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ROLE OF MINOR SUBUNITS IN THE STRUCTURAL ASYMMETRY OF THE ESCHERICHIA COLI F_1-ATPase

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SUMMARY: The β subunits of the Escherichia coli F_1-ATPase react independently with chemical reagents (Stan-Lotter, H. and Bragg, P.D. (1986) Arch. Biochem. Biophys. 248, 116-120). Thus, one β subunit is readily crosslinked to the ε subunit, another reacts with N-N'-dicyclohexylcarbodiimide (DCCD), and a third one is modified by 4-chloro-7-nitrobenzofurazan (NbfCl). This asymmetric behaviour is not due to the association of the δ and ε subunits of the ATPase molecule with specific β subunits since it is maintained in a δ, ε-deficient form of the enzyme.

The F_1-ATPase of Escherichia coli, and of other organisms, consists of five different subunits (α – ε) in a stoichiometry of $\alpha_3\beta_3\gamma\delta\varepsilon$ (1). The three β subunits show an interesting type of asymmetry (2 – 6). Thus, one-third of the β subunits react with NbfCl (at lysine residues) or IAANS but not with DCCD. Another one-third of the β subunits react with DCCD but not with NbfCl (at lysine residues) or IAANS. The remaining one-third of the β subunits does not react with these reagents but is readily crosslinked to the ε subunit by 1-ethyl-3[3-(dimethylamino)propyl]carbodiimide. The origin of the asymmetry is unclear since the α and β subunits appear to alternate in a hexagonal arrangement (7). It has been proposed that the association of the other subunits with a specific αβ subunit pair imparts asymmetry (8,9). In this paper we show that the structural asymmetry of the β subunits is maintained in the absence of the δ and ε subunits.

MATERIALS AND METHODS

The F_1-ATPases of E. coli ML308-225 and CM2786 were prepared as described previously (6). The isolation of the δ, ε-deficient enzyme from strain ML308-225

ABBREVIATIONS: DCCD, N,N'-dicyclohexylcarbodiimide; IAANS, 2-(4-iodoacetamidoaniline)naphthalene-6-sulfonic acid; NbfCl, 4-chloro-7-nitrobenzofurazan.

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followed the procedure described in reference 10 except that gel filtration through Bio-gel A 1.5 m was replaced by sucrose gradient centrifugation (see reference 6). The F_1-ATPase of CM2786 was treated with trypsin and reisolated on a sucrose gradient as described previously (11). Chemical modification by DCCD and NbfCl followed the procedures given in reference 6. SDS-polyacrylamide gel electrophoresis was performed according to the method of Laemmli (12). Isoelectric focusing gels were run in one dimension using the O'Farrell system (13) modified as described in reference 14. Proteins were stained with Coomassie blue (15). Gels containing isotopes were exposed at 20°C to Kodak XAR-5 film.

[^{14}C]DCCD and [^{14}C]NbfCl were supplied by Amersham and Research Products International respectively.

RESULTS AND DISCUSSION

Tuttas-Dörschug and Hanstein (10) have described a simple method to prepare the F_1-ATPase of E. coli in a form lacking the δ and ε subunits. The chemical modification of this enzyme by [^{14}C]DCCD and [^{14}C]NbfCl was compared with that of the normal five-subunit F_1-ATPase. SDS-polyacrylamide gel electrophoresis followed by autoradiography showed that both reagents preferentially modified the β subunits of both enzymes. A significant difference between the δ, ε-deficient and the normal ATPase was in the modification of the γ subunit by NbfCl in the former case. (See Fig. 1.)

The asymmetry of the β subunits was examined by modifying the normal and δ, ε-deficient ATPases with [^{14}C]NbfCl subsequently followed by treatment with DCCD. The β subunits were separated by isoelectric focusing. Treatment of the ATPase with NbfCl initially modified a tyrosine residue on the β subunit but the Nbf group could then be transferred to a lysine residue by adjusting the pH of the

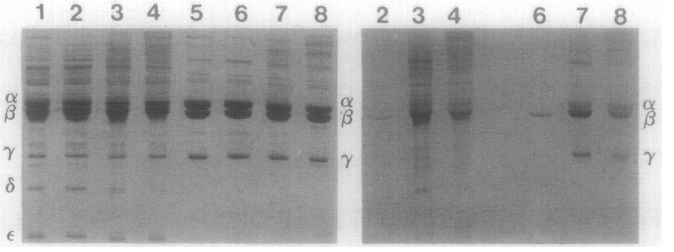

FIG. 1. SDS-polyacrylamide gel electrophoresis of normal (1 – 4) and δ,ε-deficient (5 – 8) F_1-ATPases labeled with [^{14}C]DCCD (lanes 2,6), and with [^{14}C]NbfCl at tyrosine (lanes 3,7) and lysine (lanes 4,8) residues. Untreated enzymes are in lanes 1 and 5. Left-hand panel, Coomassie blue stained. Right-hand panel, autoradiograph. The positions of migration of the subunits are indicated.

solution to pH9 (16). Modification of the β subunit at a tyrosine residue does not alter the charge of the subunit. This can be seen by comparison of lanes 1 and 5 in Fig. 2, where the major isoelectric form of the β subunit migrates in the "O" position. Modification of the lysine residue by NbfCl results in the loss of a single positive charge. This is indicated in Fig. 2, lanes 3 by an intensification of the band in the – 1 position. (Modification of the satellite β band normally found in the – 1 position is shown by an increase in the band in the – 2 position). Modification of the β subunits by [^{14}C]NbfCl at tyrosine and lysine residues can be seen more clearly by examination of the autoradiographs in the right-hand panels of Fig. 2. Treatment of the enzymes with DCCD resulted in the conversion of about one-third of the β subunits into a species which migrated, as a consequence of derivatization of a glutamic acid residue (17), in the +1 position (Fig. 2, lanes 1). Some double modification is indicated by a band in the +2 position.

Subsequent treatment of both N- and O-[^{14}C]Nbf-modified normal and δ,ε-deficient enzymes with DCCD gave new protein-staining bands in the +1 (and +2) positions indicative of reaction of the β subunits with the reagent (Fig. 2, lanes 4 and 6). Examination of the autoradiographs showed that the radioactive band of the O-Nbf modified subunit in the zero position had migrated to the +1 (and +2)

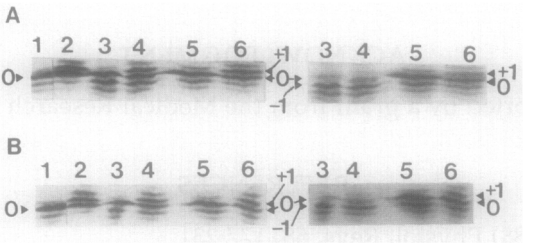

FIG. 2. Incorporation of [^{14}C]NbfCl and DCCD into β subunits of normal (A) and δ,ε-deficient (B) F_1-ATPases. The ATPases were treated as described in Materials and Methods, and the labeled subunits separated on an isoelectric focusing gel. The gel was stained with Coomassie blue (left-hand panels) or exposed to X-ray film (right-hand panels). The position of migration of the major isoelectric species of the β subunits is designated 0 (zero). The change in the number of positive or negative charges due to incorporation of labels is indicated. The lanes are 1, untreated enzyme; 2, F_1 treated with 0.5 mM DCCD; 3, F_1 treated with 1 mM NbfCl and the label transferred to a lysine residue; 4, as 3 with enzyme subsequently treated with 0.5 mM DCCD; 5, F_1 treated with 1 mM NbfCl, but with the label remaining on a tyrosine residue; 6, as 5 with the enzyme subsequently treated with 0.5 mM DCCD.

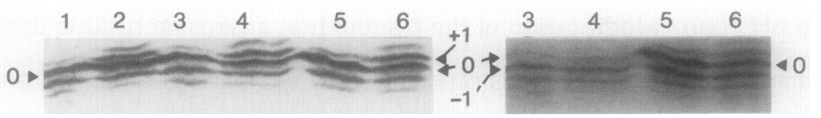

FIG. 3. Incorporation of [^{14}C]NbfCl and DCCD into β subunits of trypsin-treated F_1-ATPase. The lanes are as in Fig. 2.

position(s) indicative of double labeling of the β subunit by both reagents, as has been observed previously (18) (Fig. 2, lanes 5 and 6). By contrast, there was no shift in the positions of the radioactive bands of the N-Nbf labeled β subunits on further treatment with DCCD (Fig. 2, lanes 3 and 4).

Verification of the behaviour of the δ,ε-deficient enzyme was obtained using trypsin-treated F_1-ATPase. This form of the enzyme lacks the δ and ε subunits, and has had a portion of the α and γ subunits cleaved (11,19). Trypsin-treated ATPase behaved similarly (Fig. 3) to the normal and δ,ε-deficient enzymes examined above.

The inability of DCCD and NbfCl (at a lysine residue) to label the same β subunit, although both label β subunits individually, demonstrates the asymmetric behaviour of the β subunits in the F_1-ATPase. The fact that both normal and δ,ε-deficient enzymes behave similarly in these experiments indicates that the δ and ε subunits are not responsible for the asymmetry of the enzyme. Asymmetry must be a property of the organization of the γ subunit relative to the α and β subunits in view of the seeming symmetry of the hexagonal arrangement of the α and β subunits (7).

ACKNOWLEDGMENT

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