Stimulation by 2,4-dinitrophenol (%) is the increase in enzyme activity in the presence of 0.5 mM 2,4-dinitrophenol as a percentage of the activity with the same metal ion in the absence of 2,4-dinitrophenol. ○ - Data from Pullman et al. [2]. □ - Data from experiments described previously [5]. In these experiments the error in the percent stimulation, indicated by the height of the rectangles, is inversely proportional to the activity of the enzyme; thus it is small with metals such as Mg^{2+}, Co^{2+} and Zn^{2+} which produced high ATPase activity but very large with Ca^{2+} which produced weak activity (7% of that with Mg^{2+}). The conditions of assay were not identical for the two sets of data.

way. When the metal ion has a high rate of ligand exchange some other step is rate limiting and 2,4-dinitrophenol does not increase the rate. With ITP as substrate the ligand exchange never makes a large contribution to limiting the overall rate since stimulation by 2,4-dinitrophenol is not found.

The rate of substitution of water by other ligands is correlated with the exchange rate for water [8] and the possibility cannot be eliminated that other leaving groups could leave at rates which are similarly correlated with the rate of water exchange. Thus little can be said about the chemical nature of either the leaving or entering ligands in the ATPase reaction.

Although the nucleoside base of the substrate is a factor in determining the type of effect produced by 2,4-dinitrophenol it seems unlikely that a direct interaction between the metal ion and nucleoside base is involved because there is no relation between the pattern of metal ion interaction with the adenine ring of ATP and the effects produced by 2,4-dinitrophenol. Mg^{2+} and Ca^{2+} do not interact with the adenine ring but Mn^{2+} and Zn^{2+} do [9, 10]; there are no data for Fe^{2+}, Co^{2+} or Ni^{2+} but these would be expected to behave similarly to Mn^{2+} and Zn^{2+} rather than Mg^{2+}. A ligand exchange involving only the polyphosphate chain seems unlikely because it could not account for the difference between ATP and ITP and again the pattern of metal complexing is different from the pattern of stimulation by 2,4-dinitrophenol; Mg^{2+}, Ca^{2+} and Zn^{2+} complex with β- and γ-phosphate groups while Co^{2+} and Mn^{2+} complex with the α-, β- and γ-phosphate groups.

The difference between the reactions with ATP and ITP appears to be produced by differences in the interactions of the purine rings with the enzyme protein: these interactions produce, possibly by way of a conformational change in the protein, different catalytic interactions between the protein, metal ion and polyphosphate chain. With ATP and metal ions which have a slow rate of exchange a particular metal-ligand interaction is a relatively stable intermediate which is labilised by 2,4-dinitrophenol.

It is not improbable that the metal-ligand bond which is labilised by 2,4-dinitrophenol involves as ligand a group in the ATPase protein and this could provide an explanation for the uncoupling action of 2,4-dinitrophenol and the failure to find the non-phosphorylated high energy intermediate postulated in theoretical mechanisms for mitochondrial oxidative phosphorylation. If such a metal-ATPase interaction were the high energy intermediate and could be formed by electron transport as well as from ATP then its nature is such that it would not be isolated as a distinct chemical compound and its labilisation by 2,4-dinitrophenol would produce uncoupling of oxidative phosphorylation.

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