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ATPase OF BOVINE HEART MITOCHONDRIA

MODULATION OF ITPase ACTIVITY BY ATP, ADP, ACETYL ATP AND ACETYL AMP

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(1) Mitochondrial ATPase (F_1) is influenced by specific nucleotides in its kinetic behavior towards its substrates. In this work, initial hydrolysis rates, as well as continuous reaction progress, were measured by recording proton production (equivalent to triphosphate hydrolysis). (2) After preincubation with ATP, F_1 hydrolyzes MgITP partly as if it were MgATP, with respect to temperature dependence and 2,4-dinitrophenol inhibition/stimulation. (3) Acetyl ATP is a competitive inhibitor versus ATP on the F_1-ATPase. With F_1 which has been freed of ambient ATP by repeated precipitations with ammonium sulfate the K_i of acetyl ATP is 400 nM. (4) F_1-ATPase which was depleted of bound nucleotides in the presence of glycerol (Garret, N.E. and Penefsky, H.S. (1975) J. Biol. Chem. 250, 6640–6647) was preincubated with ADP and acetyl ATP. These preparations were assayed for hydrolytic activity with MgITP as substrate. Compared to a nonpreincubated control enzyme, the hydrolysis with these preparations was first stimulated, then inhibited. This stimulation/inhibition effect is most pronounced at 10°C, but is also observed at 20°C. (5) When nucleotide-depleted enzyme is preincubated with acetyl AMP, its ability to hydrolyze MgITP slowly decreases to approx. 50% after 60 min. This effect is reversed by further preincubation with acetyl ATP. It is speculated that under appropriate conditions AMP may exist or arise in a buried position on F_1-ATPase, and act there as an inhibitor of MgITP hydrolysis.

Introduction

No convincing function has been established for the 'tight' nucleotide binding sites on mitochondrial ATPase. The binding to these sites is highly specific, requiring positions 1 and 6 to be adenine-like [1] The structural requirements are the same as those for nucleoside triphosphate synthesis from diphosphates, and for nucleoside triphosphate-driven NAD reduction by succinate There seem to be three tight sites per molecule of F_1, but they may not all be identical [2].

Enzyme stored in ATP-containing ammonium sulfate solution at 2°C may be washed by repeated precipitation from ATP-free buffer During this brief washing procedure the nucleotide content is in some ways changed, because the washed enzyme has kinetic properties that differ from enzyme that is assayed directly from solutions containing ATP [3]. Preincubation with ADP results in yet another type of enzyme.

We have shown previously that such preincubation of F_1 with ATP (possibly constituting a transfer of the enzyme to a form containing only ATP as tightly bound nucleotide) changes the kinetics of the hydrolysis of ITP and other nonadenine-like nucleotides [3] The mechanism of this interaction between tight and catalytic sites is unknown

Product inhibition of ATPase by ADP gives convex-curved recordings of hydrolysis progress in

assay methods where ADP is not continuously removed. This inhibition is complex and time dependent, implicating both catalytic and regulatory sites in the process, with different time constants involved in the different partial processes [1,4].

When washed F_1 hydrolyses ITP no such product inhibition is seen, apparently because IDP has lower affinities for the catalytic and regulatory sites [1] When F_1 is preincubated with ATP and used for ITP hydrolysis, convex progress curves are obtained with the protonometric method, especially at low temperatures (Harris, D. and Klungsøyr, L, unpublished observations). This may be explained in several different ways. The stimulatory effect caused by ATP preincubation is released by dissociation of ATP after dilution of the enzyme into the assay mixture (This was the explanation given previously for the gradual loss during ITPase assay of the stimulation caused by preincubation with 2'-deoxy ATP [3]. Also, preincubation with ATP might change the affinity of the enzyme for IDP at some site, so that this product becomes inhibitory A third possibility is that the ATP introduced by preincubation of the enzyme is in some way changed into an inhibitory substance

Preincubation of F_1 with 2'-deoxy ATP stimulates ITP hydrolysis more than ATP preincubation [3] Hydrophobicity may be one factor in ligand binding during preincubation, and the kinetic consequences of this treatment For this reason we wanted to test the effect of preincubation of F_1 with acetylated nucleotides. Diacetyl ATP is a substrate for myosin ATPase [5], but preliminary experiments showed that this substance has little effect upon F_1-ATPase. 3'-Acetyl ADP is a competitive inhibitor versus ADP in oxidative phosphorylation [6], and we found that monoacetylated nucleotides were both competitive inhibitors of F_1 and influenced the kinetics of the enzyme after preincubation In part of the experiments described here, we used enzyme that had been depleted of most of its bound nucleotides [4,7].

Recently, Kormer et al [8] described the effects of 2'(3')-*O*-trinitrophenyl derivatives of adenine nucleotides on F_1-ATPase. These substances were found to be powerful competitive inhibitors versus ATP, and were also bound to tight sites without any change in hydrolytic activity.

Materials and Methods

ATPase was prepared from bovine heart mitochondria as described by Knowles and Penefsky [9], and stored in liquid N_2 in 50 mM Tris-HCl buffer, pH 8, containing 250 mM sucrose, 4 mM ATP and 2 mM EDTA. Portions of enzyme solution were thawed and mixed with an equal volume of neutralized saturated ammonium sulfate solution This suspension was stored at 2°C. For preparation of washed enzyme, the protein was centrifuged down in the cold, dissolved in 10 mM Tris-sulfate buffer, pH 8, containing 250 mM sucrose and 1 mM EDTA at room temperature. An equal volume of saturated ammonium sulfate solution was added, and the mixture was chilled for 10 min before centrifugation This procedure was repeated twice more.

For preparation of nucleotide-depleted enzyme the procedure of Garret and Penefsky [7] was used with the modifications of Roveri et al [4]. One such preparation was analyzed for nucleotides with high-pressure liquid chromatography on an anion-exchange column, using 1 M ammonium formeate at pH 4 as the eluting solvent 0.5 mol ADP and 0 2 mol ATP were found per mol enzyme (360 000 Da).

ATPase activity and ITPase activity were measured by the protonometric method, essentially as described by Van de Stadt et al. [10] The volume of the reaction chamber was 4.5 ml. The amount of nucleotides introduced into the assay mixture with preincubated enzyme was kept low by adjusting the enzyme concentration so that 1 μl was a suitable volume of enzyme for assay

Acetylated nucleotides were prepared as described by Frandsen and Krishna [11] 1 mmol of nucleotide was dissolved in 50 ml water and the pH adjusted to 6 3. Acetylation was carried out with 5-ml portions of a mixture of acetic anhydride (2 parts) and triethylamine (5 parts) Rapid mixing is essential. The product is checked by high-pressure liquid chromatography, on a reversed-phase column (Chrompack standard column packed with Microbondapack C 18) The mobile phase contained 80% 100 mM sodium phosphate, pH 6.0, and 20% methanol Two to three portions of acetylating mixture were usually sufficient to acetylate all the nucleotide Reagents and water

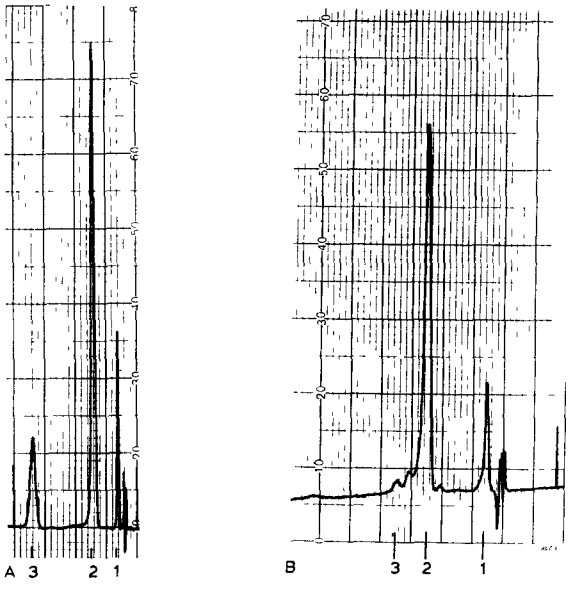

Fig 1 High-pressure liquid chromatography of acetylated nucleotides after partial hydrolysis (see Materials and Methods) Solvent 20% methanol in 100 mM sodium phosphate buffer, pH 6 0 Sensitivity 0 05 (A) Acetylated AMP Recorder speed 0 1 cm/min Peaks A – 1, 2 and 3 AMP, acetyl AMP and diacetyl AMP respectively (B) Acetylated ATP Recorder speed 0 5 cm/min Peaks B – 1, 2 and 3 ATP, acetyl ATP and diacetyl ATP, respectively

were then removed by repeated lyophilization. The product contained mostly the diacetyl derivative. It was dissolved in water and kept at pH 9.5 for several hours at room temperature. The content of the monoacetyl derivative increased to a maximum, whereupon free nucleotide increased in quantity Chromatograms of the substances used are shown in Fig 1. ATP, ADP, AMP and ITP were obtained from Sigma

Results

Effects of preincubation with ATP on F_1-ITPase. Initial rates

F_1 stored at 2°C in ATP-containing ammonium sulfate solution was washed three times by precipitation from ATP-free buffer as described in Materials and Methods. Part of the solution of enzyme in ATP-free buffer was retained as control, and the rest was made 9 mM with respect to ATP. The rates of ITP hydrolysis were determined at five different temperatures between 10 and 30°C. Suitable dilutions of the enzyme solutions were made with ATP-free or ATP-containing buffers, as the rates increased with increasing temperatures. In Fig. 2 the logarithms of the observed rates are plotted against the inverse temperature. After preincubation of F_1 with ATP the temperature dependence of ITP hydrolysis is reduced, i.e., it becomes more like that of ATP hydrolysis [3].

Preincubation with ATP also makes F_1-ITPase less sensitive to dinitrophenol at low temperatures (Fig. 3) and as has been shown previously [3] dinitrophenol actually stimulates ITPase on the ATP preincubated enzyme. It appears that with

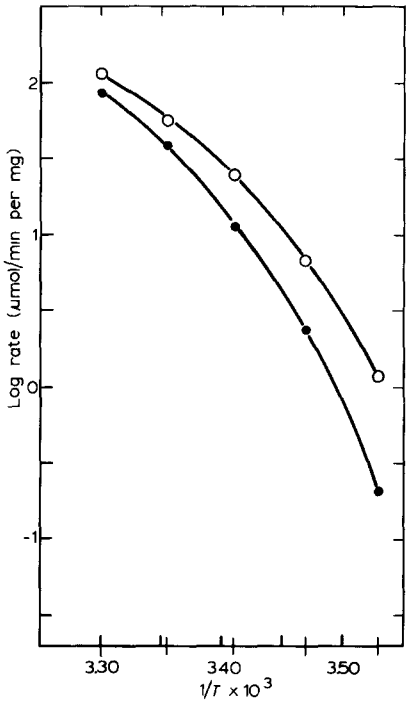

Fig 2 The logarithms of ITP hydrolysis rates plotted against the inverse temperature (●) Enzyme freed of ambient ATP by repeated precipitation with ammonium sulfate (washed enzyme, see Materials and Methods), (○) washed enzyme after preincubation with 9 mM ATP in 10 mM Tris-sulfate buffer, pH 8 0, containing 250 mM sucrose and 1 mM EDTA The reaction mixture contained 1 mM Tris-sulfate, 1 mM $MgSO_4$, 0 1 mM EDTA, 50 mM NaCl and 4 mM MgITP adjusted to pH 8 0 Initial rates were determined from a recording of proton production versus time, related to a standard of 5 mM succinic acid of which 10 μl were added to the complete reaction mixture before addition of enzyme The rates are given as μmol ITP hydrolyzed/min per mg enzyme

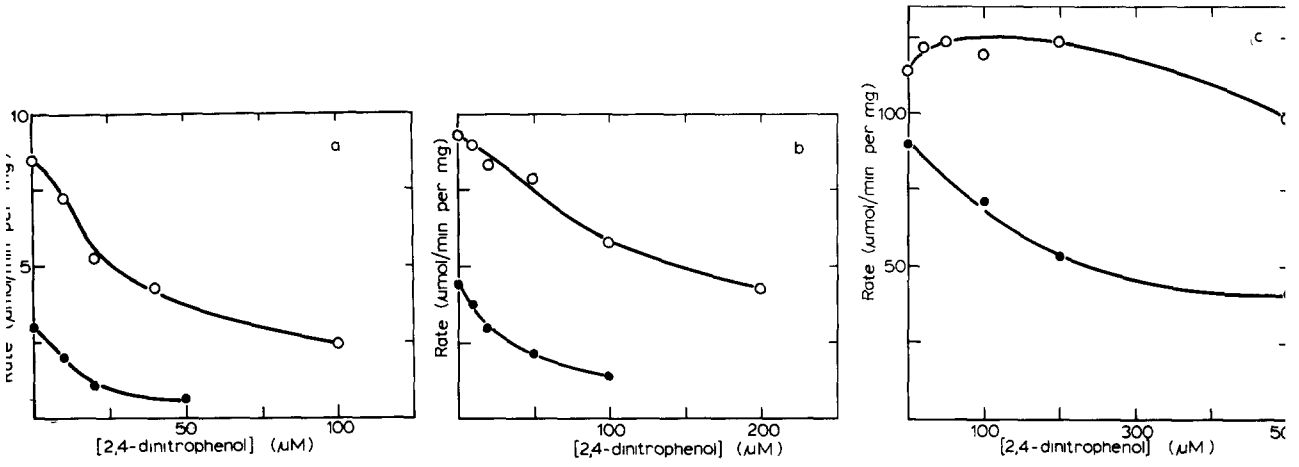

Fig 3 The rate of hydrolysis of ITP by washed ATPase, in the presence of 2,4-dinitrophenol and with (○) or without (●) preincubation with 9 mM ATP, at three different temperatures (a) 10, (b) 18 and (c) 27°C Initial rates were determined by the protonometric method as described in the legend to Fig 2 and in Materials and Methods The concentration of MgITP was 4 5 mM

respect to both temperature dependence and effect of dinitrophenol, ATP-preincubated F_1 hydrolyses ITP partly as if it were ATP

The reaction rates presented in Figs. 2 and 3 are initial velocities The preincubation effect of ATP dissipates over a period of from several seconds to a few minutes [3]. The change in velocity as the reaction proceeds must be caused by some change in the enzyme or its bound ligands, presumably at tight sites. In order to use acetyl ATP as a probe in tight site interactions, we needed to know how it affects the processes taking place at the catalytic site.

Acetyl ATP as an inhibitor of F_1-ATPase

The hydrolysis of ATP by F_1 is inhibited by low concentrations of acetyl ATP On washed enzyme (prepared as described in Materials and Methods) the inhibition had a competitive pattern with a K_i of 400 nM (Fig 4). In this experiment, which was performed at 20°C, a K_m for MgATP of 180 μM was found Neither the K_m for MgATP nor the inhibition by acetyl ATP changed much with the temperature (experiment not shown).

On nucleotide-depleted enzyme the data could not be fitted to a competitive pattern (Fig. 5) Calculated from the slope effects in the reciprocal plots, the K_i for acetyl ATP was 1.7 μM at 10°C and 4 μM at 30°C, while the K_m values for MgATP were of the order of 500 μM (30°C) and 700 μM (10°C).

Preincubation of F_1 with acetyl ATP

Nucleotide-depleted enzyme in glycerol-con-

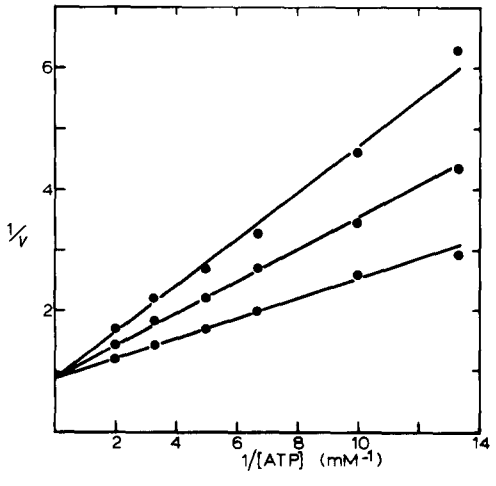

Fig 4 Double-reciprocal plots of initial rates of hydrolysis of ATP The rates are given in arbitrary units as determined by the protonometric method The enzyme was washed enzyme, and the experiments were carried out as described in the legend to Fig 2, except that MgITP was replaced by varying concentrations of MgATP (Lower trace) Control, (Middle trace) reactions carried out in the presence of 250 nM acetyl ATP, (Upper trace) reactions carried out in the presence of 500 nM acetyl ATP The temperature was 20°C

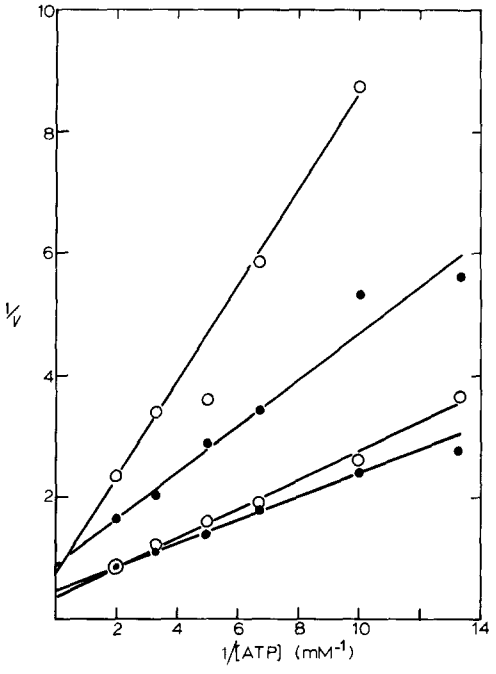

Fig 5 Double-reciprocal plots of initial rates of ATP hydrolysis at 10 (○) and 30°C (●) The rates are given in arbitrary units determined by the protonometric method as described in the legend to Fig 2, except that MgITP was replaced by varying concentrations of MgATP The enzyme was nucleotide-depleted enzyme in 50% glycerol (see Materials and Methods) Lower lines, control experiments Upper lines, experiments made in the presence of 4 μM acetyl ATP

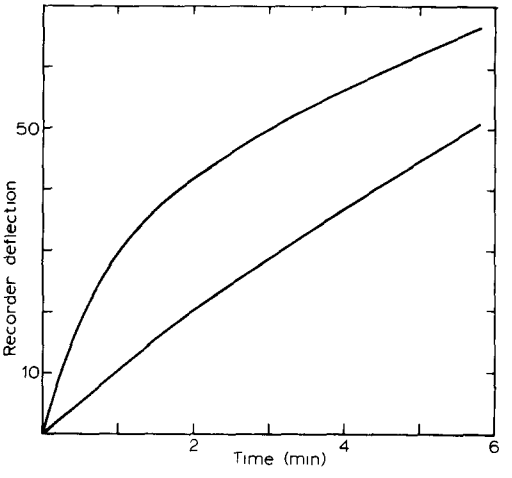

Fig 6 Copies of recorder tracings from assays of ITP hydrolysis at 10°C by the protonometric method as described in the legend to Fig 2 A recorder deflection of 10 corresponds to approx 25 nmol protons, or to the hydrolysis of 25 nmol of ITP in the 4 5 ml reaction volume Lower trace control experiment The reaction mixture contained 1 μl of 0 8 mM acetyl ATP, and the reaction was started with 1 μl of nucleotide-depleted enzyme in 50% glycerol Upper trace nucleotide-depleted enzyme in 50% glycerol was made 0 8 mM in acetyl ATP, and preincubated at room temperature for 10 min 1 μl of the preincubated enzyme was used to start the reaction in a standard reaction mixture

taining buffer was preincubated with acetyl ATP The treatment resulted in highly increased initial rates of ITP hydrolysis compared to an untreated control. The progress recording was, however, strongly curved, and after an intermediate phase the hydrolysis was inhibited compared to the control (Fig 6).

This could mean that the acetyl ATP slowly dissociates from the enzyme during the assay, it could also mean that the tightly bound triphosphate is changed while bound to the enzyme, even though the slowness of the dissipation shows that the change is unlikely to take place at the catalytic site

Nucleotide-depleted enzyme preincubated with ADP

If tightly bound ATP is changed during ITP hydrolysis, it seems reasonable that it becomes tightly bound ADP We therefore measured ITP hydrolysis with nucleotide-depleted enzyme that had been preincubated with acetyl ADP, and with ADP, at 10 and 20°C In both cases we obtained strong stimulation of the initial rates, followed by a distinct inhibition compared to the untreated control An experiment with ADP-preincubated enzyme hydrolysing ITP is shown in Fig. 7. Again the biphasic effect of ADP preincubation could be caused by loss of nucleotide to the solvent during the assay, although this could hardly explain the strong inhibition that developed. The alternative is then that also ADP is changed on the enzyme, and that the likely product, AMP, has an inhibitory effect

Preincubation of F_1 with acetyl AMP

Preincubation of nucleotide-depleted enzyme with acetyl AMP led to a slowly developing inhibition of ITP hydrolysis (Fig 8). The inhibition reached about 50% after preincubation for 1 h at

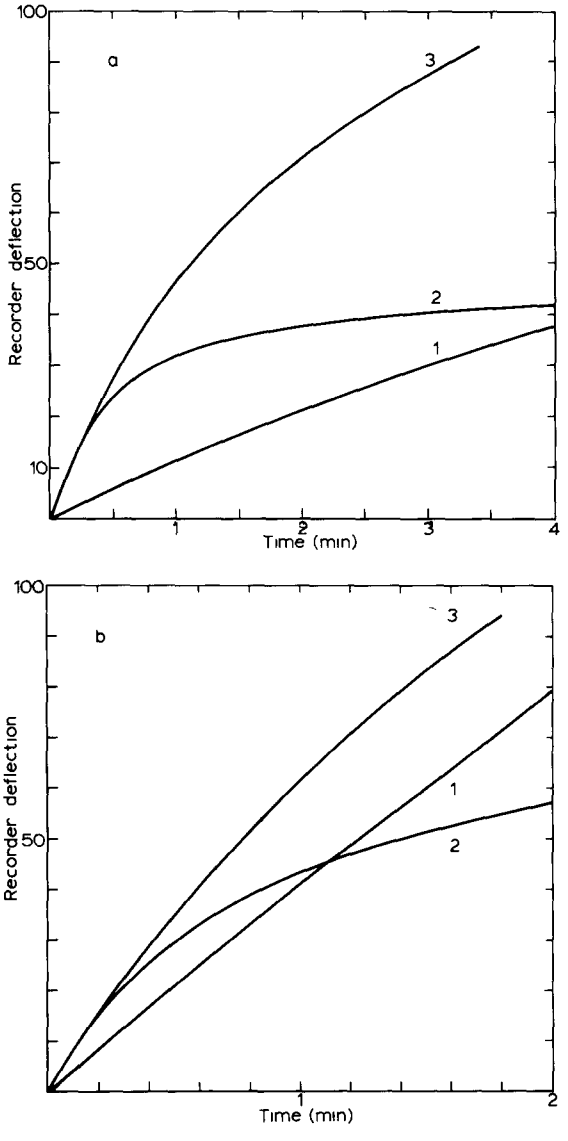

Fig 7 Copies of recorder tracings from assays of ITP hydrolysis The enzyme and conditions were as described in Fig 6, except that the temperature was 10°C in a and 20°C in b (1) Control enzyme, (2) enzyme preincubated with 2 mM ADP, (3) enzyme preincubated with 10 mM ATP

room temperature. The effect could be reversed during a second preincubation period with acetyl ATP (Fig 9). The experiments are complicated by the dual action of acetyl ATP: Immediate inhibition at the catalytic site seen when acetyl ATP is added to the reaction mixture prior to the control enzyme, and stimulation after preincubation. The

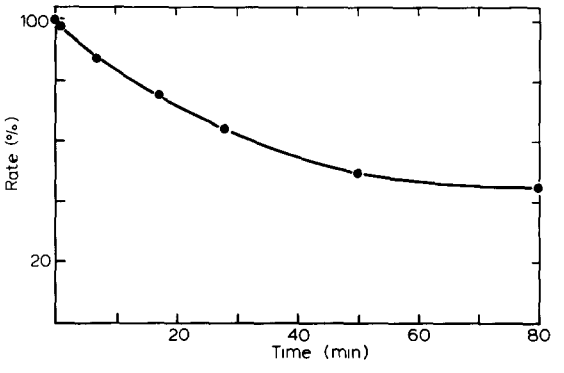

Fig 8 Effect of preincubation with acetyl AMP on ITPase activity of nucleotide-depleted F_1 Nucleotide-depleted enzyme in 50% glycerol was made 20 mM in acetyl AMP and immediately assayed for ITPase activity as described in the legend to Fig 2 except that the ITP concentration was 2 mM During the 80 min preincubation period at room temperature the enzyme was repeatedly assayed as shown Nucleotide-depleted enzyme, similarly diluted, served in control experiments, which activity was taken as 100% The assay temperature was 30°C

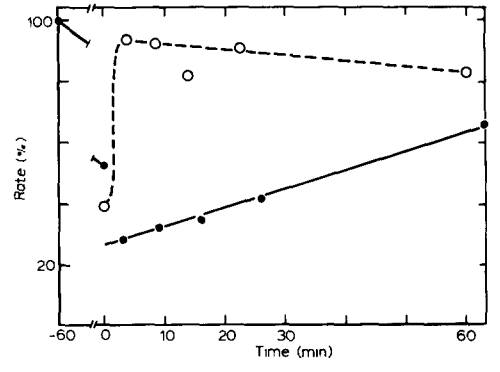

Fig 9 ITPase activity of nucleotide-depleted F_1 after preincubation with acetyl AMP, followed by treatment with acetyl ATP The assays were carried out as described in the legend to Fig 2, except that the concentration of MgITP was 2 mM, and the temperature was 30°C Stock enzyme solubtion was diluted to contain approx 1 mg protein/ml, so that 1 μl was a suitable volume to inject into the assay medium (4 5 ml) (●) A series of experiments carried out in the following way control-level ITPase activity was determined and set equal to 100% at −60 min A sample of the enzyme was made 20 mM in acetyl AMP, incubated at room temperature for 60 min, assayed at time zero and at the same time the mixture was made 8 mM in acetyl ATP This made the final concentration of acetyl ATP in the assay medium 1 8 μM and caused a further drop in activity as assayed at 3 min, while later assays show recovery of activity (○) Another sample of the same enzyme dilution was assayed for activity in the presence of 1 μl of 8 mM acetyl ATP in the reaction medium (○, at zero time) This enzyme sample was then made 8 mM in acetyl ATP and 1 μl portions were assayed at intervals

latter appears quickly when only acetyl ATP is present, slowly when the enzyme has first been treated with acetyl AMP

Discussion

When F_1 is assayed with the protonometric method, there is an almost linear relationship between the proton stoichiometry and the recorder deflection within the pH interval used for measurement. The production of protons as a function of time is therefore directly represented by the recorder tracing. Some features of the progress curves obtained under different conditions are as follows. (1) Hydrolysis of MgATP: this gives a convex progress curve, as expected from product inhibition (even if this is acting on more than one site, with different time constants) [3]. (2) Hydrolysis of MgITP. With washed F_1 the recorded curve is slightly concave, with a distinct initial lag. With nucleotide-depleted enzyme the lag is absent Temperature and ITP concentration have little effect upon the shape of the hydrolysis curve. (Harris, D and Klungsøyr, L., unpublished experiments) (3) F_1 preincubated with ATP and hydrolyzing MgITP. the hydrolysis is stimulated relative to the nonpreincubated control, most at low temperatures. The progress recording is convex, most at low temperatures, indicating loss of stimulation or even inhibition as the reaction proceeds [3] (Figs 2 and 3). (4) F_1 preincubated with acetyl ATP and hydrolyzing MgITP the reaction is more stimulated than after preincubation with ATP and deoxy ATP, and the curvature of the progress recording is pronounced, so that the reaction ends up inhibited compared to the nonpreincubated control Both stimulation and subsequent inhibition are most pronounced at low temperatures Acetyl ATP has the strongest effect on nucleotide-depleted enzyme (Fig. 6) (5) The effect of preincubation of F_1 with ADP is complex. On washed enzyme, at 30°C, the effect is inhibitory [1] while nucleotide-depleted enzyme is strongly stimulated after preincubation with ADP, when hydrolyzing ITP at 10 or 20°C. In the latter cases the progress curve is convex, so that the reaction ends up strongly inhibited compared to the nonpreincubated control, especially at 10°C, but also at 20°C (Fig 7) (6) Nucleotide-depleted F_1 preincubated with acetyl AMP is slowly converted to an inhibited form This inhibition is reversible if acetyl ATP is added to the incubated mixture of F_1 and acetyl AMP (Fig 9)

Concave hydrolysis curves may indicate release of inhibition, or an activation process that starts when assay conditions are established With washed F_1 hydrolyzing ITP, assay condition activation seems unlikely, since ITP does not bind to the tight sites [1]. A release of inhibition seems therefore to be taking place as ITP is hydrolyzed.

Similarly, convex hydrolysis curves may indicate loss of stimulation or building up of inhibition. The shape of the curve with washed enzyme hydrolyzing ITP after preincubation with 2′-deoxy ATP has been interpreted as being a loss of stimulation [3] However, such loss of stimulation during the assay should only lead to the control level of activity This is often not the case Nucleotide-depleted enzyme preincubated with acetyl ATP, although initially stimulated, reaches a final activity during the assay which is below the control level This is observed both at 10°C (Fig 6) and 20°C (data not shown) The observed change from strong stimulation to distinct inhibition during the assay may be explained if the bound stimulatory acetyl ATP is chemically changed into an inhibitory substance, possibly acetyl ADP However, when nucleotide-depleted enzyme is preincubated with ADP, the hydrolysis of ITP at 10 and 20°C is stimulated initially, and then becomes strongly inhibited (Fig. 7). The same is observed when the enzyme is preincubated with acetyl ADP or 2′-deoxy ATP (data not shown)

If inhibition builds up because of a chemical change in the initially stimulatory ADP, AMP formation at a regulatory site during ITP hydrolysis at the catalytic sites might be the mechanism.

We have at present only preliminary information on the conditions under which pretreatment of F_1 with AMP will affect the hydrolysis of ITP. It is, however, a reasonable assumption that AMP is either introduced with difficulty into the tight regulatory sites of F_1, or it is only loosely bound to these sites. Acetyl AMP, on the other hand, is quite firmly held to F_1, as is shown by its slow displacement by acetyl ATP (Fig. 9). The low rate at which inhibition of ITP hydrolysis develops by preincubation with acetyl AMP shows that the

binding of the inhibitor is a complicated process indeed (Fig. 8). Kormer et al. [8] suggest that a certain specificity is involved in the binding of the trinitrophenyl group of the trinitrophenyl nucleotides. Our experiments with much simpler derivatives would argue for rather unspecific hydrophobic effects

We therefore postulate that nucleotides made more hydrophobic by esterification of the ribose hydroxyl groups more easily than the free nucleotides gain access to and stay bound to the regulatory tight sites of F_1 (and its catalytic sites as well). If AMP is indeed the executor of the inhibition that develops on F_1 after preincubation with ADP, it would be carried to its site of action as its precursor, ADP.

Washed enzyme that has been preincubated with ATP may be assumed to contain mostly ATP in all tight sites [1]. This enzyme hydrolyzes ITP as if it were ATP with a fraction of its capacity (Figs 2 and 3) The nucleotide equipment at the tight sites seems to determine the mode of hydrolysis at the catalytic sites Possibly, therefore, the tight site nucleotides may participate in the path to orthophosphate from the triphosphates bound at the catalytic sites.

The results with nucleotide-depleted enzyme, in which preincubation with ADP strongly stimulates ITP hydrolysis, show that the stimulatory effect is not restricted to the triphosphates, even if the diphosphate stimulation is more quickly exhausted. The experiments with acetyl nucleotides add one more example to the list of changes which are tolerated by the enzyme, leaving N-1 and C-6 as practically the only positions that must be adenine-like for tight-site effects [1], even if the ligands apparently cover a wide range of affinities

Thus, the positions N-1 and C-6 are not decisive for affinity to F_1 tight sites. Is it possible that these positions play a direct role in the hydrolytic process itself? In ATP phosphoribosyltransferase (EC 2 4 2.17) N-1 acts as a nucleophile and the equilibrium of that reaction is determined by whether or not the product is protonated [12].

We suggest that when nucleotide-depleted enzyme is preincubated with ATP or ADP and then used for hydrolysis of MgITP, the phosphoanhydride groups 'wander' from ITP bound at the catalytic sites to the nucleotides at the tight sites, and on to the water acceptor. Simultaneously, a slow leakage of phosphoanhydride groups from the tightly bound nucleotides takes place Surprisingly, this leakage does not stop at the ADP stage, but goes on to form deeply buried AMP which is no longer an acceptor of phosphoanhydride groups. This will block the path to water and inhibit hydrolysis.

On nucleotide-depleted enzyme MgITP has direct access to water, and on washed enzyme after a short lag, during which inhibitory AMP may be lost

It is tempting to speculate that a wandering of phosphoanhydride groups might take place also under phosphorylating conditions. In *Rhodospirillum rubrum* chromatophores the tight-site nucleotides are not released during phosphorylation, but possibly ambient ADP may be phosphorylated from bound ATP in the absence of P_i [13].

Our model stands in apparent contradiction to the results of Webb et al [14] which predict a single displacement mechanism However, these authors use a substrate that shows no P_i-H_2O exchange, which would classify it as a nonATP-like substrate. It is implicit in our model that ATP and ITP are hydrolyzed on F_1 by different mechanisms.

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References

1 Harris, D A, Gomez-Fernandez, J C, Klungsøyr, L and Radda, G K (1978) Biochim Biophys Acta 504, 364–383
2 Slater, E C, Kemp, A, Van der Kraan, J, Muller, J L M, Roveri, O a, Verschoor, G J, Wagenvoord, R J and Wielders, J P M (1979) FEBS Lett 103, 7–11
3 Harris, D A, Dall-Larsen, T and Klungsøyr, L (1981) Biochim Biophys Acta 635, 412–428
4 Roveri, O A, Muller, J L M, Wilms, J and Slater, E C (1980) Biochim Biophys Acta 589, 241–255
5 Hasselbach, W (1965) Biochim Biophys Acta 20, 355–368
6 Schafer, G and Onur, G (1979) Eur J Biochem 97, 415–424
7 Garret, N E and Penefsky, H S (1975) J Biol Chem 250, 6640–6647
8 Kormer, Z S, Kozlov, I A, Milgrom, Y M and Novikova, I Y (1982) Eur J Biochem 121, 451–455

9 Knowles, A F and Penefsky, H S (1972) J Biol Chem 247, 6617–6623
10 Van de Stadt, R J , De Boer, B L and Van Dam, K (1973) Biochim Biophys Acta 376, 13–26
11 Frandsen, E K and Krishna, G (1976) Life Sc 18, 529–542
12 Martin, R G (1962) J Biol Chem 238, 257–268
13 Harris, D A and Baltscheffsky, M (1979) Biochem Biophys Res Commun 86, 1248–1255
14 Webb, M R , Grubmeyer, C , Penefsky, H S and Trentham, D R (1980) J Biol Chem 255, 11637–11639