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Beef-heart mitochondrial F_1-ATPase can use endogenous bound phosphate to synthesize ATP in dimethyl sulfoxide

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Beef-heart mitochondrial F_1-ATPase contained 5 mol of inorganic phosphate bound per mol of F_1, following pretreatment with ATP A portion of the phosphate, bound most likely at a catalytic site, reacted in dimethylsulfoxide with endogenous adenine nucleotide to form ATP

Mitochondrial F_1-ATPase, ATP synthesis, Bound phosphate, Dimethylsulfoxide

1 INTRODUCTION

In oxidative phosphorylation in mitochondria, the F_1F_0 complex synthesizes ATP from ADP and inorganic phosphate, coupled with proton influx across the inner membrane Isolated F_1 has ATPase activity, however, it can be induced to form ATP in the presence of ~30% (v/v) Me_2SO [1–8] This system is therefore of considerable interest, since it provides a simple model system whereby some aspects of the mechanism of oxidative phosphorylation may be studied It was recently shown that endogenous bound ADP is converted to ATP in Me_2SO at a single site [7,8] In this report, we show that synthesis of ATP by F_1 in Me_2SO can also be carried out in the absence of medium phosphate when the F_1 has been pretreated with ATP before being used in the synthesis reaction. Phosphate analyses of preloaded F_1 revealed 5 mol P_i bound per mol of F_1 Since ATP synthesis by F_1 can be carried out with endogenous bound P_i, at least one of the bound P_i molecules must be present at a catalic site

2. MATERIALS AND METHODS

2 1 *Preparation of beef heart mitochondrial F_1-ATPase and assays*

The beef heart mitochondria were a generous gift from Dr Y Hatefi (Research Institute of Scripps Clinic, La Jolla, CA) The preparation of beef heart mitochondrial adenosine triphosphatase, coupled assay of F_1-ATPase activity, and determination of protein concentration were carried out as described previously [9,10] The M_r of F_1 used in the calculations was 371 000 [11]

2 2 *ATP synthesis and measurement of bound ATP and ADP*

The F_1 stored in $(NH_4)_2SO_4$ suspension was precipitated, redissolved in a buffer of 100 mM Tris-acetate, pH 6 8, and desalted for use by passage through a Sephadex G-50-80 centrifuged column equilibrated with the same buffer The stock solutions, protocol for ATP synthesis and measurement of bound nucleotide(s) (ATP and ADP) were as described previously [8], except that in these experiments with medium nucleotide absent, three 100 µl aliquots of each 400 µl reaction mixture were desalted once only and the volume of the combined eluates made up to 300 µl with appropriate buffer, i e , 100 mM Tris-acetate, pH 6 8, ±30% (v/v) Me_2SO Note that the stock solution of 100 mM Tris-acetate, pH 6 8, containing 40% Me_2SO, was made just before use This is important, since the pH tends to fall if the solution is left standing even in the cold room The aqueous reaction mixtures were passed through centrifuged columns equilibrated with 100 mM Tris-acetate, pH 6 8, and the Me_2SO-containing reaction mixtures were passed through centrifuged columns equilibrated with the same buffer containing 30% (v/v) Me_2SO

2 3 *P_i loading of F_1 and measurement of bound P_i*

The F_1 stored in $(NH_4)_2SO_4$ suspension was precipitated, redissolved in a buffer of 100 mM Tris-acetate, pH 6 8, and desalted for use by passage through a Sephadex G-50-80 centrifuged column equilibrated with the same buffer The F_1 was preloaded with ADP/P_i by incubation with 250 µM ATP in 100 mM Tris-acetate, pH 6 8, for 30 s [7], and Mg^{2+} was not added The F_1 was then passed through a set of Sephadex G-50-80 centrifuged columns equilibrated with 100 mM Tris-acetate, pH 6 8 The centrifugates were pooled and passed through a second set of similarly prepared columns The second set of centrifugates were also pooled and the final volume made up to a desired volume such that the final protein concentration would be in the 1–2 mg/ml range Aliquots of this combined centrifugate were set aside for bound nucleotide, P_i and protein determinations.

The P_i assays were performed as described by Lanzetta et al [12] using the Malachite Green/ammonium molybdate/Sterox mixture (Note that since Sterox is no longer available commercially, we substituted Brij 35 with good results) 550 µl aliquots of the F_1 samples were heated at 70°C for 10 min, kept on ice for 10 min, and then centrifuged [8,13] The supernatant (500 µl samples) was assayed for P_i, with and without the addition of 5 nmol P_i At least two samples of each were assayed Both assays (supernatant and supernatant plus P_i) give the same answer for the nmols of P_i present in the supernatant

Abbreviations Me_2SO, dimethylsulfoxide, P_i, inorganic phosphate, F_1, F_1 ATPase portion of mitochondrial ATP synthase

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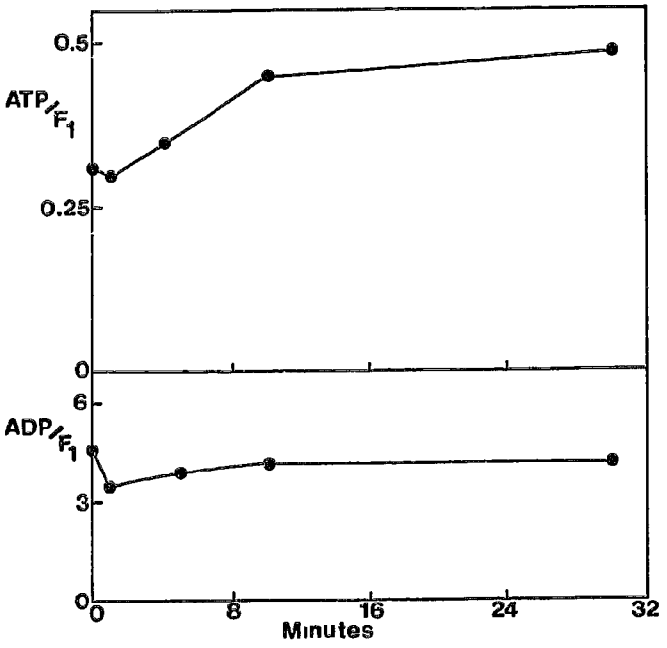

Fig 1 Changes in the concentrations of bound nucleotides in ATP-pretreated F_1 following dilution into buffer containing 30% (v/v) Me_2SO The experiment was carried out as described in section 2 Bound nucleotide content is expressed as mol/mol F_1

3. RESULTS AND DISCUSSION

In a recent study we have found that beef-heart mitochondrial F_1-ATPase pretreated with ATP contains approximately 5 mol ADP/mol F_1 [8] Others have also found at least 5 mol nucleotide bound per mol of F_1 [14,15]. Although not unambiguously established, some previous work has indicated that ADP and P_i dissociate from F_1 at similar rates both in unpromoted [16] and ATP-promoted reactions [10,17] These results suggested to us that F_1 pretreated with ATP might contain bound P_i

F_1 was incubated with 0 25 mM ATP in 0 1 M Tris-acetate, pH 6 8 for 0 5 min in the absence of Mg^{2+}. Nucleotides and any phosphate were removed by passage twice through centrifuged columns of Sephadex G-50 In 3 separate experiments with ATP-pretreated F_1, 5.0±0.5 mol P_i bound per mol F_1 were found. Thus, in our ATP-preloaded enzyme, phosphate was present in amounts stoichiometric to ADP Bound P_i has not been detected previously by other workers [14], although Penefsky and coworkers [18,19] found at least 2 binding sites for P_i on beef-heart F_1 under certain conditions. They suggested on the basis of indirect evidence that the high-affinity P_i binding site might be a catalytic site for ATP formation Our results are consistent with those of Penefsky [18,19], particularly if the 3 non-catalytic adenine nucleotide binding sites on F_1 [20] also contain bound P_i, as suggested by our data. The inability of other workers [14] to detect bound P_i on F_1 may be due to (i) the use of a phosphate assay less sensitive than the malachite green assay used here, or (ii) the inhibition of P_i binding to F_1 by EDTA present in their buffers [18]

F_1 normally functions as an ATPase It can be induced to form ATP from added ADP and P_i by carrying out the reaction in the presence of Me_2SO [1–8] We have shown recently [8] that endogenous bound ADP can be converted to ATP We therefore examined the ability of F_1 to convert the endogenous P_i to ATP F_1, after pretreatment with ATP and subsequent removal of adenine nucleotides and P_i, was incubated in 30% Me_2SO without the further addition of P_i or nucleotide. As shown in Fig. 1, ATP (0 15 mol/mol F_1) was formed from endogenous P_i and ADP, thus indicating that both endogenous P_i and ADP are bound at a catalytic site The level of bound ADP decreased in the first 2 min of incubation in Me_2SO We have found (Beharry and Bragg, unpublished results) that this decrease is due to the release of adenine nucleotide from catalytic sites on F_1 when the enzyme is transferred to 30% Me_2SO. Subsequently, there is rebinding of the nucleotide prior to ATP formation. Loss of nucleotide is facilitated by high (10 mM) concentrations of exogenous P_i These conditions favour ATP formation with about 0.5 mol ATP being formed/mol F_1 (also see [8]) In summary, beef-heart mitochondrial F_1-ATPase contains about 5 mol of bound P_i/mol enzyme following pretreatment with ATP. Part of the bound P_i will react with endogenous ADP in the presence of 30% Me_2SO to form ATP, and thus must be at a catalytic site

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