OLIGOMYCIN-SENSITIVE ATPase FROM BEEF HEART MITOCHONDRIA: REACTION WITH 2-MERCAPTOPROPIONYLGLYCINE

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1. Introduction

Evidence is accumulating that oligomycin sensitivity is dependent on the functional state of mitochondrial sulfhydryl groups [1].

Previous experiments have shown that with ADP an increase of mitochondrial reactive SH groups was observed, while, in contrast, there was a decrease when oligomycin was present [2]. The increase of SH group reactivity with ADP was found to be abolished by oligomycin [3,4].

The thiol reagent 2-mercaptopropionylglycine has also been found to produce an increase of reactive SH groups in the mitochondrial membrane, which similarly was abolished by oligomycin (1 μg/mg protein) at ⩽ 9 nmol thiol reagent/mg protein [5]. A recoupling activity has been described for the reagent 2-mercaptopropionylglycine [6,7].

We report here an increase in intensity of band 4 (30 000–32 000 daltons, nomenclature from [8]), apparently identical with band 7 (nomenclature from [9]) after reaction of oligomycin-sensitive beef heart mitochondrial ATPase with 7 nmol 2-mercaptopropionylglycine/mg protein. This increase is completely reversed by raising to 14 nmol/mg protein. The ATPase activity is decreased at 3.5–7 nmol thiol reagent/mg protein. Oligomycin sensitivity of the ATPase activity is increased at 10 μM reagent.

It is proposed that a sulfhydryl–disulfide interchange between ATPase subunits (or correlated proteins) is induced by the thiol reagent.

2. Materials and methods

2.1. *Isolation of beef heart mitochondria*

For the isolation of beef heart mitochondria the method in [10] was employed. Preparation of submitochondrial particles was carried out as in [11]. Protein concentrations were estimated by the method in [12].

2.2. *Preparation of oligomycin-sensitive ATPase complex*

The preparation of the OS ATPase complex followed the procedure in [8] except for the purification step by sucrose gradient centrifugation, which was omitted. In some cases (fig.2a,b) reduction of the disulfides in submitochondrial particles was carried out with 0.5 mM 2-mercaptopropionylglycine instead of 0.5 mM dithiothreitol. The final pellets of OS ATPase were suspended in Tris–sulfate 10 mM, EDTA 0.5 mM, $MgSO_4$ 1.0 mM, sucrose 50 mM (pH 7.5) and stored at −70°C.

2.3. *Estimation of ATPase activities*

The estimation followed the prescription given by [8]. A crude commercial preparation of phosphatidylcholine was used for the activation step (Sigma, no. P-5638).

2.4. *Polyacrylamide slab-gel electrophoresis*

The gradient slab-gel electrophoresis was carried out with 5–15% gel. Electrode buffer: Tris 50 mM,

glycine 0.38 mM, sodium dodecylsulfate 0.1%, EDTA 2 mM, pH 8.8. About 0.11 mg protein (not heated!) was applied to the gels. Electrophoresis was carried out with 30 mA constant current for about 2 h. Staining with Coomassie blue, destaining with 10% acetic acid. Absorbance of the gels was measured with a Zeiss PMQ II spectrophotometer at 560 nm.

2.5. *Substances*

All buffer substances were of highest purity available, oligomycin and ATP were purchased from Sigma, München, 2-mercaptopropionylglycine was obtained from Santen Pharmaceutical Co., Osaka. Standards (mol. wt): crystalline bovine serum albumin, 67 000; egg albumin, 45 000; chymotrypsin, 25 000; cytochrome *c*, 13 500.

3. Results

In fig.1a,2a a pattern of oligomycin-sensitive ATPase reveals protein peaks analogous to those in

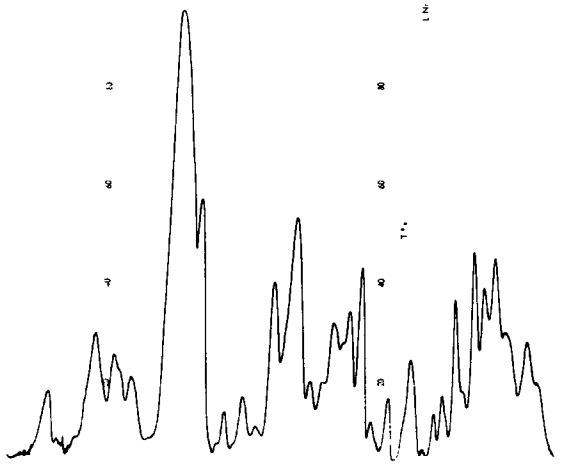

Fig.1a

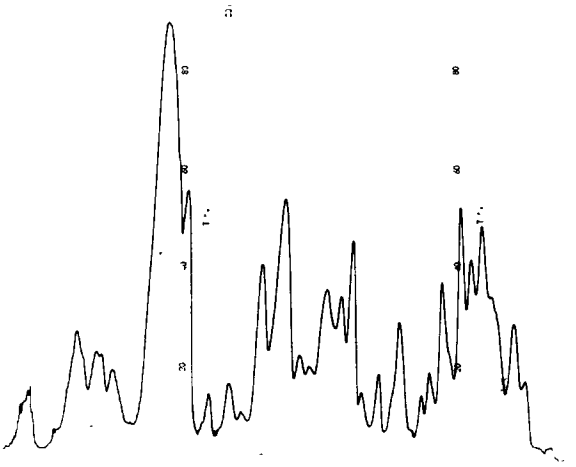

Fig.1b

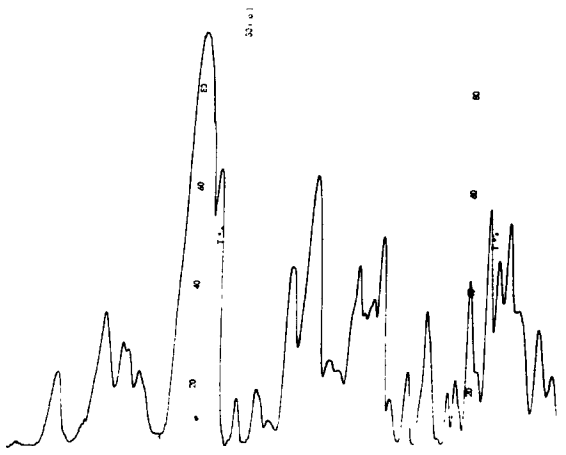

Fig.1c

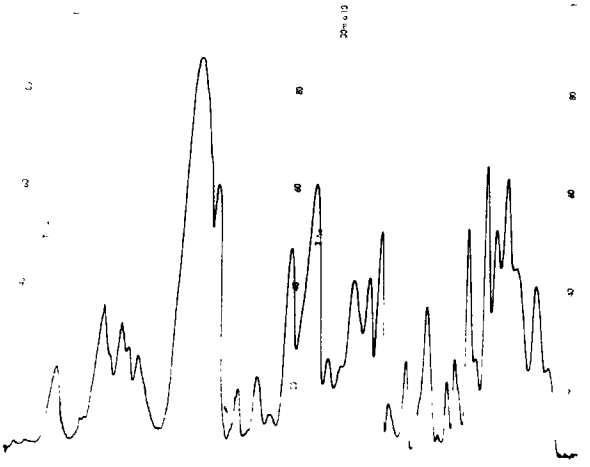

Fig.1d

Table 1
Peak height ratios bands 4/γ[a]

MPG[b] (nmol/mg prot.)	Ratio
none	1.343
3.72	1.335
7.44	1.455
14.88	1.320

[a] Nomenclature from [8]
[b] 2-Mercaptopropionylglycine

[8,9]. In presence of 7.44 nmol/mg protein of 2-mercaptopropionylglycine distinct changes can b found at mol. wt 31 000 and 34 000 (fig.1c). The increase in band intensity at 31 000 mol. wt compared to the band at 34 000 is also revealed by table 1, where the ratios of these bands are formed.

There also is some change in the region of 8000–15 000 mol. wt, less well defined, which apparently is not overcome by higher concentrations of 2-mercaptopropionylglycine.

A slight difference in preparative procedure was used for the ATPase shown in fig.2a,b (see legend). Apart from the drastic change in amount of 31 000 mol. wt protein (fig.2b) there also is, analogous to fig.1a–d a change in intensities of the (splitted) proteins of ~20 000 mol. wt. These proteins may represent OSCP (cf. [9]). Reversal of such changes at higher concentrations of 2-mercaptopropionylglycine is clearly revealed from fig.1d and from table 1.

Determinations of ATPase activity in the same preparation shown in fig.1 have revealed that the activities are inhibited at 3.5–7 nmol 2-mercaptopropionylglycine/mg protein, while, at 14 nmol, the activity of the control is reached (table 2).

Fig.2a

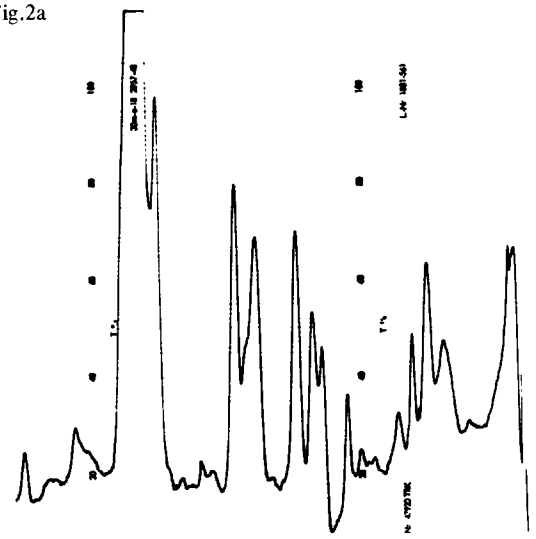

Fig.2b

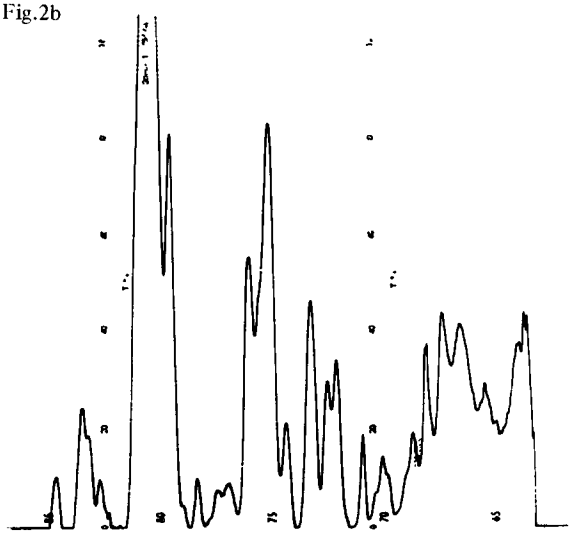

Fig.2. Electrophoresis of OS ATPase from beef heart mitochondria. Preparation as in [8] except for the addition of 0.5 mM 2-mercaptopropionylglycine instead of 0.5 mM dithiothreitol to submitochondrial particles. Other conditions similar to those in fig.1. (a) Control; (b) incubated with 7.3 nmol 2-mercaptopropionylglycine/mg protein.

Fig.1. Electrophoresis of OS ATPase from beef heart mitochondria. OS ATPase from beef heart mitochondria (14.75 mg protein/ml) was incubated at 22°C for 30 min in Tris–sulfate 10 mM, EDTA 0.5 mM, $MgSO_4$ 1.0 mM, sucrose 50 mM (pH 7.5). Thereafter dialysis against distilled water was performed for 60 min and at 5°C. The milieu for electrophoresis was attained by suitable additions, so that final concentrations were: sodium phosphate 0.1 M (pH 7.0), 10% (v/v) glycerol, 2.5% (w/v) sodium dodecylsulfate and 1.5% (v/v) mercaptoethanol. (a) Control; (b) incubated with 3.72 nmol 2-mercaptopropionylglycine/mg protein; (c) incubated with 7.44 nmol 2-mercaptopropionylglycine/mg protein; (d) incubated with 14.88 nmol 2-mercaptopropionylglycine/mg protein.

Table 2
Activities of OS ATPase

MPG[a] (nmol/mg prot.)	(μmol P_i/min/ mg prot.)[b]
None	3.93 ± 0.185 [9]
3.5	3.36 ± 0.06 [9]
7.0	3.52 ± 0.08 [9]
14.0	3.91± 0.195 [9]

[a] 2-Mercaptopropionylglycine
[b] The activation of the ATPase was carried out by addition of phosphatidylcholine (no. P-5638, Sigma)

As was found [1] thiol reagents increase the effect of oligomycin on ATPase preparations. In agreement with this finding, we show in fig.3 that 2-mercaptopropionylglycine also increases oligomycin sensitivity of the ATPase.

4. Discussion

Important progress has been made during recent years toward an understanding of the molecular architecture of ATPase systems like F_1 and CF_1 [13–15]. In CF_1 the outstanding role of the γ subunit in energy transduction by means of the proton channel and, moreover, the interaction of two adjacent SH groups in that subunit has been investigated [14]. The γ subunit thus should form a gate of the proton channel [15].

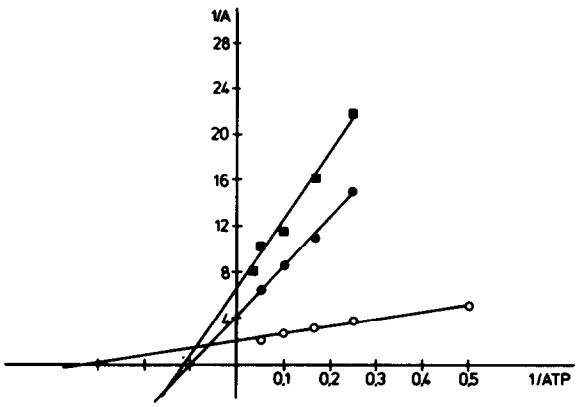

Fig.3. Increase of oligomycin sensitivity of ATPase by 2-mercaptopropionylglycine. The incubations were carried out as in [8] with 15 μg ATPase protein (○) and in presence of 66 μg oligomycin/mg protein (●) in presence of 66 μg oligomycin/mg protein and 10 μM 2-mercaptopropionylglycine (■). The experimental points represent means of double determinations.

In comparison, much less is known about oligomycin-sensitive ATPase and its subunits. In SDS gel electrophoresis the 31 000 mol. wt band of OS ATPase is adjacent to the γ subunit of 34 000 mol. wt [13]. Oligomycin-insensitive ATPase was reported [9] to be lacking the band at 31 000 mol. wt. Oligomycin sensitivity thus should be correlated with this band. The intensity of the 31 000 mol. wt band is increased by ~7 nmol 2-mercaptopropionylglycine/mg protein; also the γ subunit appeared somewhat broader in fig.1c and the peak height ratio of bands 4/γ was changed.

The changes in peak height observed may either mean that the amount of polypeptides has changed, or alternatively, as pointed out to us by one of the reviewers, that the polypeptides changed shape and reactivity. Which one of these possibilities is the correct one cannot be decided at present. Independent of interpretation, however, a distinct structural change in this region has occurred. A step is thus made toward a later identification of oligomycin-sensitive SH proteins in the ATPase.

All evidence obtained centers around the paramount importance of the 31 000 mol. wt band which comprises at least two polypeptides [8] (see the shoulders in fig.2a,b).

For a possible mechanism, we consider a concentration-dependent cleavage and reformation of disulfide bonds by means of SH–S–S– interchange reaction [16–18]. Analogous to [16–18] this generally implies reversibility of the reaction, as demonstrated in fig.1d and tables 1,2. In accordance is also the observation that pretreatment of the submitochondrial particle preparation with 2-mercaptopropionylglycine instead of dithiothreitol resulted in a further increase in intensity of polypeptides 31 000 mol. wt (fig.2b).

Future work should enlarge our knowledge of the interrelations between the γ subunit, the 31 000 mol. wt band and the lower molecular weight polypeptides.

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