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Biochimica et Biophysica Acta 1186 (1994) 243–246

BBA Biochimica et Biophysica Acta

Rapid Report

# Complementation of *Escherichia coli uncD* mutant strains by a chimeric $F_1$-$\beta$ subunit constructed from *E. coli* and spinach chloroplast $F_1$-$\beta$

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Received 29 March 1994

**Abstract**

ATP-synthesizing $F_0F_1$-ATPases are complex enzymes consisting of at least eight different subunits. These subunits are conserved during evolution to a very variable degree ranging in pairwise comparison between, for example, *Escherichia coli* and spinach chloroplast from 20% to 66% identical residues. It was surprising to find that some of the less well conserved subunits like $\delta$ and $\epsilon$ could replace their *E. coli* counterparts, whereas the highly conserved $\beta$ subunit, which carries the active site, in the *E. coli* enzyme could not be substituted by spinach chloroplast $\beta$ (Lill et al. (1993) Biochim. Biophys. Acta 1144, 278–284). We constructed a chimeric $F_1$-$\beta$ subunit consisting of spinach $\beta$ in which the 96 N-terminal amino acids were replaced by the respective residue sequence from *E. coli* $\beta$. Whereas spinach $\beta$ did not complement *E. coli uncD* mutant strains, the chimeric $\beta$ subunit restored growth under conditions of oxidative phosphorylation.

*Key words:* ATPase, $F_0F_1$-; ATPase, $F_1$-; Complementation; Chimeric protein

$F_0F_1$-ATPases synthesize ATP at the expense of protonmotive force [1–6]. They are found in bacteria, mitochondria and chloroplasts. The enzyme consists of two distinct subcomplexes, the membrane-embedded proton channel $F_0$, and the extrinsic, water-soluble part, $F_1$, which carries the catalytic sites. $F_1$ of *Escherichia coli* and chloroplasts consists of five different subunits, $\alpha$ (56 kDa), $\beta$ (54 kDa), $\gamma$ (31–36 kDa), $\delta$ (21 kDa), and $\epsilon$ (15 kDa) witha stoichiometry of 3 : 3 : 1 : 1 : 1 and nucleotide binding sites on or between $\alpha$ and $\beta$.

While the general architecture of $F_0F_1$-ATPases from different sources is very similar, their subunits are conserved to very variable degrees. The $\beta$ subunits contain the catalytic nucleotide binding sites and it is not surprising that $F_1$-$\beta$ is the best conserved subunit: 66% of the residues are identical in pairwise comparison between spinach and *E. coli* $\beta$. Subunits $\delta$ and $\epsilon$, on the other hand, share only about 25% of identical residues in pairwise comparison.

This degree of conservation does not, however, correlate with the 'exchangeability' of the subunits: Table 1 summarizes the data which have been published so far.

It was surprising to find that the highly conserved subunit $\beta$ from spinach did not complement the respective *E. coli unc* mutant strain, whereas cyanobacterial $\beta$ from *Synechocystis* sp. PCC6803 did [7]. The percentages of identical residues are 71% (*E. coli* ⇔ *Synechocystis*), 66% (*E. coli* ⇔ spinach) and 80% (*Synechocystis* ⇔ spinach). The degree of identity is thus very similar, with *Synechocystis* $\beta$ resembling *E. coli* $\beta$ just a little more than spinach $\beta$ resembles *E. coli* $\beta$. We tried to take advantage of this small difference and constructed a chimeric $\beta$ subunit consisting of ~ 20% of the *E. coli* sequence (at the N-terminus) and the remaining ~ 80% of the spinach $\beta$ sequence. The chimeric protein allowed for significant growth of $\beta$-defective *E. coli* mutant strains under conditions of oxidative phosphorylation, thus indicating that the N-terminal part of subunit $\beta$ at least in *E. coli* $F_0F_1$ has to fulfill some rather restricted structural requirements.

Abbreviations: $F_0F_1$, $F_0F_1$-ATPase; $F_0$, proton channel (membrane-embedded); $F_1$, ATPase (soluble part).

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*SSDI* 0005-2728(94)00052-7

Table 1
Summary of complementations of *E. coli unc* mutant strains by the respective spinach $CF_0CF_1$ subunits

| Subunit | Identical residues | Complementation | Reference |
|---|---|---|---|
| $\alpha$ | 52 | yes | [7] |
| $\beta$ | 67 | no | [7,8] |
| $\gamma$ | 39 | no | [7] |
| $\delta$ | 23 | yes | [7] |
| $\epsilon$ | 26 | yes | [7] |
| I/II-b | 19/20 | no/no | [9–11] |
| III-c | 29 | no | [12] |
| IV-a | 26 | no | B [a] |

[a] Burkovski, A., unpublished data.

Enzymes and reagents for molecular biology were obtained from Bethesda Research Laboratories, Boehringer-Mannheim, and New England Biolabs. Oligonucleotides were synthesized on a Pharmacia Gene Assembler. All chemicals were of analytical grade.

For complementation experiments, strains RH344 ([13] *uncD*244, *recA*56, *srl*::Tn*10*, *bglR*, *thi1*,*rel1*, HfrP01) and JP17 ([14] *ΔuncD*, *argH*,*pyrE*, *entA*, *recA*::Tn10) were used. Growth on minimal medium containing the respective supplements and 0.4% succinate or, as a control, glucose as the sole carbon and energy source was monitored. Complementation assays were carried out at least in triplicate from independent transformations as described earlier [7].

Recently we cloned the genes for the five subunits from spinach chloroplast $CF_1$ and from *Synechocystis* sp. PCC 6803 into pJLA expression vectors [7]. For the experiments presented here we used plasmid pJLA-$\beta$, which encodes the spinach $F_1$-$\beta$ subunit, as starting material. In order to construct the desired hybrid gene we used an *Sst*I restriction site at position 283 in the *atpb* gene. The sequence between this site and the ATG start codon was removed and substituted for by the respective nucleotide sequence from *E. coli* $\beta$. The required part of the *E. coli* $\beta$ gene was amplified from plasmid pDP31 ([15], encoding *E. coli* $F_1$-$\beta$ and -$\epsilon$) by the polymerase chain reaction using Vent Polymerase and with primers containing the appropriate restriction sites.

In order to unravel the reason for the lack of function of chloroplast $F_1$-$\beta$ in *E. coli* $F_1$, the amino acid sequences of the subunits were compared. Fig. 1 shows an alignment of the amino acid sequences of $F_1$-$\beta$ from spinach, *E. coli* and *Synechocystis* sp. PCC6803 together with a secondary structure prediction for spinach $\beta$. Identical residues between all three proteins are indicated by stars, *pairwise* identities are indicated by reverse printing. It is evident that the central nucleotide-binding region is best conserved, whereas both the amino and carboxy termini in a number of positions contain residues where only *E. coli* and *Synechocystis* $\beta$ are identical while spinach $\beta$ is different. We decided to substitute the N-terminal part of spinach $\beta$ by the respective sequence from *E. coli*. The spinach $\beta$ gene contains a *SstI* restriction site at position 283 of the nucleotide sequence. We substituted these 283 nucleotides by the respective sequence from *E. coli* and upon expression obtained a chimeric protein which consisted of 74 N-terminal amino acids from the *E. coli* $F_1$-$\beta$ sequence and 402 residues from the remaining spinach $\beta$ sequence. The resulting chimeric $\beta$ subunit was shorter by 22 residues than spinach $\beta$ due to gaps and an a priori shorter *E. coli* $\beta$ sequence (cf. Fig. 1). Upon expression the chimeric $\beta$ subunit precipitated into inclusion bodies.

Table 2 shows the results of complementation experiments with strain RH344, which is defective in subunit $\beta$ and therefore unable to grow under oxidative phosphorylation. Whereas wild-type spinach $\beta$ did not complement, the chimeric $\beta$ subunit allowed for significant growth on succinate. Similar results were obtained with strain JP17 (data not shown). In conclusion, a chimeric protein consisting of the N-terminal fifth of the amino acid sequence from *E. coli* $F_1$-$\beta$ and the remainder from the not closely related spinach chloroplast $F_1$-$\beta$ was able to functionally substitute *E. coli* $\beta$ in *E. coli* $F_0F_1$. This finding is remarkable in view of the fact that the chimeric protein precipitated into inclusion bodies (just as spinach $\beta$) upon expression in *E. coli*. Obviously, some molecules were incorporated into the nascent $F_0F_1$-ATPase before they could precipitate. This process was temperature-dependent, it worked more effectively if larger amounts of chimeric $\beta$ were produced at higher temperature. The failure of spinach $\beta$ in complementation was thus due not to an unfavourable kinetic competition between precipitation and folding/assembly, but to a 'misfit'.

The sequence alignment (Fig. 1) shows some additional amino acid residues both within and at the N-terminus of the spinach $\beta$ sequence as compared to the *E. coli* sequence. With the exception of the additional N-terminal 10-amino-acid stretch in spinach $\beta$, *Synechocystis* $\beta$ and spinach $\beta$ are comparable and, because *Synechocystis* $\beta$ was effective in complementation [7], the additional residues within the spinach $\beta$ sequence can be excluded as reason for the failure of spinach $\beta$ in complementation.

The 10 N-terminal residues in spinach $\beta$ might cause some sterical hindrance. The space being occupied by these residues would have to fit into some complementary cavity on another subunit. A possible candidate for such a cavity, however, is not evident from inspection of the primary structures of the other $F_1$ subunits.

Another explanation for the activity of the chimeric $\beta$ is some, possibly subtle, change in tertiary structure

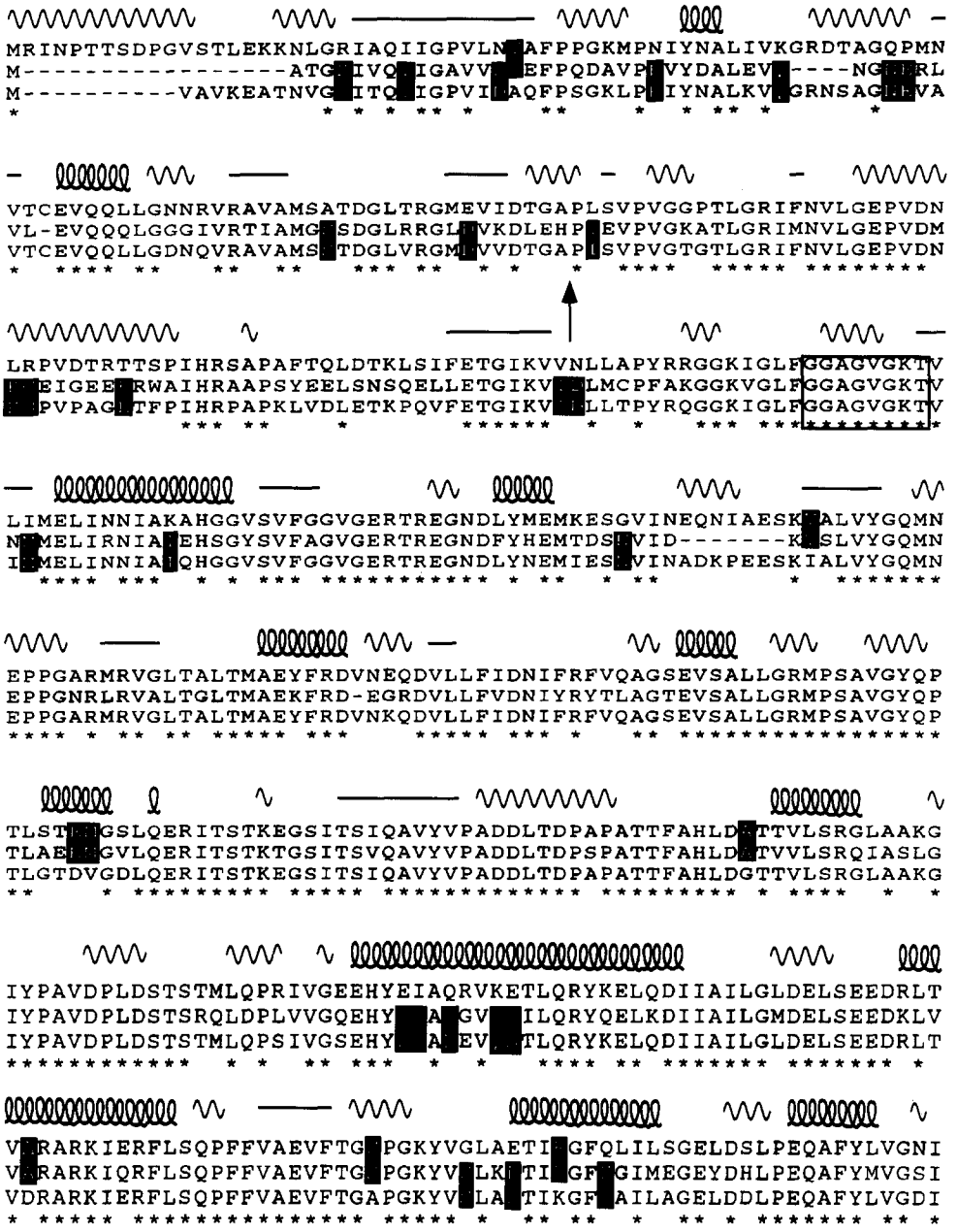

Fig. 1. Amino acid sequence alignment (obtained with ClustAl V running under the DKFZ (Heidelberg) HUSAR package) of spinach chloroplast $F_1$-$\beta$ (sw: atpb – spiol, top), *E. coli* $F_1$-$\beta$ (sw: atpb – ecoli, center), and *Synechocystis* sp. PCC 6803(sw: atpb – syny3, bottom). Stars below the alignment indicate identical residues; pairwise identities are indicated by reverse printing. The boxed region shows the so-called 'glycine-rich loop', which is involved in binding of the ATP-$\gamma$-phosphate moiety. The arrow indicates the point where the chimeric $\beta$ subunit was merged from the *E. coli* N-terminal part and the spinach chloroplast $\beta$ remainder. The uppermost row shows a secondary structure prediction for spinach $\beta$ as obtained with the program Predict Protein [18]. ⌇⌇ = helices, ——— = strands, ∿∿ = loops.

in the N-terminal region. As the remainder of the sequence was left untouched and the center part of $\beta$ has been shown to be involved in nucleotide binding, it is probable that the protein consists of several domains with specific functions. It must be expected then that these building blocks fold in very much the same manner, irrespective of the N-terminal 74 amino acids.

A comparison of structurally resolved nucleotide-binding proteins (elongation factor Tu, (Protein Data Bank [16] ident code: 1ETU); H-*ras* oncogene protein p21, (PDB: 521p); nitrogenase Fe protein, (PDB: 1NIP); *E. coli* recA protein, (PDB: 1REA), adenylat kinase, (PDB: 1AK3); inspection of structures with the program 'O' [17]) reveals a common fold around the so-called glycine-rich phosphate binding loop (residues $G_{172}$GAGVGK$T_{179}$ in spinach $\beta$, cf. boxed region in Fig. 1). This fold, characterized by a $\beta$-loop-$\alpha$...$\beta$-loop-$\alpha$ structure in all nucleotide-binding proteins

Table 2
Growth of *E. coli* strain RH344 [13] under different conditions

| Plasmid | Succinate | | | Glucose | | |
|---|---|---|---|---|---|---|
| | 30°C | 37°C | 42°C | 30°C | 37°C | 42°C |
| pJLA 503 | − | − | − | + | + | + |
| pJLA-β | − | − | − | + | + | +/− |
| pJLA-β1 | − | +/− | + | + | + | +/− |
| pDP 31 | + | ++ | +/− | + | ++ | + |

Cells were grown as detailed in Materials and Methods. Symbols are: −, no growth; +/−, poor, but significant growth; +, growth; ++, fast growth with high yields. Data were obtained at least in triplicate and from independent transformations.

structurally resolved so far, is located near the N-terminus, only RecA contains additional N-terminal helices and strands. $F_1$-β, on the other hand, contains 171 residues preceding the glycine-rich loop. Substitution of the first 96 of these residues by the respective residues from *E. coli* makes spinach β *E. coli*-$F_0F_1$ 'compatible'. We conclude that the amino-terminus of $F_1$-β is involved in inter-subunit interactions. In view of the fact that conformational changes play a substantial role for the function of $F_0F_1$-ATP synthases and in view of the probable location of the active sites between subunits α and β subunit α would be the most promising candidate to interact with the N-terminal part of subunit β. Such an interaction is not evident from the available primary structures but has to await three-dimensional structural resolution.

We gratefully acknowledge the excellent technical assistance of Martina Blomeier and a generous gift of plasmid pDP31 and *E. coli* strain JP17 by Dr. Alan Senior. This work was supported by a grant from the Deutsche Forschungsgemeinschaft (SFB 171-B3/B4/B8).

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