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Comparison of F_1's of Oxidative Phosphorylation from *Escherichia coli* and *Salmonella typhimurium* and Demonstration of Interchangeability of Their Subunits†

Shih-Yuan Hsu, Mototaka Senda,‡ Hiroshi Kanazawa, Tomofusa Tsuchiya, and Masamitsu Futai*

ABSTRACT: The peripheral membrane portion (SF_1) of proton-translocating ATPase of *Salmonella typhimurium* and its α, β, and γ subunits were purified and compared with the same portion (EF_1) from *Escherichia coli*. The α, β, and γ subunits of these F_1's were found to be mutually interchangeable, and all possible combinations of the three subunits from EF_1 and SF_1 showed ATPase activity. Both F_1's could bind functionally to the integral membrane part (F_0) of either bacterium, suggesting that F_0 and F_1 are interchangeable in these two bacteria and thus that the two F_1's are closely similar at the level of subunit structure. However, SF_1 differed from EF_1 in some enzymological properties such as its specific activity and susceptibilities to sodium dodecyl sulfate and methanol. The specific ATPase activity of EF_1 was more than twice that of SF_1, and hybrid enzymes containing the β subunit of EF_1 had higher activity than other hybrids. Amino acid analysis suggested that the primary structures of the α subunits of the two F_1's are less homologous than those of the β subunits. Thus, the primary structure of the α subunit may be more species specific than that of the β subunit.

The proton-translocating ATPases (F_0F_1)[1] in membranes of chloroplasts, mitochondria, and bacteria catalyze ATP synthesis coupled with electron transport [for reviews, see Futai & Kanazawa (1983), Maloney (1983), and Senior & Wise (1983)]. The catalytic portion of the enzyme, F_1, is composed of five different subunits (α, β, γ, δ, and ϵ), while the intrinsic membrane portion, F_0, which functions as a proton pathway

† From the Department of Microbiology, Faculty of Pharmaceutical Sciences, Okayama University, Okayama 700, Japan. *Received June 29, 1983.* This research was supported by grants from the Ministry of Education, Science and Culture of Japan and the Toray Science Foundation.

‡ Present address: Nippon Roche Research Center, Kamakura, Japan.

[1] Abbreviations: F_0, integral membrane portion of the proton-translocating ATPase; F_1, peripheral membrane portion of the proton-translocating ATPase; F_0F_1, entire proton-translocating ATPase; EF_1, F_1 from *E. coli*; TF_1, F_1 from the thermophilic bacterium PS3; SF_1, F_1 from *S. typhimurium*; $NaDodSO_4$, sodium dodecyl sulfate; α, β, γ, δ, and ϵ, subunits of F_1; a, b, and c, subunits of F_0; Tris, tris(hydroxymethyl)-aminomethane; EDTA, ethylenediaminetetraacetic acid; Tricine, *N*-[tris(hydroxymethyl)methyl]glycine.

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in *Escherichia coli*, has three subunits, a, b, and c. Individual subunits of *E. coli* F_1 (EF_1) have been isolated, and the ATPase activity can be reconstituted from a combination of the α, β, and γ subunits (Dunn & Futai, 1980; Futai, 1977). Similar results were obtained with F_1 (TF_1) from the thermophilic bacterium PS3 (Kagawa & Nukiwa, 1981; Yoshida et al., 1977). Binding sites for nucleotides were found in the α and β subunits in studies in which the subunits were isolated or affinity labeled with nucleotide analogues [for reviews, see Futai & Kanazawa, (1983) and Senior & Wise (1983)]. These sites have been implicated as catalytic or regulatory sites.

ATPase activity was reconstituted on mixing combinations of the α, β, and γ subunits from EF_1 and TF_1, and the three hybrid enzymes $\alpha^E\beta^T\gamma^E$ (formed from α and γ of EF_1 and β of TF_1), $\alpha^E\beta^E\gamma^T$, and $\alpha^T\beta^T\gamma^E$ were obtained (Futai et al., 1980; Takeda et al., 1982). However, no activity was observed on mixing the α subunit of either EF_1 or TF_1 with the β and γ subunits of the other bacterium. These results suggested that the β and γ subunits of the F_1's from the two bacteria are homologous both in structure and function but that the α subunit shows little homology in the two species. It is noteworthy that these two bacteria are only distantly related: the thermophilic bacterium PS3 is a Gram-positive organism found in a hot spring while *E. coli* is Gram-negative and is of intestinal origin. Thus it seemed interesting to obtain subunits from more closely related organisms and study formation of hybrid ATPase complexes containing heterogeneous α subunits. However, it is not easy to prepare all these three subunits from F_1's in reconstitutively active forms, and so far this has been acheived only with EF_1 and TF_1.

Recently, the primary structures of all the subunits of F_0 and F_1 from *E. coli* (Gay & Walker, 1981a,b; Kanazawa et al., 1981a,b,c, 1982a,b; Mabuchi et al., 1981; Nielsen et al., 1981; Saraste et al., 1981), the β and ϵ subunits of chloroplast F_1 (Krebbers et al., 1982; Zurawski et al., 1982), the β subunit of beef heart (Runswick & Walker, 1983), and the a subunit of mitochondrial F_1 (Anderson et al., 1981; Macino & Tzagoloff, 1980; Bibb et al., 1981) have been determined. The primary structures of the c subunit from nine organisms have also been determined (Sebald et al., 1983). About 70% homology was found in the primary structure of the β subunits of chloroplasts, mitochondria, and bacteria (Futai & Kanazawa, 1983; Walker et al., 1982), but less homology was found in other subunits: about 20% homology was observed between the ϵ subunits of *E. coli* and chloroplasts (Futai & Kanazawa, 1980; Krebbers et al., 1982; Zurawski et al., 1982). Thus, homology of the primary structure seems to differ in different subunits, although comparison for the homologies of all the subunits is not yet possible.

These two lines of approach suggest that the subunits of F_1 are homologous in function and structure in different organisms, although the extent of homology varies in different subunits. In this study we compared the F_1's from two closely related organisms, *E. coli* and *Salmonella typhimurium*. We purified F_1 and the α, β, and γ subunits from both organisms. All possible combinations of the three subunits gave complexes with ATPase activity, and the β subunit of SF_1 (*S. typhimurium* F_1) was found to have a similar amino acid composition to that of EF_1. Furthermore, EF_1 and SF_1 could bind to F_0 from either *S. typhimurium* or *E. coli*. However, the two F_1's differed significantly in the amino acid composition of their α subunit, specific ATPase activity, and other enzymological properties.

Experimental Procedures

Bacteria and Growth Conditions. *E. coli* KY7485 (λ*asn*-5) (Kanazawa et al., 1979), ML308-225, and AN120 (*uncA*, defective in the α subunit of EF_1) (Butlin et al., 1971) and *S. typhimurium* LT2 were grown aerobically in minimal medium (Tanaka et al., 1967) supplemented with glucose as a sole carbon source.

Preparations. EF_1 and SF_1 were prepared from 300 g of wet cells as described previously (Futai et al., 1974). The α, β, and γ subunits of EF_1 and SF_1 were dissociated by cold treatment in buffer of high salt concentration and purified by hydroxylapatite and DEAE-Sepharose column chromatographies. The purities of the subunits of SF_1 were as reported previously (Dunn & Futai, 1980; Futai, 1977). The purities of the subunits of SF_1 are shown in Figure 1. The procedure originally developed for the preparation of subunits of EF_1 gave comparable purities and yields of SF_1 subunits. Inverted membrane vesicles were prepared by passing cells of *E. coli* or *S. typhimurium* through a French press (Futai, 1974). Membrane vesicles depleted of F_1 were prepared by suspending the membranes in 1.0 mM Tris-HCl, pH 8.0, 0.5 mM EDTA, and 10% glycerol and centrifuging the suspension at 105000*g* for 1.5 h. The resulting pellet (depleted membranes) was suspended in 10 mM Tris-HCl, pH 8.0, containing 0.14 M KCl, 2.0 mM β-mercaptoethanol, and 10% glycerol. Polyacrylamide gel electrophoresis in the presence of $NaDodSO_4$ was carried out as described previously (Laemmli, 1970). Antibodies were prepared by immunizing albino rabbits with SF_1 or EF_1 in complete Freund's adjuvant.

Assays. The standard reaction mixture for ATPase assay consisted of 4.0 mM ATP (Na salt), 2.0 mM $MgCl_2$, 50 mM Tris-HCl, pH 8.0, and enzyme in a total volume of 0.6 mL (Futai et al., 1974). One unit of enzyme was defined as the amount hydrolyzing 1 μmol of ATP/min at 37 °C under the standard assay conditions. Formation of a protonmotive force in the membrane vesicles was examined qualitatively by measuring quenching of quinacrine fluorescence as described previously (Kanazawa et al., 1979). Membrane vesicles (100–200 μg of protein) were suspended in 1.0 mL of 10 mM Tricine–choline, pH 8.0, containing 140 mM KCl, 1.0 mM $MgCl_2$, and 1.0 μM quinacrine, and fluorescence (emission, 500 nm; excitation, 420 nm) was monitored in a Hitachi spectrofluorometer, Model 650-10S. Then, 10 μL of 0.2 M ATP was added, and ATP-dependent quenching of fluorescence was monitored. Amino acid analysis was carried out by the routine procedure in an amino acid analyzer, Model A-3300 (Irika Kogyo) equipped with an integrator, Model 7,000A. Samples of 1.0 mg of isolated subunits were hydrolyzed in vacuo in 6.0 N HCl for 24, 48, and 72 h at 110 °C, and the hydrolysates were subjected to amino acid analysis. Reconstituted ATPase complexes were centrifuged in a sucrose gradient as described previously (Futai, 1977). Protein was assayed as described by Lowry et al. (1951).

Materials. Chemicals were obtained from the following companies: ATP, Daiichi Pharmaceutical Co.; hydroxylapatite (Bio-Gel HTP), Bio-Rad Co.; DEAE-Sepharose CL-6B, Pharmacia Co.; quinacrine, Sigma Chemical Co.; complete Freund's adjuvant, Difco Laboratories; reagents for polyacrylamide gel electrophoresis, Wako Chemical Co. (Tokyo, Japan). Other reagents were the highest grade commercially available.

Results

Preparation of F_1 and Its Subunits from S. typhimurium. Bragg & Hou (1975) reported that $F_1(SF_1)$ from *S. typhimurium* could be purified by essentially the same procedure as that applied to *E. coli*. We obtained SF_1 by essentially the same procedure, with a recovery of 25%, confirming their

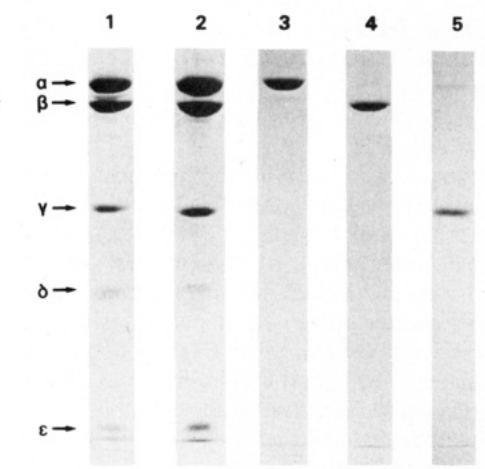

FIGURE 1: $NaDodSO_4$–polyacrylamide gel electrophoresis of EF_1, SF_1, and α, β, and γ subunits of SF_1. Samples were incubated with 1% $NaDodSO_4$ and 2% β-mercaptoethanol for 5 min in a boiling water bath and subjected to electrophoresis in a discontinuous gel system (20 min). The samples applied were as follows: (lane 1) 20 μg of EF_1; (lane 2) 40 μg of SF_1; (lane 3) 7 μg of α subunit of SF_1; (lane 4) 5 μg of β subunit of SF_1; (lane 5) 3 μg of γ subunit of SF_1.

results. In agreement with their results, we found that the subunits of SF_1 had slightly different molecular weights from those of EF_1 as estimated by polyacrylamide gel electrophoresis: as shown in Figure 1, the γ subunit of SF_1 had slightly higher mobility, and the ϵ and δ subunits had slightly lower mobilities than the corresponding subunits of EF_1. However, actual differences of molecular weights were within 1000–2000, as calculated from the mobilities on gel electrophoresis. Gels with the overloaded samples are shown in Figure 1. With smaller samples, the α and β subunits of the two F_1's had essentially the same mobilities on the gel. The specific activity of SF_1 was lower than that of EF_1: the final specific activity of SF_1 was about 30 units/mg of protein, whereas that of EF_1 was 90–120 units/mg of protein (Futai et al., 1974). No attempt has previously been made to prepare subunits of SF_1. In this study, α, β, and γ subunits of comparable purity were prepared essentially by the procedure used for preparing subunits of EF_1 (Figure 1).

Interchangeability of F_1 and F_0 of the Two Bacteria. We studied the functional binding of F_1 to F_0 by testing the formation of a protonmotive force depending on hydrolysis of ATP. Quenching of the fluorescence of quinacrine was assayed as a measure of the protonmotive force. Membrane vesicles from *E. coli* or *S. typhimurium* showed ATP-dependent quenching, whereas membranes of either bacteria depleted of F_1 showed no activity. With depleted membranes of *E. coli* ML308-225, ATP-dependent quenching was restored to the control level with addition of EF_1 or SF_1 (not shown). Furthermore, either EF_1 or SF_1 restored the ATP-dependent quenching in depleted membranes of an F_1 mutant (AN120) (Figure 2). As shown previously (Butlin et al., 1971), this mutant has active F_0 but inactive F_1. The restored level of quenching in the mutant membrane was essentially the same as the level in the wild type. These results indicate that SF_1 could bind to F_0 of *E. coli* and that hydrolysis of ATP by SF_1 drove proton translocation through F_0 of *E. coli*. Essentially the same results were obtained by using membranes of *S. typhimurium*: SF_1 or EF_1 restored ATP-dependent quenching of depleted membranes from *S. typhimurium*. These results indicate that the F_1 and F_0 of the two bacteria are interchangeable, suggesting that the subunit structure of F_0 required for functional binding of F_1 is essentially the same in the two organisms.

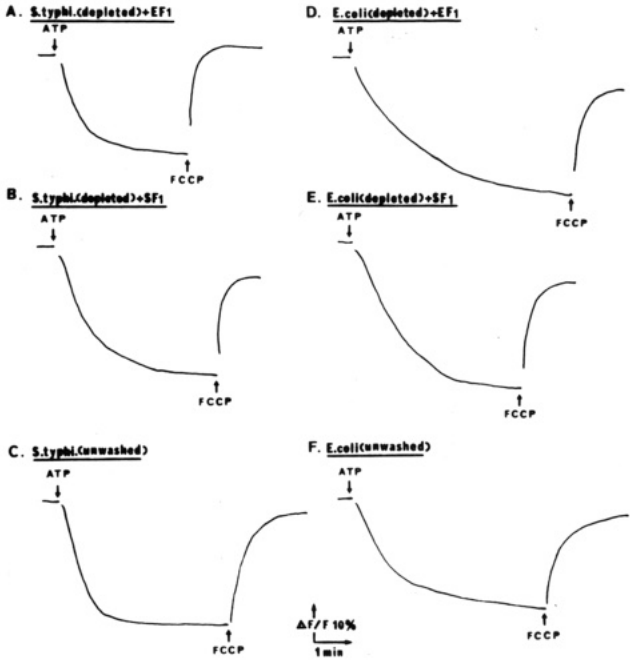

FIGURE 2: Formation of protonmotive force in membrane vesicles of *S. typhimurium* and *E. coli*. Membrane vesicles were obtained from *S. typhimurium* LT2 (A and B) or *E. coli* AN120 (D and E) and washed with dilute buffer to remove F_1. Depleted membrane vesicles (100 μg of protein) were preincubated for 10 min with 55 μg of EF_1 (A and D) or with 145 μg of SF_1 (B and E) in 1.0 mL of 10 mM Tricine–KOH buffer, pH 8.0, containing 1 μM quinacrine, 140 mM KCl, and 10 mM $MgCl_2$. This mixture was introduced into a cuvette, and its fluorescence (emission, 500 nm; excitation, 420 nm) was monitored. At the indicated time, 10 μL of 0.2 M ATP or 3 μL of 1 mM FCCP [carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone] was added. As controls, essentially the same experiments were carried out with unwashed membrane vesicles (unwashed) from *S. typhimurium* LT2 (C) and *E. coli* (F). No quenching of fluorescence was observed in washed membranes without addition of F_1. Other procedures were as described under Experimental Procedures.

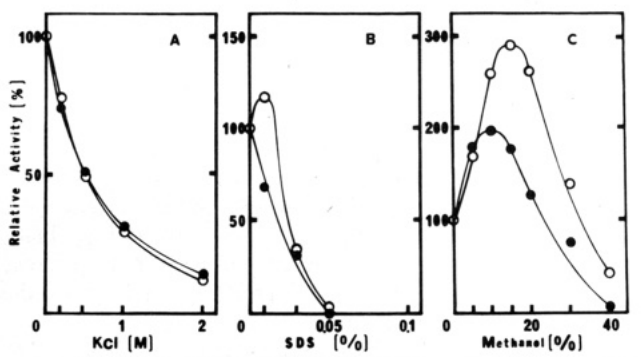

FIGURE 3: Effects of KCl, $NaDodSO_4$ (SDS), and methanol on EF_1 and SF_1. The ATPase activities of EF_1 (○) and SF_1 (●) were assayed in the presence of different concentrations of KCl (A), $NaDodSO_4$ (B), or methanol (C). The results are expressed as relative activities: the specific ATPase activities of EF_1 and SF_1 were 100 and 30 units/mg of protein, respectively. Other conditions were as described under Experimental Procedures.

Comparison of the Properties of SF_1 and EF_1. SF_1 showed essentially the same pH optimum (pH 8.5) and ATP/Mg^{2+} ratio (0.5) for maximal activity as EF_1 (Futai et al., 1974; Kanazawa et al., 1980) (data not shown). Both F_1's were inhibited by a high concentration of KCl (Figure 3A). SF_1 was slightly more resistant than EF_1 to the denaturating agent $NaDodSO_4$ (Figure 3B): a low concentration (0.01%) of $NaDodSO_4$ even activated SF_1 about 10%, although a higher concentration was inhibitory. On the other hand, the same

Table I: Reconstitution of ATPase Activity from Combinations of Subunits of TF_1 and EF_1[a]

expt no.	subunit α	subunit β	subunit γ	ATPase activity (units/mg of protein)
1	S	S	S	5.7
2	S	S	E	4.2
3	S	E	S	11.0
4	E	S	S	4.2
5	E	E	S	11.0
6	E	S	E	8.4
7	S	E	E	10.5
8	E	E	E	19.4

[a] α, β, and γ subunits from SF_1 (indicated as S) or EF_1 (indicated as E) were mixed in 200 μL of 10 mM succinate–Tris, pH 6.0, containing 0.2 M KCl, 10% glycerol, and 0.3 mM β-mercaptoethanol, and the mixture was dialyzed at 23 °C for 8 h against 50 mM succinate–Tris, pH 6.0, containing 10% glycerol, 0.3 mM β-mercaptoethanol, 0.1 mM EDTA, 2 mM ATP, and 2 mM $MgCl_2$. Amounts of subunits used were (α) 10, (β) 10, and (γ) 2.0 μg. After dialysis, ATPase activity was assayed as described under Experimental Procedures. Negligible ATPase activity (less than 0.1 unit/mg of protein) was formed in each case without ATP.

low concentration (0.01%) of $NaDodSO_4$ inhibited EF_1 30%. Methanol stimulated EF_1 and SF_1 2- (at 10%) and 3-fold (at 15%), respectively, although it was inhibitory at higher concentration (Figure 3C). SF_1 was inhibited by dicyclohexylcarbodiimide under essentially the same conditions as described for EF_1 (Pougeois et al., 1980). Antibodies against EF_1 or SF_1 inhibited the ATPase activities of both SF_1 and EF_1 completely, suggesting that the two F_1's have the same exposed antigenic sites.

Formation of Hybrid Enzymes from the α, β, and γ Subunits of EF_1 and SF_1. The ATPase activity of SF_1 was reconstituted by dialyzing a mixture of the α, β, and γ subunits against buffer containing Mg^{2+} and ATP (Table I). The specific activity (5.7 units/mg of protein) of the reconstituted ATPase was about 20% of that of the original SF_1. The reconstituted complex gave a single peak of activity on sucrose gradient centrifugation, and the molecular weight of the complex was calculated to be about 300 000, suggesting that a single complex with ATPase activity was reconstituted. A mixture of the three subunits dialyzed against buffer without ATP or Mg^{2+} gave essentially no ATPase activity (less than 0.1 unit/mg of protein). None of the isolated subunits of SF_1 had ATPase activity when assayed individually, and ATPase could not be reconstituted from a mixture of any two of them, confirming results on EF_1 (Dunn & Futai, 1980; Futai, 1977). These results are also consistent with previous results on TF_1 (Kagawa & Nukiwa, 1981; Yoshida et al., 1977), except that TF_1 ATPase could be reconstituted without ATP.

Formation of hybrid enzymes was studied by mixed combinations of the three subunits from EF_1 and SF_1. In this study one subunit of the combination of α, β, and γ of SF_1 or EF_1 was replaced by the corresponding subunit of the other F_1. As shown in Table I, all possible combinations gave ATPase activity, suggesting that the subunits from the two F_1's are homologous in structure and function. As shown in Table I, hybrid enzymes containing β from EF_1 had higher ATPase activities (about 11 units/mg of protein) than hybrids containing β from SF_1 (about 4 units/mg), except the combination of α and γ from EF_1 and β from SF_1 which had an activity of 8.4 units/mg. As shown above, methanol stimulated EF_1 and SF_1 2- and 3-fold, respectively (Figure 3C). Thus, it was of interest to test the effect of methanol on hybrid enzymes. However, 10% methanol stimulated hybrid enzymes only about 1.5-fold except that a hybrid enzyme formed from α of *E. coli* and β and γ of *S. typhimurium* was stimulated 2-fold. Furthermore, methanol (at 20%) inhibited all the hybrid enzymes about 50% except a complex formed from α of *E. coli* and β and γ of *S. typhimurium* was stimulated 1.2-fold. On the other hand, the ATPase reconstituted from the homologous combination showed essentially the same properties as EF_1 of SF_1. These results suggest that the properties of hybrid enzymes, probably their subunit interactions, have changed to some extent from original F_1 complex.

Table II: Amino Acid Compositions of α and β Subunits from EF_1 and SF_1[a]

residue	α (residues/mol) SF_1	α (residues/mol) EF_1	β (residues/mol) SF_1	β (residues/mol) EF_1
Asp/Asn	47.9	44	39.5	38
Thr	26.4	23	26.6	24
Ser	33.1	32	22.5	25
Glu/Gln	62.7	59	50.9	59
Gly	52.7	47	42.9	45
Ala	53.4	57	42.1	34
Cys	3.3	3	3.2	1
Val	38.5	41	38.1	47
Met	9.4	11	14.0	14
Ile	29.8	40	28.2	27
Leu	37.2	47	39.0	42
Tyr	5.3	15	13.5	15
Phe	26.2	14	15.7	14
Lys	36.1	24	24.6	20
His	13.6	7	8.4	7
Arg	15.7	30	26.1	26
Pro	21.9	18	23.5	20
Trp	ND	1	ND	1

[a] Amino acid compositions of α and β subunits of *S. typhimurium* determined in the acid hydrolysate are shown. Residues per mole were calculated by assuming that *S. typhimurium* subunits have the same numbers of residues as those of *E. coli*. As controls, the amino acid compositions of *E. coli* subunits deduced from DNA sequences are shown. SF_1 and EF_1 differed in 105 of 513 residues in the α subunit and in 53 of 459 residues in the β subunit. Although not shown here, the amino acid contents of the *E. coli* subunits were also determined protein chemically, and values were similar to those deduced from DNA sequences. Values obtained from the DNA sequence and amino acid analysis differed in 27 residues of the α subunit of EF_1 and in 35 residues of its β subunit. ND, not determined.

Amino Acid Compositions of α and β Subunits of SF_1. As discussed above, SF_1 differed from EF_1 in specific activity and other enzymological properties, although the subunits of the two F_1's were interchangeable and showed similar interactions with F_0. Furthermore, hybrid enzymes containing β from EF_1 had higher ATPase activity. Thus it became of interest to compare the primary structures of the α and β subunits of these F_1's. As an initial step, we compared the amino acid compositions of these subunits from the two F_1's. As shown in Table II, the amino acid composition of the β subunit of SF_1 is similar to that of the β subunit of EF_1, with differences in only 53 of 459 residues (11.5%). The amino acid composition of the α subunit of SF_1 was not so similar to that of α from EF_1, showing differences in 105 of 513 residues (20.5%). In this comparison, values from the DNA sequence were used as numbers of amino acid residues in EF_1 subunits. The values obtained from the DNA sequence and from amino acid analysis for EF_1 subunits differed less than 8%, as shown in the legend of Table II. These results suggest that the α subunits of the two F_1's may be less homologous in primary structure than the β subunits. However, the polarities of α from SF_1 and EF_1 were similar when calculated as described previously (Capaldi & Venderkooi, 1972): the polarity indices

of the α subunits from EF_1 and SF_1 were 42.7 and 44.4%, respectively. These results suggest that during evolution amino acids were replaced conserving the polarity of the residues.

Discussion

In this study we compared the enzymological properties of EF_1 and SF_1. These F_1's were found to be the first known F_1's in which the α, β, and γ subunits are mutually interchangeable. All possible combinations of the three subunits from EF_1 and SF_1 gave ATPase activity. Formation of hybrid ATPases has so far been reported only with some subunits of EF_1 and TF_1 (Futai et al., 1980; Takeda et al., 1982). It must be recalled that α of EF_1 (or TF_1) could not form ATPase with β and γ of TF_1 (or EF_1). Moreover, β of TF_1 could not form ATPase (with α and γ of EF_1), although β of EF_1 formed hybrid enzyme (with α and γ of TF_1). Thus, EF_1 seems to be closely similar to SF_1 but less similar to TF_1. The following additional evidence also supports this notion. ATP is required for reconstitution of EF_1 (Futai, 1977; Dunn & Futai, 1980) and SF_1 but not for reconstitution of TF_1. Both SF_1 and EF_1 were inhibited by a high concentration of KCl, whereas TF_1 was not inhibited even by 2 M KCl (Takeda et al., 1982). Antibodies against SF_1 or EF_1 formed precipitin lines with both SF_1 and EF_1 and inhibited their ATPase activities, but no immunochemical cross-reactions were observed between TF_1 and EF_1. Furthermore, F_1 and F_0 of *E. coli* and *S. typhimurium* were interchangeable in vitro, whereas TF_1 could not bind to F_0 of *E. coli* AN120 (B. P. Rosen and M. Futai, unpublished observation). These results suggest that F_0F_1 of *E. coli* is closely similar to F_0F_1 of *S. typhimurium* and less like F_0F_1 of the thermophilic bacterium. These results are consistent with the fact that *E. coli* is much more closely related to *S. typhimurium* than to the thermophile. Recent genetic evidence suggests that the a subunits of the F_0's of *E. coli* and *S. typhimurium* are also interchangeable (Zyskind & Smith, 1980): a mutant *E. coli* defective in subunit a (*uncB402*) could be complemented with a recombinant plasmid carrying *S. typhimurium* DNA near the origin of replication. It is noteworthy that the genes for F_0F_1 in the two bacteria are both near the origin of replication and that the physical maps of this region in the two are similar (Miki et al., 1978; Zyskind & Smith, 1980).

Although the F_1's from the two bacteria are closely similar, they differ in several respects. Their amino acid compositions suggest that the primary structures of the α subunits of the two F_1's are less homologous than those of their β subunits. These results suggest that the α subunit is more species specific or less evolutionally conserved than the β subunit. In agreement with this suggestion, the α subunits of TF_1 and EF_1 are not interchangeable, as discussed above. Thus comparison of the primary structures of the α subunits of the three bacteria may indicate which region is essential.

The β subunits of EF_1 and SF_1 were closely similar in amino acid composition. This finding is consistent with the high homology found in primary structure of this subunit from different organelles (Futai & Kanazawa, 1983; Walker et al., 1982): the β subunits from bovine mitochondria, spinach chloroplasts, and *E. coli* showed about 70% homology. Furthermore, immunological studies indicated that similar antigenic groups are exposed on β subunits of F_1's of different origins: antibodies against the β subunit of *Rhodospirillum rubrum* inhibited ATP-linked reactions not only in *R. rubrum* chromatophores but also in lettuce chloroplasts (Philosoph & Gromet-Elhanan, 1981). Furthermore, antibodies against β from rat and yeast mitochondria, Swiss chard chloroplasts, and *E. coli* membranes reacted with the corresponding subunits of all four F_1's (Rott & Nelson, 1981).

As shown above, the specific activity of SF_1 was lower than that of EF_1, and hybrid enzymes containing β from EF_1 had higher specific activities. Biochemical studies, including experiments using photoaffinity label, have suggested that the catalytic site is in the β subunit or the interface between the α and β subunits [for reviews, see Futai & Kanazawa (1983) and Senior & Wise (1983)]. Thus, the β subunit may be responsible for determining the final specific ATPase activity, although contribution of the α and γ subunits cannot be excluded since ATPase activity can be reconstituted only from a combination of all three subunits.

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Registry No. ATPase, 9000-83-3.

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Experimental Identification of a Theoretically Predicted "Left-Sided" Binding Mode for $(GlcNAc)_6$ in the Active Site of Lysozyme†

Sandra J. Smith-Gill, John A. Rupley, Matthew R. Pincus, Robert P. Carty, and Harold A. Scheraga*

ABSTRACT: Using conformational energy calculations, we previously predicted that there are two distinct binding modes for hexasaccharide substrates of hen egg white lysozyme (HEWL), a "left-sided" binding mode and a "right-sided" one. The former involves such residues as Arg-45, Asn-46, and Thr-47, while the latter involves such residues as Asn-113 and Arg-114. The left-sided binding mode was predicted to predominate for $(GlcNAc)_6$. We now present two lines of experimental evidence that indicate that left-sided binding occurs for this substrate. First, we show that ring-necked pheasant lysozyme (RNPL), in which Lys and His replace Asn and Arg at positions 113 and 114, respectively, has the same affinity for $(GlcNAc)_6$ as does HEWL, indicating that the "right" side is *not* involved in equilibrium binding to the substrate. Second, we show that a monoclonal antibody, HyHEL-5, which binds specifically to an epitope including residues Arg-45, Asn-46, Thr-47, Asp-48, and Arg-68 on the far "left" side of HEWL, is competitively displaced by $(GlcNAc)_5$ and $(GlcNAc)_6$ but not by GlcNAc, $(GlcNAc)_2$, or $(GlcNAc)_4$. Only the former two substrates can bind in site F in the lower active site. Since these two substrates are the only ones that competitively displace HyHEL-5, our results suggest that the terminal saccharide residues of these substrates bind to the left side of the active site cleft, as predicted from theory.

On the basis of theoretical considerations (Pincus & Scheraga, 1979, 1981a,b; Scheraga et al., 1982), we found that hexasaccharide substrates, including the homopolymer $(GlcNAc)_6$[1] and copolymers of GlcNAc and MurNAc, exhibit two distinct binding modes to the active site of hen egg white lysozyme, a "left-" and "right-sided" mode. For the homopolymer $(GlcNAc)_6$, the preferred binding mode is one involving the left side of the active site in which the terminal (reducing) sugar binds to a β-sheet region formed in part by Arg-45, Asn-46, and Thr-47. In this binding mode, the sugar residue in the D site adopts the full-chair conformation (i.e., no distortion of the D-site saccharide residue is necessary) and occupies a position somewhat removed from the deep-cleft region between the two acid catalytic residues, Glu-35 and Asp-52. The first three residues from the nonreducing end in sites A–C occupy positions quite similar to those determined by X-ray diffraction analysis (Imoto et al., 1972; Pincus et

† From the Laboratory of Genetics, National Institutes of Health, Bethesda, Maryland 20205 (S.J.S.G.), the Department of Biochemistry, University of Arizona, Tucson, Arizona 85721 (J.A.R.), the Department of Pathology, Columbia College of Physicians and Surgeons, New York, New York 10032 (M.R.P.), the Department of Biochemistry, SUNY Downstate Medical Center, Brooklyn, New York 11203 (R.P.C.), and the Baker Laboratory of Chemistry, Cornell University, Ithaca, New York 14853 (H.A.S.). *Received August 16, 1983.* This work was supported by research grants from the National Science Foundation (PCM 79-20279), from the National Institute of General Medical Sciences of the National Institutes of Health (GM-14312), and from the National Foundation for Cancer Research.

[1] Abbreviations: GlcNAc, *N*-acetylglucosamine; MurNAc, *N*-acetylmuramic acid; $(GlcNAc)_n$, n β-1,4-linked *N*-acetylglucosamine units; HEWL, hen egg white lysozyme; RNPL, ring-necked pheasant lysozyme; HyHEL-5, hybridoma anti-hen egg white lysozyme, clone number 5; mAb, monoclonal antibody; BSA, bovine serum albumin, Pentex fraction V; ELISA, enzyme-linked immunosorbant assay.

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