Vol. 137, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 29, 1986 Pages 468-473

An antibody-binding site in the native enzyme between amino acid residues 205 - 287 of the γ -subunit of F_1 from Escherichia coli

Karin Ehrig, Jürgen Hoppe, Peter Friedl and Hans Ulrich Schairer

Dept. of Cytogenetics, GBF - Gesellschaft für Biotechnologische Forschung mbH., Mascheroder Weg 1, D-3300 Braunschweig, F.R.G.

Received April 15, 1986

A monoclonal antibody was isolated specific for the isolated denatured γ-subunit of F_1 from Escherichia coli and binding to native F_1. The binding site of this antibody was identified between amino acid residues 205-287 of the polypeptide chain thus being located at the surface of the F_1 complex.

The membrane bound ATP synthases of different organisms have common functional and structural properties (for review see 1). The enzymes are composed of two parts, F_1 and F_0; F_1 is membrane associated and bears the catalytic center for ATPase activity; F_0 is membrane integrated and catalyzes H^+ conduction across the membrane. Both parts are necessary for energy-transducing reactions, i.e., reactions coupled to H^+ translocation across the membrane.

The intact ATP synthase of Escherichia coli has been purified (2,3). In agreement with genetic complementation studies (4) and direct DNA sequencing of the operon (5,6,7) it consists of eight subunits of which five ($\alpha, \beta, \gamma, \delta, \epsilon$) can be easily assigned to F_1. The characterization of defined mutations (8,9) as well as of active highly purified F_0 (10,11) confirmed that three different subunits a, b and c contribute to the formation of F_0. The stoichiometry of F_0 is believed to be a:b:c (1:2:9-15) (33,34) of $F_1\alpha_3\beta_3\ \gamma\delta\epsilon$ (12,13).

Though the primary structure is known, very little is confirmed about the catalytic sites of the enzyme. In order to elucidate the quaternary structure and the functional properties of the enzyme various techniques have been used: sophisticated dissociation and reconstitutuon experiments for F_1 (for review see 14) and F_0 (15,16), characterization

0006-291X/86 $1.50

of defined mutants (17-22), characterization of nucleotide binding sites by analogues (23,24), electron microscopy (25,26) and X-ray analysis (27) for elucidation of the quaternary structure of the enzyme.

Antibodies against the individual subunits of the enzyme provided a valuable tool in some of these studies. A further refinement was achieved by monoclonal antibodies which were used to visualize the stoichiometry of the α and ß subunits by electron microscopy (12, 13). It should be possible to construct a topological map of the enzyme by identifying the peptide segment to which a monoclonal antibody binds. If the antibody also binds to the native enzyme, this segment is accessible from the surrounding water phase. An example for such an approach is shown in this paper with a monoclonal antibody against the γ subunit.

Material and Methods

Native F_1 (28), the monoclonal antibodies against the α-subunit (12), the denatured γ subunit (5) and the peptides derived from the γ subunit by bromocyan cleavage (17) or treatment with o-iodosobenzoic acid (29) were prepared as described previously. SDS-polyacrylamide gelelectrophoresis, blotting of separated polypeptides onto nitrocellulose sheets and immunostaining of blotted proteins was performed as described previously (30), as well as solid phase amino acid sequencing of isolated peptides (17).

The peptides obtained by treatment of the γ subunit with o-iodosobenzoic acid were separated by chromatography on a Si 100 Polyol 3µm (0.8x50cm) column (Serva, Heidelberg) equilibrated in 60 % formic acid, flow rate was 0.5 ml/min and 0.5 ml fractions of 0.5 ml were collected.

Results and Discussion

The hybridoma clone γ 26 was one of several hybridoma clones obtained by fusion of NS1 myeloma cells with spleen cells from mice immunized with native F_1 (12). The monoclonal antibody γ 26 binds to native F_1. F_1 was incubated with monoclonal antibody γ 26 and α 83 and the antibody-F_1 complexes were precipitated with a polyvalent rabbit anti-mouse immunoglobulin serum. The polypeptides of the precipitates were separated by SDS-polyacrylamide gel electrophoresis and stained by $AgNO_3$. Fig. 1 shows that with non-immune mouse IgG there is no F_1 in the precipitate (lane C) in contrast to antibodies γ 26 (lane B) and α 83 (lane A). The disappearance of the γ and ϵ subunits in the precipitates of γ 26 is unique and was

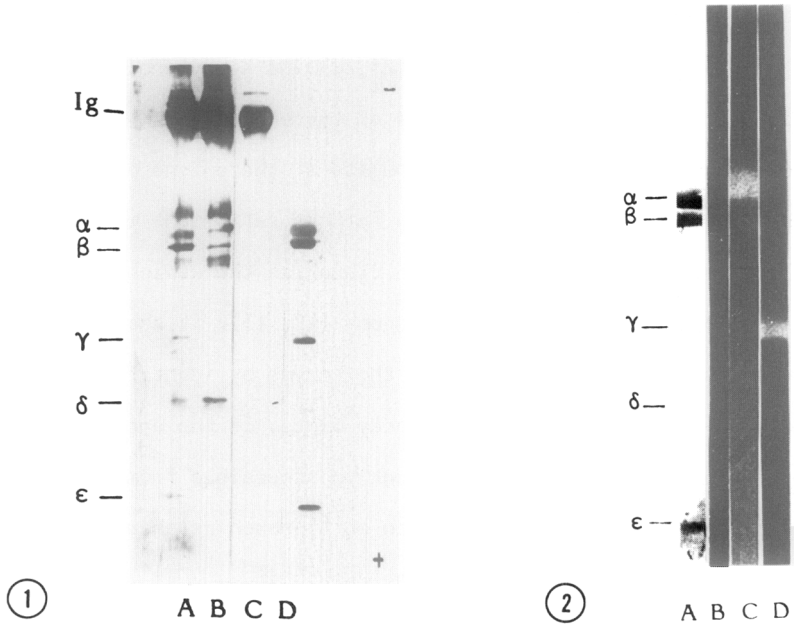

Figure 1: Immunoprecipitation by monoclonal antibodies.
10 µg F_1 was incubated with 40 µg monoclonal antibodies in 100 µl 200 mM Tris-Cl pH 7.6, 500 mM Glycine, 2 % butanol for 3 hours at room temperature. The immune complexes were precipitated by addition of 100 µl of an appropriately diluted rabbit (anti mouse immunoglobulin) antiserum. The precipitates were analyzed by SDS-PAGE. Lane A, F_1 + monoclonal antibody α 83, lane B, F_1 monoclonal antibody γ 26, lane C, F_1 + 40 µg non immune mouse IgG, lane D, F_1 standard.

Figure 2: Immunoblot of F_1.
10 µg F_1 was subjected to SDS-PAGE. The separated subunits were blotted onto a nitrocellulose sheet, which was subsequently incubated with α or γ monoclonal antibodies and FITC-labelled rabbit (anti mouse IgG) antibodies. Lane A, nitrocellulose strip stained for protein with amido black, lane B, control with 40 µg non immune mouse IgG as primary antibody, lane C, antibody α 83, lane D, antibody γ 26.

never observed with monoclonal antibodies against other subunits; as an example of a typical pattern the precipitate with monoclonal antibody against the α subunit was included. The peculiar behaviour of the monoclonal anti-γ antibody in immunoprecipitation has also been reported by Dunn et al. in a recent publication (31). Electron microscopic studies indicated that binding of antibody γ 26 disrupted F_1 (Ehrig, unpublished results).

Antibody γ 26 was specific for the γ subunit. F_1 was subjected to SDS-polyacrylamide gelelectrophoresis, the separated subunits blotted onto nictrocellulose sheets and stained immunochemically. Fig. 2 shows

in lane A the blotted F_1 subunits stained with amido black. The other lanes show the results of immunostaining: in lane B a control with non immune mouse IgG as primary antibody, in lane C staining of the α subunit by monoclonal antibody α 83 and in lane D the selective immunostaining of γ by the monoclonal antibody γ 26.

The antibody γ 26 was further characterized by its binding to peptides derived from the γ subunit by treatment with either BrCN or o-iodosobenzoic acid. BrCN cleaves polypeptide chains after methionine residues and o-iodosobenzoic acid after tryptophan residues thus generating different sets of peptides. The peptides were subjected to SDS-polyacrylamide gel-electrophoresis, blotted onto nitrocellulose and assayed for immunostaining with antibody γ 26 and a second alkaline phosphatase labelled goat antibody against mouse immunoglobulin. Fig. 3 shows the peptides separated by SDS-gelelectrophoresis and stained with silver nitrate. The o-iodosobenzoic acid treated sample contained aside of residual γ and incomplete degradation products the three peptides expected from the known amino acid sequence: I (residue 1-106), II (residue 107-204) and III (residue 205-287). In the BrCN treated sample only the three major peptides VI (residue 50-103), XI (residue 180-244) and XIII (residue 248-287) could be resolved by SDS-polyacrylgelelectrophoresis and could thus be tested for immunostaining. None of the BrCN fragments bound antibody γ 26 (data not shown) whereas the peptide III from o-iodosobenzoic acid cleavage still bound antibody γ 26 (Fig. 4, lane B).

The identity of the immunostained peptide with peptide III from o-iodosobenzoic acid cleavage was proven by isolation and aminoacid sequencing of the peptide. The peptide fragments from o-iodosobenzoic acid treatment were subjected to HPLC-chromatography on a Si 100 Polyol column. Peptide III was isolated in two fractions and its identity was verified by solid phase amino acid sequencing revealing a N-terminal sequence asn-tyr-leu-tyr-glu identical with the theoretically expected sequence of peptide III. Fig, 4

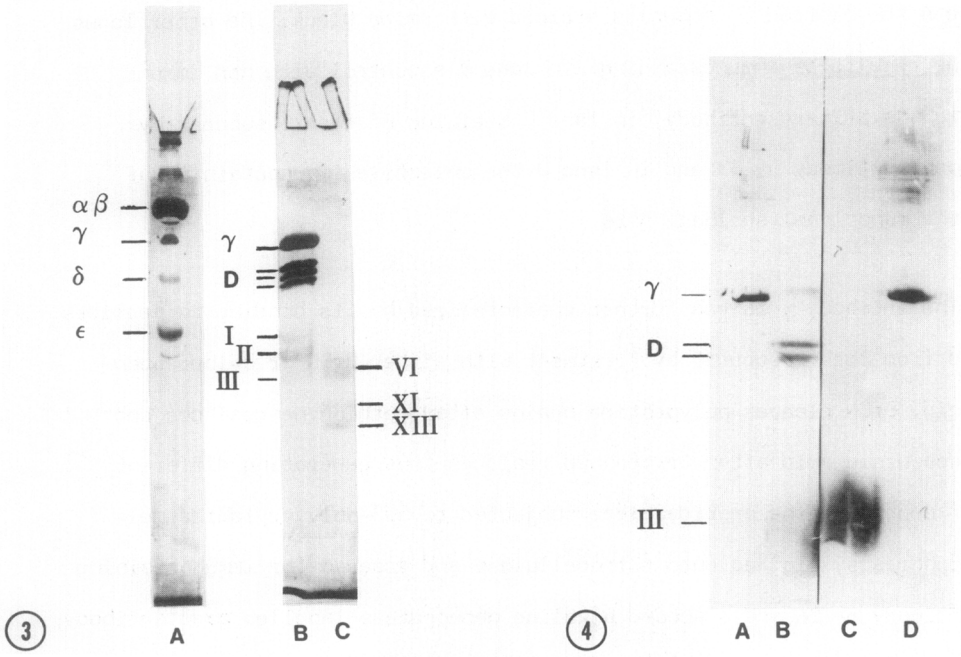

Figure 3: SDS-PAGE of peptides from subunit γ.
Subunit γ was treated with BrCN or o-iodosobenzoic acid, the generated peptides subjected to SDS-PAGE on a 20 % (w/v) acrylamide gel and stained with $AgNO_3$. Lane A, F_1, lane B, γ treated with o-iodosobenzoic acid, lane C, γ treated with BrCN. D = incomplete degradation products.

Figure 4: Immunoblot of peptides from subunit γ.
F_1, subunit and peptides generated from subunit γ were subjected to SDS- PAGE, blotted onto nitrocellulose and immunostained with monoclonal antibody γ 26 and alkaline phosphatase labelled rabbit (anti mouse IgG) antibodies. Lane A, subunit γ isolated from F_1, lane B, peptides of γ generated by o-iodosobenzoic acid, lane C, isolated peptide III, lane D, F_1.

(lane C) shows that the purified peptide could be immunostained with monoclonal antibody γ 26.

These experiments unequivocally locate the antigenic determinant of antibody γ 26 between residue 205-287 at the C-terminal end of the γ subunit. This part of γ must thus be located at the surface of the F_1 complex. The BrCN fragments could not be immunostained indicating that the antigenic determinant was destroyed by this treatment. Peptide III of o-iodosobenzoic acid cleavage has methionines at residue 244, 246. It may be speculated that the antigenic determinant of antibody γ 26 located on peptide III is near these residues.

References

1. Hoppe, J., and Sebald, W. (1984) Biochim. Biophys. Acta 768, 1-27.
2. Friedl, P., Friedl, C., and Schairer, H.U. (1979) Eur. J. Biochem. 100, 175-180.
3. Foster, D.L., and Fillingame, R.H. (1979) J. Biol. Chem. 254, 8230-8236.
4. Gibson, F., Downie, J.A., Cox, G.B., and Radik, J. (1978) J. Bacteriol. 134, 728-736.
5. Nielsen, J., Hansen, F.G., Hoppe, J., Friedl, P., and von Meyenburg, K. (1981) Mol. Gen. Genet. 184, 33-39.
6. Kanazawa, H., Mabuchi, K., Kayano, T., Moumi, T., Sekiya, T., and Futai, M. (1981) Biochem. Biophys. Res. Commun. 103, 613-620.
7. Gay, J.N., and Walker, J.E. (1981) Nucleic Acids Res. 9, 3919-3926.
8. Hansen, F.G., Nielsen, J., Riise, E., and von Meyenburg, K. (1981) Mol. Gen. Genet. 183, 463-472.
9. Downie, J.A., Cox, C.B., Langman, L., Ash, G., Becker, M., and Gibson, R. (1981 J. Bacteriol. 145, 200-210.
10. Friedl, P., and Schairer, H.U. (1981) FEBS Lett. 128, 261-264.
11. Schneider, E., and Altendorf, K. (1982) Eur. J. Biochem. 126, 149-153.
12. Lünsdorf, H., Ehrig, K., Friedl, P., and Schairer, H.U. (1984) J. Mol. Biol. 173, 131-136.
13. Ehrig, K., Lünsdorf, H., Friedl, P., and Schairer, H.U. (1986) Methods Enzymol. Vol. 126, in press.
14. Futai, M., and Kanazawa, H. (1983) Microbiol. Rev. 47, 285-312.
15. Schneider, E., and Altendorf, K.H. (1984) Proc. Natl. Acad. Sci. U.S.A. 81, 7279-7283.
16. Schneider, E., and Altendorf, K.H. (1985) EMBO J. 4, 515-518.
17. Hoppe, J., Schairer, H.U., and Sebald, W. (1980) FEBS Lett. 109, 107-111.
18. Hoppe, J., Schairer, H.U., Friedl, P., and Sebald, W. (1982) FEBS Lett. 145, 21-24.
19. Hoppe, J. Schairer, H.U., and Sebald, W. (1980) Eur. J. Biochem. 112, 17-24
20. Friedl, P., Bienhaus, G., Hoppe, J.,and Schairer, H.U. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 6643-6646.
21. Friedl, P., Hoppe, J., Gunsalus, R.P., Michelsen, O., von Meyenburg, K., and Schairer, H.U. (1983) EMBO J. 2, 99-103.
22. Friedl, P., Friedl, C., and Schairer, H.U. (1980) FEBS Lett. 119, 254-256.
23. Bragg, P.D., Stan-Lotter, H., and Hou, C. (1982) Arch. Biochem. Biophys. 213, 669-679.
24. Ting, L.P., and Wang, J.H. (1982) Biochemistry 21, 269-275.
25. Tiedge, H., Schäfer, G., and Mayer, F. (1983) Eur. J. Biochem. 132, 37-45.
26. Akey, C.W., Crepeau, R.H., Dunn, S.D., McCarty, R.E., and Edelstein, S.J. (1983) EMBO J. 2, 1409-1415.
27. Amzel, L.M., McKinney, M., Narayanan, P., and Pedersen, P.L. (1982) Proc. Natl., Acad. Sci. U.S.A. 79, 5882-5856.
28. Vogel, G., and Steinhard, R. (1976) Biochemistry 15, 208-216.
29. Fontana, A., Dalzoppo, D., Grandi, C., and Zambonin, M. (1983) Meth. Enz. 91, 311-318.
30. Friedl, P., Hoppe, J., and Schairer, H.U. (1984) Biochem. Biophys. Res. Commun. 120, 527-533.
31. Dunn, S.D., Tozer, R.G., Antczak, D.F., and Heppel, L.A. (1985) J. Biol. Chem. 260, 10418-10425.
32. Foster, D.L., and Fillingame, R.H. (1982) J. Biol. Chem. 275, 2009-2015.
33. von Meyenburg, K., Jørgensen, B.B., Nielsen, J., Hansen, F.G., and Michelsen, O. (1982) Tokai J. Exp. Clin. Med., Special Symp. Issue 1982.