Vol. 194, No. 1, 1993 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 15, 1993 Pages 483-489

THE EFFECT OF DEPLETION OF NUCLEOTIDE AND OF δ AND ε SUBUNITS ON ATP SYNTHESIS IN DIMETHYL SULFOXIDE BY F_1-ATPase OF ESCHERICHIA COLI

Seelochan Beharry and Philip D. Bragg

Department of Biochemistry & Molecular Biology, University of British Columbia, 2146 Health Sciences Mall, Vancouver, B.C., Canada V6T 1Z3

Received June 5, 1993

SUMMARY: The F_1-ATPase of Escherichia coli contains 3 mol bound adenine nucleotide/mol F_1. It is of the F_1[2,1] type based on the ability of GTP to displace 1 mol adenine nucleotide/mol F_1 (Kironde, F.A.S. and Cross, R.L. (1986) J. Biol. Chem. 261, 12544-12549). A portion of the adenine nucleotide (2 mol/mol F_1) is not displaceable by GTP. F_1[2,1] was converted to F_1[1,0]. This form of the enzyme synthesized ATP from endogenous ADP and inorganic phosphate in a medium containing 30% (v/v) dimethyl sulfoxide (Me_2SO). A δ,ε subunit-depleted form of the F_1-ATPase was shown to be predominantly in the F_1[0,1] form. ATP was not synthesized from endogenous ADP by the subunit-depleted enzyme in Me_2SO unless additional molecules of ADP were bound. It is concluded that ATP synthesis from endogenous ADP in Me_2SO occurs at GTP-nondisplaceable adenine nucleotide binding sites.

Escherichia coli F_1F_o, the proton-translocating ATP synthase, is the terminal enzyme of oxidative phosphorylation, in which process it synthesizes ATP from ADP and inorganic phosphate. F_1 may be separated from F_o as a soluble enzyme consisting of five different subunits (α-ε) in a stoichiometry ($\alpha_3\beta_3\gamma\delta\varepsilon$) (1-3). F_1 is able to hydrolyse ATP to ADP and phosphate. It can be induced to reverse this process in a medium containing 30-40% (v/v) dimethyl sulfoxide (Me_2SO) (4-16).

Normally an isolated, F_1 contains three mol bound adenine nucleotide/mol of F_1 (17-18). Three other binding sites for adenine nucleotide can be filled by incubation with ATP (19-21). Of these six sites present on F_1, three are specific for adenine nucleotide while the remaining three sites can bind guanine and inosine nucleotides also (22). Incubation of F_1 (preloaded with adenine nucleotide by incubation with ATP) with GTP will displace the adenine nucleotides from the adenine nucleotide non-specific sites. This observation permitted Kironde and Cross (23,24) to show that isolated F_1 contains two mol adenine nucleotide bound at specific (i.e., GTP-nondisplaceable) sites and one mol adenine nucleotide/mol F_1 at a nonspecific (i.e., GTP-displaceable) site. They introduced the symbol F_1[2,1] to

0006-291X/93 $4.00

describe this state of site occupancy. We have recently shown that the E. coli F_1 has a similar site occupancy (16).

Previous studies have led to the assumption that in the ATP synthase ATP synthesis occurs at the nonspecific sites on F_1 and that the specific sites are catalytically inert (22-28). Recently, we have shown that F_1[3,0] and F_1[2,0] in a Me_2SO medium can synthesize ATP from endogenous ADP bound at adenine nucleotide-specific sites (16). These results suggest that equating nucleotide-nonspecific sites with catalytic sites for ATP synthesis is not correct under all circumstances.

In the present paper we show that F_1[1,0] can form ATP from endogenous ADP in the presence of 30% (v/v) Me_2SO. We extend this observation to demonstrate that F_1 lacking the δ and ε subunits is predominantly in the F_1[0,1] form and cannot make ATP from endogenous ADP until further sites are filled with adenine nucleotide. These results support our proposal (16) that the GTP-nondisplaceable adenine nucleotide-binding sites of F_1 are the sites of ATP synthesis in Me_2SO.

MATERIALS AND METHODS

The F_1-ATPase of E. coli CM2786 was prepared as described previously (29). F_1 lacking δ and ε subunits was purified from strain ML308-225 as before (30,31). ATPase assays, ATP synthesis on F_1, measurements of bound ATP and ADP, and of protein concentration were performed as described previously (12-14). The M_r of F_1 used in calculations was 381000 (1).

Preparation of F_1[1,0]

Desalted F_1[2,1] in 100 mM Tris acetate, pH 6.8 containing 30% (v/v) Me_2SO was incubated with 4 mM $MgCl_2$ and 10 mM potassium phosphate at 30°C. ATP synthesis was stopped after 30 min by passing the reaction mixture through centrifuged columns of Sephadex G-50-80 equilibrated with 100 mM Tris acetate/30% Me_2SO, pH 6.8. The centrifugate (containing Me_2SO) was incubated with 1 mM GTP-Mg^{2+} for 5 min at 30°C before application to centrifuged Sephadex G-50-80 columns equilibrated with 100 mM Tris acetate, pH 6.8 (Note: Me_2SO is absent). The centrifugates were pooled and an equal volume of saturated $(NH_4)_2SO_4$ solution, pH 8, was added. The precipitate was stored at 4°C. Before use, the enzyme was desalted on centrifuged columns of Sephadex G-50-80 equilibrated with 100 mM Tris acetate, pH 6.8.

GTP-chase experiments

F_1 was incubated for 5 min in 100 mM Tris acetate, pH 6.8 containing 4 mM $MgCl_2$, 4 mM potassium phosphate and 4 mM GTP. F_1 was freed of released adenine nucleotide and GTP by centrifugation through appropriate buffer-equlilibrated columns of Sephadex G-50-80 (15).

RESULTS

ATP synthesis by F_1[1,0] in Me_2SO

F_1 as isolated by our method is the [2,1] enzyme (16). It was converted to F_1[1,0] by the procedure described in Materials and Methods. Analysis of three different

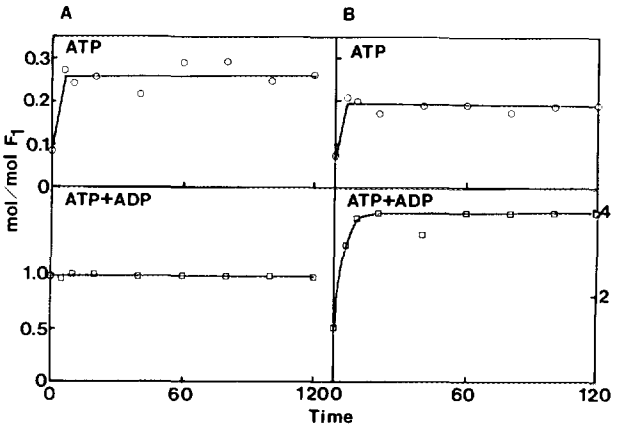

Fig. 1. Changes in the concentrations of bound adenine nucleotides in F_1[1,0] during incubation in a 10 mM phosphate buffer containing $MgCl_2$ and 30% (v/v) Me_2SO. A: The buffer contains no added ADP. B: 10 μM ADP present. The experiment was performed as described in Materials and Methods. Time is expressed in minutes.

preparations of the enzyme gave 0.13 ± 0.09 mol ATP/mol F_1 and 1.02 ± 0.16 mol ADP/mol F_1.

Fig. 1A shows that incubation of the F_1[1,0] enzyme with Mg^{2+} and phosphate in the presence of 30% (v/v) Me_2SO resulted in the synthesis of ATP. The ATP, and unconverted ADP, remained bound to the enzyme. No ATP was formed in the absence of Me_2SO (results not shown). Addition of 10 μM ADP to the incubation mixture in Me_2SO did not increase the extent of formation of ATP although additional ADP (3 mol/mol F_1) was bound by the enzyme (Fig. 1B).

ATP synthesis by subunit-depleted F_1 in Me_2SO

Tuttas-Dorshüg and Hanstein (31) have described a simple method to prepare F_1 of *E. coli* in a form lacking the δ and ε subunits. Analysis of two different preparations of the subunit-depleted enzyme for bound adenine nucleotide gave 0.05 ± 0.04 mol ATP/mol F_1 and 1.3 ± 0.1 mol ADP/mol F_1. That the enzyme was predominantly F_1[0,1] was shown by treatment with GTP-Mg^{2+} which displaced about 0.84 mol ADP/mol F_1 (Table 1).

Incubation of the subunit-depleted F_1 with Mg^{2+} and phosphate in 30% (v/v) Me_2SO did not result in ATP synthesis (Fig. 2B). There was transient loss, followed by rebinding, of ADP. This phenomenon has been observed previously with other forms of F_1 and has been attributed to a change in the conformation of the enzyme on placing it in a Me_2SO medium (15,16,32,33).

The subunit-depleted F_1 was incubated with ATP in the absence of added Mg^{2+} and the unbound adenine nucleotide removed by chromatography on a centrifuged column of Sephadex G-50. Analysis of two different preparations of the preloaded enzyme gave 0.06 ± 0.05 mol ATP/mol F_1 and 4.8 ± 0.2 mol ADP/mol F_1.

TABLE 1
Adenine nucleotide content and deduced site occupancy of F_1 lacking δ and ε subunits

GTP chase	mol bound/mol F_1			Deduced site occupancy
	ATP	ADP	Total	
-	0.004 ± 0.006	1.28 ± 0.05	1.28 ± 0.05	[0,1]
+	0.002 ± 0.001	0.45 ± 0.01	0.45 ± 0.01	

The analysis of the F_1 for adenine nucleotides and protein and the procedure for performing the GTP-chase experiment are described in Materials and Methods. Total: ATP + ADP.

Incubation of this enzyme with $Mg^{2+}$and phosphate in 30% (v/v) Me_2SO resulted in the conversion of 0.45 mol ADP/mol F_1 to ATP (Fig. 2A). The newly-formed ATP was readily displaced on incubation with exogenous Mg-ATP (4 mM) (Fig. 3).

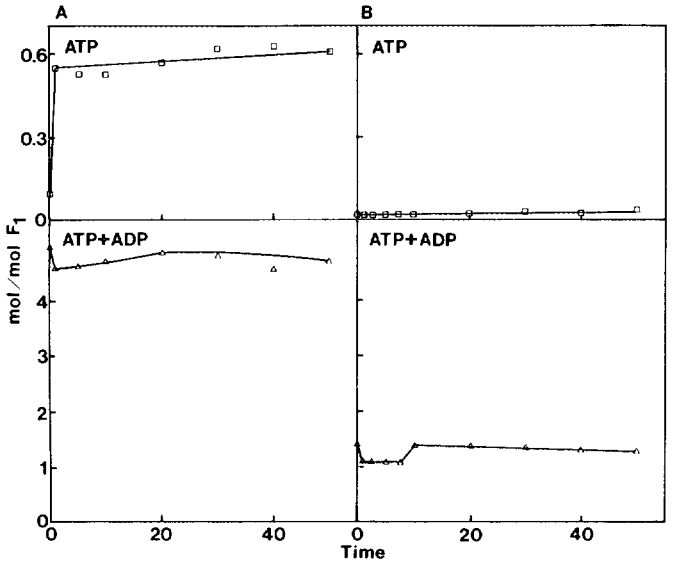

Fig. 2. Changes in the concentrations of bound adenine nucleotides in F_1 lacking δ and ε subunits during incubation in a 10 mM phosphate buffer containing $MgCl_2$ and 30% (v/v) Me_2SO. A: Enzyme preloaded with nuceotide by incubation with 250 μM ATP-Mg^{2+} in 100 mM Tris acetate, pH 6.8, for 5 min. Unbound nucleotide and phosphate were removed by passage of the incubation mixture through centrifuged columns of Sephadex G-50-80. B: Non-preloaded enzyme. Time is expressed in minutes.

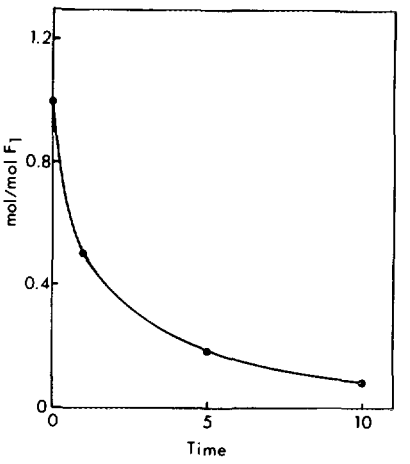

Fig. 3. Displacement of newly synthesized [^{32}P]ATP from F_1 lacking δ and ε subunits on incubation with non-radioactive ATP. Synthesis of ATP in 30% (v/v) Me_2SO in the presence of [^{32}P]phosphate was carried out for 45 min as described in reference 12 and in the legend to Fig. 2. The enzyme was preloaded with nucleotide. Unbound nucleotide and phosphate were removed by passage of the incubation mixture through centrifuged columns of Sephadex G-50-80. Non-radioactive ATP and $MgCl_2$ were added to a final concentration of 4 mM in the effluent. Samples were removed at intervals following addition of Mg-ATP and the concentration of bound [^{32}P]ATP determined following removal of displaced radiolabeled ATP on centrifuged columns of Sephadex G-50-80. The concentration of remaining bound [^{32}P]ATP is plotted versus time (in minutes) after addition of Mg-ATP.

DISCUSSION

The ability of F_1[1,0] to form ATP in Me_2SO strongly supports our previous suggestion (16) that ATP formation can occur at the adenine nucleotide-specific binding sites of the E. coli F_1. Thus, the view that adenine nucleotide-specific binding sites are structural not catalytic sites is no longer tenable, at least for ATP synthesis in Me_2SO. The ability of GTP and ITP to bind to the nonspecific binding sites and to be hydrolyzed (34,35) raises the possibility that F_1 has two classes of sites: adenine nucleotide-specific binding sites involved in ATP synthesis and adenine nucleotide-nonspecific sites which carry out ATP hydrolysis. This possibility has been proposed previously by others (36).

ATP formation in Me_2SO with F_1 from a thermophilic bacterium containing a single molecule of bound ADP had been demonstrated by Yoshida and Allison (10). It is difficult to relate their work to that described here since the binding sites of the thermophilic F_1 have not been characterized in the same manner as those of E. coli or the mitochondrion. However, the stability of the bound ADP of the thermophilic F_1, and its lack of exchange with medium adenine nucleotide in the absence of turnover, suggest that it occupies an "adenine nucleotide-specific" site.

The lack of ATP formation in Me_2SO by the δ,ε-depleted F_1, which is predominantly in the F_1[0,1] form, agrees fully with our hypothesis that ATP

formation occurs at adenine nucleotide-specific (GTP-nondisplaceable) sites. The ability of the δ,ε-depleted F_1 to form ATP in Me_2SO after preloading with ADP is expected since adenine nucleotide-specific sites will have been loaded with ADP which is then available for ATP formation. The newly-formed ATP is displaceable by exogenous Mg-ATP with a half-time for release of less than one minute. The ready exchangeability of the newly-formed ATP suggests that nucleotide binding at adenine nucleotide-specific sites is weaker in δ,ε-depleted F_1 than in the native enzyme. This is consistent with the finding that the purified subunit-depleted enzyme lacks bound nucleotide at the adenine nucleotide-specific sites. Much remains to be learned regarding the role of the minor subunits in nucleotide-binding and catalysis. Only α and β subunits are required for ATP hydrolysis in a thermophilic F_1 ATPase (37).

In conclusion, that data above obtained from use of $F_1[1,0]$ and $F_1[0,1]$ strongly support the concept that adenine nucleotide-specific (GTP-nondisplaceable) sites are catalytic sites for ATP formation in Me_2SO.

ACKNOWLEDGMENT

This work was supported by a grant from the Medical Research Council of Canada.

REFERENCES

1. Boyer, P.D. (1993) Biochim. Biophys. Acta 1140, 215-250
2. Futai, M., Noumi, T., and Maeda, M. (1989) Annu. Rev. Biochem. 58, 111-136
3. Penefsky, H.S., and Cross, R.L. (1991) Adv. Enzymol. Relat. Areas Mol. Biol. 64, 173-214
4. Sakamoto, J., and Tonumura, Y. (1983) J. Biochem. (Tokyo) 93, 1601-1614
5. Yohda, M., Kagawa, Y., and Yoshida, M. (1986) Biochim. Biophys. Acta 850, 429-435
6. Sakamoto, J. (1984) J. Biochem. (Tokyo) 96, 475-481
7. Sakamoto, J. (1984) J. Biochem. (Tokyo) 96, 483-487
8. Cross, R.L., Cunningham, D., and Tamura, J.K. (1984) Curr. Top. Cell. Regul. 24, 335-344
9. Gomez-Puyou, A., Tuena de Gomez-Puyou, M., and De Meis, L. (1986) Eur. J. Biochem. 159, 133-140
10. Yoshida, M., and Allison, W.S. (1986) J. Biol. Chem. 261, 5714-5721
11. Kandpal, R.P., Stempel, K.E., and Boyer, P.D. (1987) Biochemistry 26, 1512-1517
12. Beharry, S., and Bragg, P.D. (1991) Biochem. Cell. Biol. 69, 291-296
13. Beharry, S., and Bragg, P.D. (1991) FEBS Lett. 291, 282-284
14. Beharry, S., and Bragg, P.D. (1992) Biochem. Biophys Res. Commun. 182, 697-702
15. Beharry, S., and Bragg, P.D. (1992) Biochem. J. 286, 603-606
16. Beharry, S., and Bragg, P.D. (1992) Biochemistry 31, 11472-11476
17. Maeda, M., Kobayashi, H., Futai, M., and Anraku, Y. (1976) Biochem. Biophys. Res. Commun. 70, 228-234
18. Bragg, P.D., and Hou, C. (1977) Arch. Biochem. Biophys. 178, 486-494
19. Bragg, P.D., Stan-Lotter, H., and Hou, C. (1982) Arch. Biochem. Biophys. 213, 669-679
20. Bragg, P.D. (1984) Can. J. Biochem. Cell. Biol. 62, 1190-1197
21. Issartel, J.P., Lunardi, J., and Vignais, P.V. (1986) J. Biol. Chem. 261, 895-601

22. Perlin, D.S., Latchney, L.R., Wise, J.G., and Senior, A.E. (1984) Biochemistry 23, 4998-5003
23. Kironde, F.A.S., and Cross, R.L. (1986) J. Biol. Chem. 261, 12544-12459
24. Kironde, F.A.S., and Cross, R.L. (1987) J. Biol. Chem. 262, 3488-3495
25. Cross, R.L., and Nalin, C.M. (1982) J. Biol. Chem. 257, 2874-2881
26. Nalin, C.M., and Cross, R.L. (1982) J. Biol. Chem. 257, 8055-8060
27. Cunningham, D., and Cross, R.L. (1988) J. Biol. Chem. 263, 18850-18856
28. Dunn, S.D., and Futai, M. (1980) J. Biol. Chem. 255, 113-118
29. Bragg, P.D., and Hou, C. (1989) Biochim. Biophys. Acta 974, 24-29
30. Bragg, P.D., and Hou, C. (1990) Biochem. Biophys. Res. Commun. 166, 431-435
31. Tuttas-Dorshüg, R., and Hanstein, W.G. (1989) Biochemistry 28, 5107-5113
32. Beharry, S., and Bragg, P.D. (1989) FEBS Lett 253, 276-280
33. Beharry, S., and Bragg, P.D. (1992) J. Bioenerg. Biomembr. 24, 507-514
34. Senior, A.E., Lee, R.S.F., Al-Shawi, M.K., and Weber, J. (1992) Arch. Biochem. Biophys. 297, 340-344
35. Perlin, D.S., Latchney, L.R., Wise, J.G., and Senior, A.E. (1984) Biochemistry 23, 4998-5003
36. Schuster, S.M., Ebel, R.E., and Lardy, H. (1975) J. Biol. Chem. 250, 7848-7853
37. Kagawa, Y., Ohta, S., Harada, M., Sato, M., and Ito, Y. (1992) Ann. N.Y. Acad. Sci. 671, 366-376