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Conformational change in beef-heart mitochondrial F_1 ATPase to ATP synthesis mode induced by dimethylsulfoxide and ATP revealed by sulfhydryl group labeling

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Treatment of beef-heart mitochondrial F_1 ATPase with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) results in the incorporation of 1 mol DTNB/mol F_1 without loss of ATPase activity. Incorporation is not prevented by ATP. Labeling occurs predominantly on an α-subunit, but also with a significant degree of modification of γ- and ε-subunits. It is suggested that the modified sulfhydryl groups of the α-, γ- and ε-subunits are in proximity so that only one can be modified by the reagent. Guanidine hydrochloride (0.3 M) dissociates F_1 into its subunits. Eight sulfhydryl groups/mol F_1 can be modified under these conditions. Guanidine hydrochloride does not cause dissociation of F_1 in the presence of 30% (v/v) dimethylsulfoxide (Me_2SO) and 2 mM ATP. Under these conditions a second molecule of DTNB is incorporated into F_1 with nearly equal modification of the ε-subunit and an α-subunit. It is proposed that Me_2SO and ATP induce a more stable conformation of F_1, which is resistant to dissociation by guanidine hydrochloride, but in which the site of reaction with DTNB is made more accessible by the guanidine hydrochloride to permit the simultaneous modification of an α-subunit and the ε-subunit. Ths conformation is probably that which occurs during ATP synthesis by F_1 in the presence of Me_2SO.

Mitochondrial F_1 ATPase; Conformational change; ATP synthesis

1. INTRODUCTION

In oxidative phosphorylation in mitochondria the F_1F_0 complex synthesizes ATP from ADP and 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 [1–5]. Thus, this system is of interest in studies of the mechanism of oxidative phosphorylation.

In order to study the role of Me_2SO in favouring the formation of ATP over its hydrolysis, the possibility that this compound induces a conformational change in F_1 has been examined. We have used the degree to which Me_2SO changes the exposure of sulfhydryl groups to the sulfhydryl modifying agent DTNB as an indication of conformational change. Although previous work [6,7] suggested that twelve cysteine residues were present in beef-heart mitochondrial F_1, recent amino acid sequence data has indicated that eight residues are present [8]. Two cysteine residues occur in each of the three α-subunits, with one each in the γ- and ε-subunits. Previous studies have suggested that one sulfhydryl group is accessible to DTNB in native yeast mitochondrial F_1 and two sulfhydryl groups are available in the mammalian enzyme [6,9,10]. The subunit location of the accessible sulfhydryl groups has not been determined.

In the present paper we confirm that eight cysteine residues are present in beef-heart mitochon-

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Abbreviations: DTNB, 5,5'-dithiobis(2-nitrobenzoic acid); Me_2SO, dimethylsulfoxide

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drial F_1. In the absence or presence of 30% (v/v) Me_2SO and/or 2 mM ATP only one sulfhydryl group reacts with DTNB. An additional sulfhydryl group is revealed by guanidine hydrochloride in the presence of 30% (v/v) Me_2SO and 2 mM ATP. One of the sulfhydryl groups reacting with DTNB under these conditions is on an α-subunit. The other reactive sulfhydryl group is on the ϵ-subunit. It is proposed that Me_2SO and ATP induce a more stable conformation of F_1 which is resistant to dissociation by guanidine hydrochloride. This conformation is probably that which occurs during ATP synthesis by F_1 in the presence of Me_2SO.

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 system of F_1-ATPase activity and determination of protein concentration were carried out as described previously [11].

2.2. *Sulfhydryl group determination*

The sulfhydryl groups of the F_1-ATPase were measured with 5,5'-dithiobis(2-nitrobenzoic acid) using the spectrophotometric assay [12] as described by Adade et al. [13], except that no sucrose was used in the buffer mentioned. The absorption coefficient of the compound formed between DTNB and cysteine·HCl was found to be 9466 $M^{-1}\cdot cm^{-1}$ in 30% (v/v) Me_2SO compared with 13600 $M^{-1}\cdot cm^{-1}$ for the aqueous system.

2.3. *Inhibition studies*

Inhibition studies of ATPase activity by DTNB were carried out using the coupled assay system of Penefsky [14]. The F_1 was incubated with DTNB (0.3 mM) in a buffer of 10 mM Tris-sulphate and 1 mM EDTA, pH 7.5 [13], with and without 30% (v/v) Me_2SO. After the indicated times (usually 30 min), guanidine hydrochloride (0.3 M, final concentration) was added to the incubation mixture. Samples were taken from each reaction mixture and assayed for ATPase activity in the appropriate buffer system. F_1 from the aqueous reaction mixtures was assayed in the aqueous buffer system, whereas F_1 from 30% Me_2SO reaction mixtures was assayed in the 30% Me_2SO buffer assay system. Under our experimental conditions 30% Me_2SO did not affect the coupled assay system provided that the amount of pyruvate kinase was increased three-fold. For investigations with ATP, the enzyme was incubated with ATP (2 mM, final concentration) for 2 min before addition of DTNB (0.3 mM, final concentration).

2.4. *Gel electrophoresis*

SDS-polyacrylamide gel electrophoresis was performed as described by Laemmli [15]. Non-denaturing polyacrylamide gel electrophoresis studies were carried out with a 4% stacking gel on a 15% separating slab gel by the method of Davis [16]. Proteins were stained with Coomassie blue [16].

2.5. *Radiolabeling*

F_1 (0.8–1.0 mg/ml) was reacted with [^{14}C]DTNB (Research Products International Corp., IL, USA) in the presence or absence of 30% (v/v) Me_2SO in the appropriate buffer with and without 2 mM ATP. After 20 min a portion (125 μl) of the reaction mixture was desalted through a centrifuged 1 ml column of Sephadex G-50 in the appropriate buffer with and without Me_2SO [18]. This was done in triplicate and the samples combined. Guanidine·HCl was added to the remaining reaction mixture to a final concentration of 0.3 M. This reaction mixture was allowed to stand for 60 min before desalting. Samples were run on SDS-polyacrylamide gels without 2-mercaptoethanol. After staining and drying, the gels were placed against X-ray film (XAR-5). The intensity of the bands on the radioautograph were scanned using a laser densitometer. The area under the peaks was measured and the relative ratios of the labeling calculated.

3. RESULTS

The effect of Me_2SO on the conformation of F_1 was examined by determining the exposure of the sulfhydryl groups to the aqueous medium.

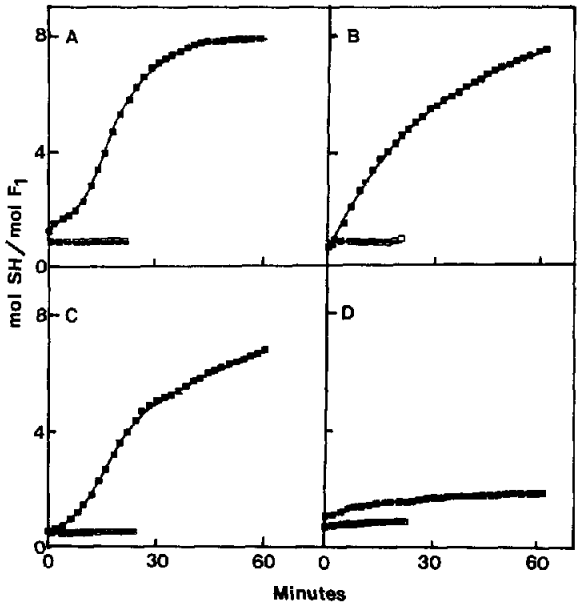

Fig.1. Assay of sulfhydryl groups of F_1 following exposure of the enzyme to 0.3 M guanidine hydrochloride in the absence (A,B) and in the presence of 30% (v/v) Me_2SO (C,D). In B and D, 2 mM ATP was present. Sulfhydryl groups were assayed with 0.3 M DTNB. The concentration of F_1 was 0.32 mg protein/ml. Open points, without guanidine hydrochloride. Closed points, guanidine hydrochloride present.

Sulfhydryl groups were determined by reaction with DTNB. It was found that the absorption coefficient of the sulfhydryl adduct with this reagent was diminished in Me_2SO. The absorption coefficient in 30% Me_2SO was determined using cysteine as the sulfhydryl compound. A value of 9466 $M^{-1} \cdot cm^{-1}$ was found in this solvent compared with 13600 $M^{-1} \cdot cm^{-1}$ for the entirely aqueous system.

As shown in fig.1, only one sulfhydryl/mol F_1 was available for reaction in the native F_1 both in the absence and presence of Me_2SO. Full exposure of the eight sulfhydryl groups present in F_1 [9] was produced by treatment of the enzyme with 0.3 M guanidine hydrochloride. ATP (2 mM) or the presence of 30% Me_2SO slowed the rate of exposure of sulfhydryl groups. A combination of ATP and Me_2SO stabilized the F_1, permitting the reaction of only one additional sulfhydryl group with the sulfhydryl reagent (fig.1D).

The reaction of one sulfhydryl group with DTNB had little effect on enzyme activity, at least initially (fig.2). Addition of ATP to the entirely aqueous system increased the stability of the enzyme since the slow loss of activity observed in its absence was prevented (fig.2A,B). Addition of 0.3 M guanidine hydrochloride resulted in immediate loss of enzyme activity. This was not prevented by ATP and/or Me_2SO. However, a significant difference was observed between the samples treated with guanidine hydrochloride. As shown by electrophoresis in non-denaturing polyacrylamide gels, the presence of ATP or Me_2SO alone did not prevent dissociation of the enzyme into subunits. The F_1 did not dissociate when both Me_2SO and ATP were present (data not shown).

The subunit location of the sulfhydryl groups reacting with DTNB under the conditions of figs 1 and 2 was examined using [^{14}C]DTNB (fig.3). In

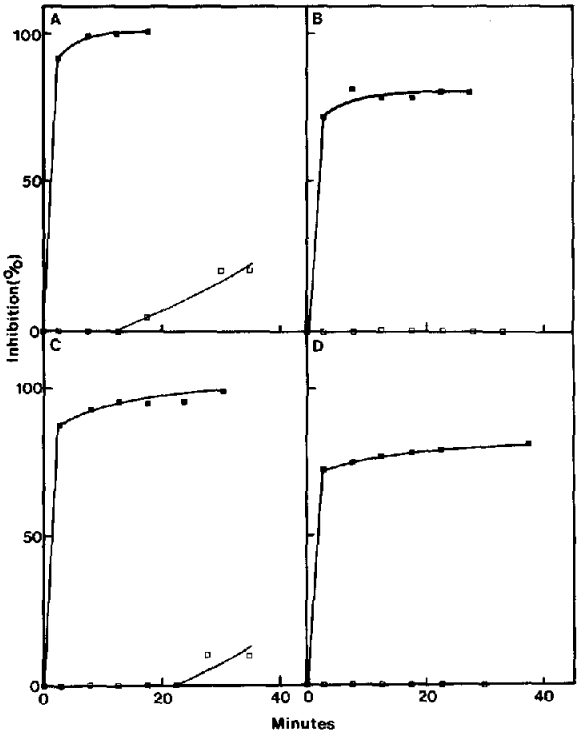

Fig.2. Inhibition of ATPase activity of F_1 by DTNB following exposure of the enzyme to 0.3 M guanidine hydrochloride in the absence (A,B) and in the presence of 30% (v/v) Me_2SO (C,D). In B and D, F_1 was incubated with ATP (2 mM) for 2 min before addition of DTNB. The concentration of F_1 was 0.23 mg protein/ml. Open points, without guanidine hydrochloride. Closed points, guanidine hydrochloride present.

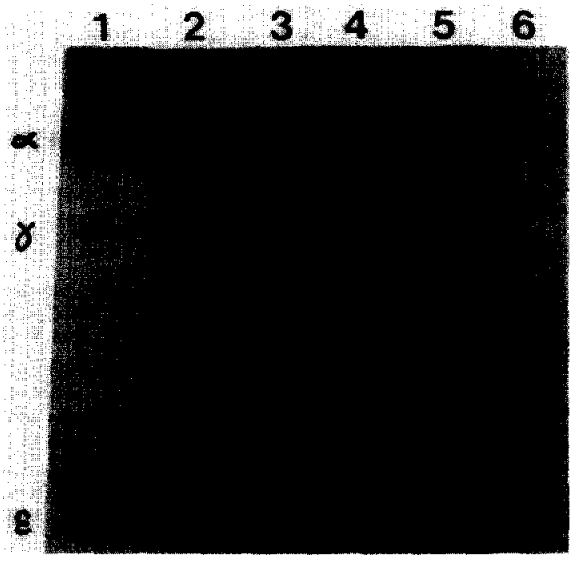

Fig.3. Radiolabeling of subunits of F_1 by [^{14}C]DTNB. The experiment was carried out as described in section 2. Lanes: 1, F_1 reacted with [^{14}C]DTNB in the presence of 0.3 M guanidine hydrochloride; 2, F_1 reacted with DTNB; 3, F_1 reacted with DTNB in the presence of 30% (v/v) Me_2SO; 4, F_1 reacted with DTNB in the presence of 2 mM ATP; 5, F_1 reacted with DTNB in the presence of 30% (v/v) Me_2SO, 2 mM ATP and 0.3 M guanidine hydrochloride. The positions of migration of the subunits of F_1 on SDS gel electrophoresis are indicated.

Table 1

Extent of labeling of sulfhydryl groups of F_1 by [^{14}C]DTNB

Reaction conditions			mol SH labeled/ mol F_1	Distribution of label on subunits		
Me_2SO	ATP	Guanidine·HCl		α	γ	ϵ
−	−	−	0.95	0.52	0.27	0.16
−	−	+	8.0	6.4	0.6	1.1
−	+	−	0.95	0.52	0.21	0.21
+	−	−	0.60	0.42	0.11	0.06
+	+	−	0.90	0.66	0.01	0.22
+	+	+	2.0	1.2	0.09	0.70

This experiment was carried out as described in section 2. Labeling by [^{14}C]DTNB was performed in a buffer with or without 30% (v/v) Me_2SO and/or 2 mM ATP as indicated in the table. Guanidine·HCl (0.3 M) was added to the reaction mixtures, where indicated, and labeling allowed to proceed for a further 60 min. −, absent; +, present. The extent of labeling of F_1 was determined spectrophotometrically (see fig.1). The distribution of radiolabeling on the subunits was measured by densitometry of radioautographs

agreement with the amino acid sequence data of Walker et al. [8] only α-, γ- and ϵ-subunits were labeled by the reagent. Under the reaction conditions which gave 1 mol DTNB reacting/mol F_1, the α-subunit was predominantly labeled by [^{14}C]DTNB, although significant labeling of γ- and ϵ-subunits also occurred (table 1). In the presence of Me_2SO, ATP and guanidine hydrochloride, conditions which led to the reaction of 2 mol DTNB/mol F_1, significant extra labeling of both α- and ϵ-subunits was observed. DTNB under these reaction conditions labeled a sulfhydryl group on one of the α-subunits, and the sulfhydryl group of the ϵ-subunit.

4. DISCUSSION

In agreement with the amino acid sequence data of Walker et al. [8], eight sulfhydryl groups were detected by DTNB in fully denatured beef-heart mitochondrial F_1. These were present in the α-, γ- and ϵ-subunits only. In the absence of the denaturant in entirely aqueous media, with and without 2 mM ATP and/or 30% (v/v) Me_2SO, only 1 mol DTNB reacted per mol F_1. Incorporation of label from [^{14}C]DTNB was predominantly in the α-subunit, although significant labeling of γ- and ϵ-subunits was observed. It is interesting that labeling stabilizes the incorporation of a single molecule of the reagent. This suggests that reaction of one molecule in the native enzyme effectively prevents reaction of a second molecule of DTNB, possibly for steric reasons.

Crosslinking has revealed that α- and γ-, and γ- and ϵ-subunits are in proximity [19,20]. Sulfhydryl groups on the α- and γ-subunits are close enough for a disulfide bridge to be formed under oxidizing conditions. Perhaps DTNB reacts at an interface between α-, γ- and ϵ-subunits. Modification of this site does not affect enzyme activity.

Guanidine hydrochloride (0.3 M) in the presence of 2 mM ATP and 30% Me_2SO does not affect the intactness of the enzyme. ATPase activity is inhibited and a second molecule of DTNB can react. The incorporation of label from [^{14}C]DTNB occurs equally at α- and ϵ-subunits. These results are consistent with a relaxation of the previous site of modification to permit simultaneous reaction of two sulfhydryl groups one on an α-subunit and the other on the ϵ-subunit. This site is not protected by ATP from modification by DTNB.

Since there are three α-subunits in F_1, the labeling of only one subunit by DTNB under these conditions implies that there is a degree of asymmetry among the α-subunits. This could be permanent or be induced by the act of modification. The conformation occurring with ATP and Me_2SO is probably that of F_1 when it is in the ATP synthesis mode.

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