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A DIFFERENCE IN THE AFFINITY LABELING OF CA^{2+}, MG^{2+}-ACTIVATED ATPASES OF NORMAL AND UNC A STRAINS OF ESCHERICHIA COLI BY THE 2',3'-DIALDEHYDE DERIVATIVE OF ADENOSINE 5'-DIPHOSPHATE

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SUMMARY

The 2',3'-dialdehyde of ADP, obtained by periodate oxidation of ADP, inhibited the hydrolytic activity of the purified Ca^{2+}, Mg^{2+}-activated ATPase of Escherichia coli. In the initial stages of the reaction inhibition was due to the reaction of 1 mol inhibitor/active site. When non-specific labelling of amino groups by the dialdehyde was lowered by the simultaneous presence of 15 mM ATP in the reaction mixture, 3 mol "ATP-protectable" binding sites/mol ATPase were found. "ATP-protectable" binding of the dialdehyde was not observed when the hydrolytically inactive ATPase of an unc A mutant of E. coli was used although binding of the inhibitor to non-protected amino groups still occurred. This suggests that the mutant ATPase is unable to bind ATP or that the amino groups with which the dialdehyde reacts in the native enzyme are absent or masked.

INTRODUCTION

The Ca^{2+}, Mg^{2+}-activated adenosine triphosphatase plays an important role in energy metabolism in Escherichia coli being involved both in the formation of ATP by oxidative phosphorylation and in its utilization to energize various energy-requiring processes associated with the cell membrane (1). A series of mutants have been obtained in which this enzyme is defective. In unc A and unc D mutants loss of ATPase activity is associated with modification of the α and β subunits of the enzyme, respectively (2,3). The nature of these modifications has not been characterized.

Oxidation of nucleoside di and triphosphates with periodate generates 2',3'-dialdehyde derivatives. These derivatives have been used as affinity probes to investigate nucleotide-binding sites in several enzymes (4-6) including the ATPases of Mycobacterium phlei and ox-heart mitochondria (7,8).

Abbreviation: oADP, 2',3'-dialdehyde of ADP

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In this paper we show that the 2',3'-dialdehyde of ADP ("oADP") will bind to the purified Ca^{2+}, Mg^{2+}-activated ATPase of E. coli. With the ATPase from normal cells a portion of this binding was prevented by the simultaneous presence of ATP in the incubation mixture. However, ATP-protectable binding of oADP was not observed when the ATPase from an unc A mutant was used.

METHODS

Preparation of ATPases

The ATPases of the normal strain (E. coli ML308-225) and the unc A mutant strain (E. coli AN120) were purified to homogeneity as described previously (9,10). E. coli AN120 was a generous gift from Professor Frank Gibson, Canberra, Australia.

Preparation of oADP

The 2',3'-dialdehyde of ADP was prepared by periodate oxidation of ADP as described by Easterbrook-Smith et al. (4). The product was stored as a 50 mM stock solution at -75°C. For the preparation of [^{14}C]oADP 50 μCi [U-^{14}C]ADP (0.1 μmol) was mixed with 33 μmol ADP prior to treatment with periodate as above.

Binding of [^{14}C]oADP by ATPase

Purified ATPase (0.7-2.0 mg protein) was incubated at 22°C with 2 mM oADP (containing 2.4 μCi [U-^{14}C]oADP) in a buffer containing 0.106 M triethanolamine-HCl, pH 7.5, 1 mM $CaCl_2$ and 0.22 mM EDTA. The total volume of the system was 0.8 ml. A similar system containing 15 mM calcium ATP was set up. Following addition of oADP, 2 μl samples were removed at intervals for assay of ATPase activity. At the same time intervals 75 μl samples from the incubation mixtures were mixed with 25 μl 1.2 M KBH_4. After 5 min, 75 μl of these solutions were freed from unbound oADP and salts by the centrifugation-column procedure of Penefsky (11). The protein contents of the column effluents were determined by the method of Bradford (12) using bovine serum albumin as a protein standard. ATPase-bound oADP was measured by scintillation counting of samples of the column effluents. For calculation of the extent of binding the molecular weight of the ATPase was taken as 365,000 (13).

Assay

Calcium-dependent ATPase activity was assayed as described previously (15).

RESULTS AND DISCUSSION

The 2',3'-dialdehyde derivative oADP, obtained by periodate oxidation of ADP, inhibited the hydrolytic activity of the purified F_1 ATPase of E. coli. As shown in Fig. 1 (lower panel), the kinetics were more complex

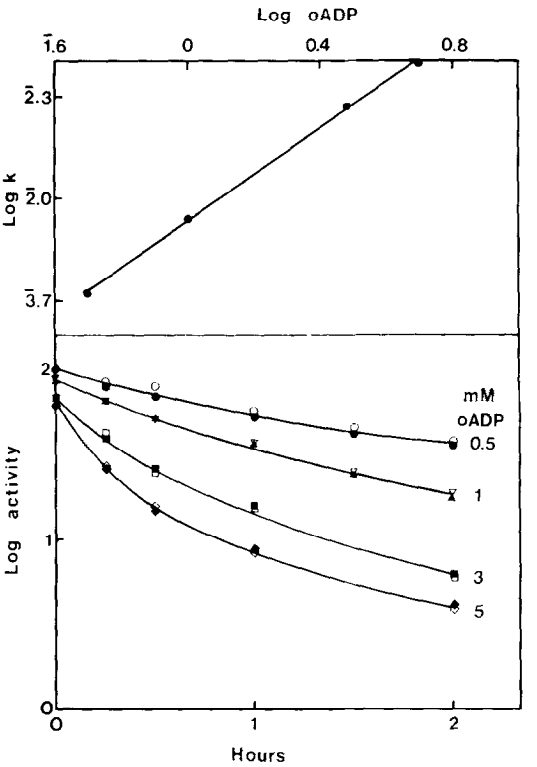

FIGURE 1. Inhibition of ATPase activity by oADP. Purified ATPase from ML308-225 was incubated at 22°C in 50 mM triethanolamine buffer, pH 7.5, with the indicated concentrations of oADP in the absence (open points) or presence (closed points) of $CaCl_2$ at a molar ratio of $CaCl_2$ to oADP of 0.5. Samples were removed at intervals for assay of ATPase activity. The upper panel shows the relationship between the pseudo-first-order rate constant of inactivation (k), calculated for the first 15 min of reaction, and the inhibitor concentration (expressed as mM). k is expressed as min^{-1}. Activity is expressed as a percentage of the control activity taken at the onset of the reaction.

than those of a first-order reaction since curved plots were obtained. However, if the logarithm of the pseudo-first-order rate constant calculated for the first 15 min of reaction is plotted versus the logarithm of the concentration of oADP (Fig. 1, upper panel), a straight line with a slope of 0.7 is obtained. This suggests that during the initial stages of the reaction inhibition was due to the reaction of 1 mol oADP/active site. A K_m of 2 mM can be calculated for this initial phase. Calcium (or magnesium) ions were not required for inhibition by oADP (Fig. 1).

It is likely that the complexity of the kinetics of inhibition was due to the ability of the aldehyde groups of oADP to form a Schiff's base

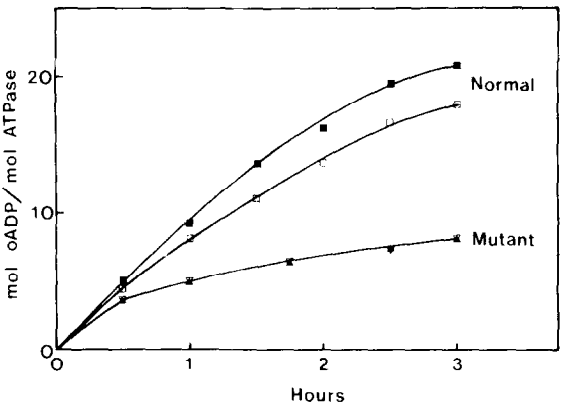

FIGURE 2. Binding of [^{14}C]oADP to the purified ATPases from normal (ML308-225) and unc A mutant (AN120) cells in the presence (open points) and absence (closed points) of 15 mM ATP. The reaction was carried out as described in Methods.

with available amino groups on the protein molecule. In order to use oADP as a probe of nucleotide-binding sites on the ATPase, it was necessary to correct for this non-specific labelling reaction. This was achieved by measuring the binding of [^{14}C]oADP in the presence and absence of 15 mM ATP. As shown in Fig. 2, the extent of non-specific labelling of the ATPase from a normal strain was considerable (>20 mol oADP bound/mol ATPase) whereas the ATP-protectable labelling by [^{14}C]oADP reached a constant value of about 3 mol oADP bound/mol ATPase.

The relationship between the extent of ATP-protectable binding of [^{14}C]oADP and the extent of inhibition of ATPase activity is shown in Fig. 3. Although most of the ATPase activity was lost during the binding of 1 mol "ATP-protectable" oADP/mol ATPase, this was accompanied by the non-specific (non-protectable) binding of about 8 mol oADP/mol ATPase. Moreover, complete inhibition of ATPase activity occurred when 3 mol "ATP-protectable" oADP/mol ATPase were bound. Thus, it is not clear if ATP-protectable binding measures binding at the active site of the ATPase.

In contrast to the enzyme from the normal strain, no ATP-protectable binding of [^{14}C]oADP was observed with the ATPase from the unc A mutant of E. coli (Fig. 2). The extent of non-specific (non-protectable) labeling

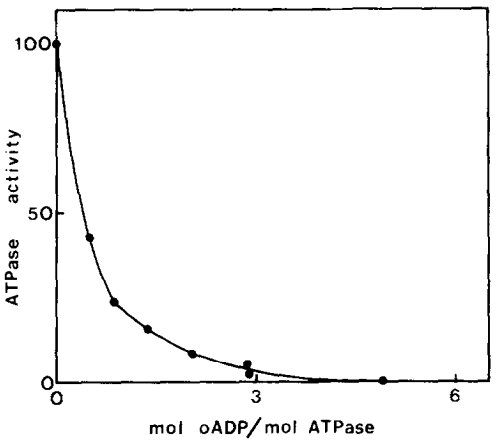

FIGURE 3. Relationship between ATPase activity and the extent of ATP-protectable binding of [^{14}C]oADP. The experiment was performed as described in Methods. ATPase activity is expressed as a percentage of the control value measured at the onset of the reaction.

of the mutant enzyme was also less than with the normal enzyme. The significance, if any, of this last observation is not clear at the present time. There are several possible explanations for the absence of ATP-protectable binding with the mutant ATPase. (a) The mutant enzyme may lack the amino groups with which oADP reacts in the normal ATPase and which are protectable by ATP. (b) The mutant enzyme may lack those ATP-binding sites which afford protection from oADP modification in the normal ATPase. (c) The conformation of the mutant ATPase may differ from that of the normal enzyme with the result that the ATP-protectable amino groups are masked. None of the evidence presently available enables a choice to be made between these possibilities since any of the above modifications in the mutant enzyme could account for its lack of hydrolytic activity.

In common with other F_1 ATPase (15), the enzyme of E. coli contains tightly-bound adenine nucleotides. However, the unc A and the normal enzyme contain 1-2 mol of both ADP and ATP/mol ATPase and have at least one additional binding site for ADP on the enzyme molecule (10,16). Thus, it is unlikely that the difference in ATP-protectable [^{14}C]oADP binding between the normal and mutant strains involves these sites.

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