Subunit equivalence in *Escherichia coli* and bovine heart mitochondrial F_1F_0 ATPases

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F_1-ATPases from bovine mitochondria and *Escherichia coli* both contain 5 subunits named α, β, γ, δ and ε. Sequence analysis shows that the δ subunits are not related, nor are the ε subunits. The counterpart of mitochondrial δ is bacterial ε. The subunit equivalent to bacterial δ is mitochondrial oligomycin sensitivity conferral protein.

F_1F_0-ATPase, of bovine heart mitochondria *F_1F_0-ATPase, of* Escherichia coli *Subunit α, β, homology*
Subunit δ, ε, non-equivalence

1. INTRODUCTION

The F_1F_0 ATPases of bovine mitochondria and *Escherichia coli* have many common structural features (reviews [1–5]). They appear to be made up of a membrane-bound domain F_1 which is attached to a transmembrane structure, F_0. In both cases a soluble particle, F_1, can be detached intact from the membrane. It is an ATPase comprising 5 subunits called α, β, γ, δ and ε in both species (see fig.1). Equivalence of subunits with the same names (α–ε) in mitochondria and *E.coli* is often assumed despite considerable size differences between the δ-subunits and between the ε-subunits (fig.1). The bacterial F_0 is made of 3 subunits a, b and c; mitochondrial F_0 appears to be more complex: extra subunits have been detected in this sector, the best characterised being the oligomycin sensitivity conferral protein (oscp) [6–9] and factor 6 (F_6) [10]. The oscp is a basic, water-soluble protein of ~18 000 M_r [5]. It facilitates binding of F_1 to F_0, and is essential for restoration of energy-linked functions in reassembly experiments. It is usually absent from preparations of F_1-ATPase (cf. [11]). The δ-subunit of the *E.coli* enzyme is also required for interaction of bacterial F_1 with the membrane [12]. It binds to the N-terminal region of α-subunits [13] and probably also makes important contacts with the amphiphilic membrane protein b [14]. The amounts of δ in preparations of bacterial F_1 depend upon the method employed for F_1 release; in some preparations it appears to

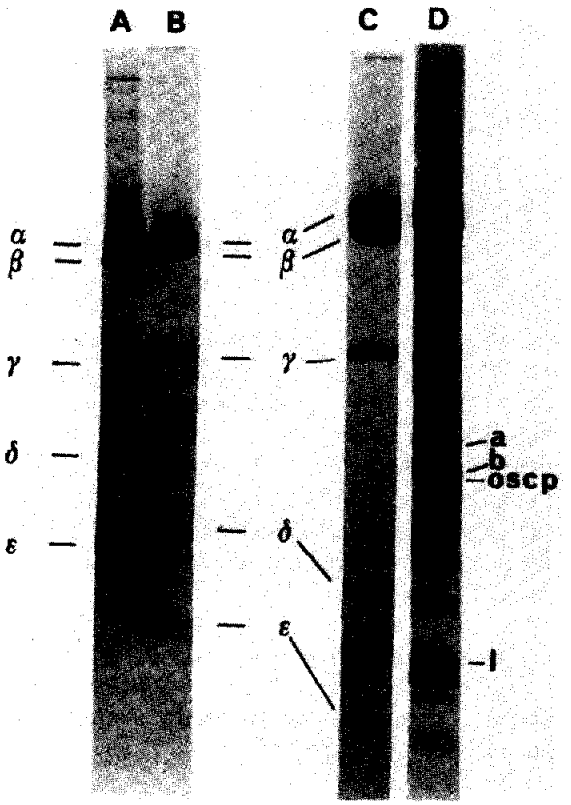

Fig.1. Polyacrylamide gels of (A) *E.coli* F_1 ATPase, (B) and (C) bovine F_1 ATPase and (D) bovine F_1F_0 ATPase. Gels were 10–25% acrylamide gradients [24] and run in the presence of sodium dodecyl sulphate [32]. Proteins were stained with Coomassie blue. a and b are F_0 proteins. I is the inhibitor protein.

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be almost absent [15]; in others it is present in a 1:1 stoichiometry with the γ- and ε-subunits [16]. Bacterial ε-subunit is also important for binding F_1 to F_0 [17,18]. It appears to interact with the γ-subunit [18] and probably with the subunit b [14]. An equivalent role has not been demonstrated for mitochondrial ε. Sequence analysis helps to define the relationships between the bacterial and mitochondrial subunits. As reported here, it is apparent that bacterial δ and mitochondrial δ are probably not related to each other. However, mitochondrial δ is homologous to bacterial ε, and bacterial δ is related to mitochondrial oscp.

2. MATERIALS AND METHODS

2.1. *Purification of enzymes*

Bovine F_1 ATPase and F_1F_0 ATPase were prepared as in [19]. Subunits were isolated from F_1 essentially as in [20]. *Escherichia coli* F_1 was a gift from Dr J. Deatherage, having been donated to him by Dr L.A. Heppel.

2.2. *Protein sequence analysis*

The sequence analysis of *E.coli* δ and ε [21–24] and of parts of bovine oscp [25] have been described. Bovine δ (150 nmol) was reacted with a 50-fold molar excess of succinic anhydride as in [26]. Peptides were isolated from a tryptic digest of it by gel filtration through Sephadex G-75, superfine, in 0.5% ammonium bicarbonate. Peptide δR1 eluted as a pure peptide as judged by N-terminal analysis. Its N-terminal sequence was determined by automated sequencer analysis in an extensively modified Beckman 890B sequencer [26]. Phenylthiohydantoins were identified by high-pressure liquid chromatography [27].

2.3. *Detection of sequence homologies*

Sequences of *E.coli* δ and ε and partial sequences of bovine oscp and δ were compared with the aid of the interactive computer program DIAGON [28].

3. RESULTS

3.1. *Equivalence between oscp and* E.coli δ

Sequences representing the N-terminal and C-terminal regions of oscp [25] are homologous to equivalent regions in *E.coli* δ as illustrated in table 1. By contrast no homology can be detected with sequences of bovine δ including δR1 that represent > 50% of its entire chain.

3.2. *Equivalence between* E.coli ε *and bovine* δ

The sequence determined in bovine δ is homologous to the *E.coli* ε sequence as illustrated in table 2. No clear homologies with other *E.coli* F_1F_0 ATPase subunits were detected.

4. DISCUSSION

The above results strongly indicate that the superficial similarity between bacterial and mitochondrial F_1 ATPase illustrated by the presence of 5 subunits in both complexes is somewhat misleading. The δ-subunits are not equivalent to each other in sequence and are also probably not equivalent in function. Similarly, ε-subunits are probably non-equivalent. The available sequence information suggests that the counterpart of the *E.coli* δ-subunit is the bovine mitochondrial oscp, a basic protein known to play an important part in binding F_1 to F_0 [8]. The bacterial δ-subunit has a similar role [12]. It is noteworthy that rat liver F_1 and bovine F_1 have been prepared with 6 subunits [29]. The extra subunit in the rat liver enzyme has a M_r of 26 500 and has properties in common with the bovine oscp. Complexes diminished in δ have been prepared from *E.coli* [15] and chloroplasts [30]. So it appears that the partitioning of the δ-subunit in *E.coli* and chloroplasts or of the oscp in beef heart (and other) mitochondria during extraction of F_1 is dependent upon the experimental conditions.

The counterpart of bovine δ is probably *E.coli* ε; recently the wheat chloroplast ε protein has been found to be homologous to the bacterial protein (I.A. Fearnley, C.J. Howe, J.C. Gray and J.E. Walker, unpublished) and so in this respect at least the chloroplast and bacterial enzymes are similar. For the moment the bovine ε has no known counterpart in *E.coli* (or chloroplasts). No specific function has been established for mitochondrial ε [5]; and in [30] it was proposed that it should be referred to as the ε′-subunit, ε being reserved for the inhibitor protein.

In contrast to the bacterial and mitochondrial δ- and ε-subunits the α- and β-subunits of bovine mitochondrial F_1 ATPase are strongly homologous to their *E.coli* counterparts. For example, 75% of

Table 1

Homologies between *E.coli* δ-subunit and bovine mitochondrial oligomycin sensitivity conferral protein of F_1F_0 ATPase

	Protein	Residues	Sequence
(a)	*E. coli* δ	1–30	M S E F I T V A R P Y A K A A F D F A V E H Q S V E R W Q D . . .
	oscp	1–37	F A K L V R P P V Q I Y G I Q G R * Y A T A L Y S A A S K Q N K L E Q V E K . . .
(b)	*E. coli* δ	143–177	I D K S V M A G V I I R A G D M V I D G S V R G R L E R L A D V L Q S
	oscp	1′–37′	I D P S I M G G M I V R I G E K Y V D M S A K T K I E K L S R A M R Q I L

In (a) the N-terminal sequence and in (b) the C-terminal sequences of the two proteins are aligned. Identities and conservative substitutions are boxed. * = deletion. Sequences of *E.coli* δ are from [21] and oscp from [25]

Table 2

Homology between the *E.coli* ε-subunit and the bovine mitochondrial δ-subunit of F_1F_0 ATPase

Protein	Residues	Sequence	Ref.
E. coli ε	21–65	V E K I Q V T G S E G E L G I Y P G H A P L L T A I K P G M I R I V K Q H G H E E F I Y L	[22]
Bovine δR1	–	Q V D V P T Q T * G A F G I L A A H V P T L Q V L R P G L V V * * * H A E D G T T S K	This work

* = deletion

amino acid residues in β-subunits are identical [31] and α-subunits also show extensive homologies (V.L.J. Tybulewicz and J.E. Walker, unpublished).

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