Subunit II (b') and Not Subunit I (b) of Photosynthetic ATP Synthases is Equivalent to Subunit b of the ATP Synthases from Nonphotosynthetic Eubacteria. Evidence for a New Assignment of b-Type F_o Subunits

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Chloroplast, Coupling Factor, Stalk, Cyanobacteria

Subunit I of chloroplast ATP synthase is reviewed until now to be equivalent to subunit b of *Escherichia coli* ATP synthase, whereas subunit II is suggested to be an additional subunit in photosynthetic ATP synthases lacking a counterpart in *E. coli.* After publication of some sequences of subunits II a revision of this assignment is necessary. Based on the analysis of 51 amino acid sequences of b-type subunits concerning similarities in primary structure, isoelectric point and a discovered discontinuous structural feature, our data provide evidence that chloroplast subunit II (subunit b' of photosynthetic eubacteria) and not chloroplast subunit I (subunit b of photosynthetic eubacteria) is the equivalent of subunit b of nonphotosynthetic eubacteria, and therefore does have a counterpart in e.g. *E. coli.* In consequence, structural features essential for function should be looked for on subunit II (b').

Introduction

The energy-transducing membranes of chloroplasts, mitochondria and eubacteria contain an F-type H^+-translocating ATP synthase (EC 3.6.1.34) which catalyzes formation and release of ATP depending on an electrochemical proton gradient generated by electron transport across the respective membrane. In each case, a membrane-embedded moiety (F_o) translocates protons and a peripheral moiety (F_1) bears the three catalytic sites. Electron microscopic studies have revealed that the subcomplexes seem to be connected through a slender stalk (for recent reviews, see van Walraven and Bakels, 1996; Weber and Senior, 1997). X-ray analysis of the isolated mitochondrial F_1 (Bianchet *et al.,* 1991; Abrahams *et al.* 1994), spectroscopic studies on isolated CF_1 from spinach chloroplasts (Sabbert *et al.*, 1996; Sabbert and Junge, 1997) and single molecule fluorescence microscopic observation of a recombinant eubacterial F_1 subcomplex containing subunits α, β and γ (Noji *et al.,* 1997) have yielded more detailed information about the F_1 structure and the mechanism of ATP hydrolysis. To understand the mechanism of the holoenzyme, including proton-driven ATP synthesis, knowledge of the structure and function also of the F_o subunits is essential, since they are involved in coupling proton translocation to ATP synthesis/release.

It is generally assumed that the basic mechanism of the holoenzyme is the same among F-type ATP synthases from different sources, and that therefore knowledge about the ATP synthase of one source can be applied to the enzyme from another source, in detail, if equivalent subunits are considered. Therefore, the equivalence of subunits from the enzymes from different sources has to be analyzed.

The term 'equivalent' has a different meaning than the term 'homologous'; homologous subunits, that share a common ancestor, may not be equivalent in function. F_1 subunits α and β, for example, show more than 20% identical residues in corresponding positions, and therefore are assumed to have resulted from gene duplication of an ancestral gene (Walker *et al.,* 1984); they are homologous, but not considered to be equivalent. The term 'equivalent' is used if homologous subunits are expected to perform essentially the same function within holoenzymes from different sources.

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The simpliest composition of the F_o moiety is found in nonphotosynthetic eubacteria like *E. coli* with three different subunits, called a, b and c (Foster and Fillingame, 1979); each of them is essential for function (Schneider and Altendorf, 1984, 1985). The F_o of chloroplasts and photosynthetic eubacteria is an assembly of four different subunits, called I, II, III and IV (Hennig and Herrmann, 1986) and a, b, b' and c (Cozens and Walker, 1987), respectively. Mitochondrial F_o is built up of even more nonidentical subunits – at least 9 in bovine heart mitochondria (Collinson *et al.*, 1994) – and will not be discussed here. Also the products of gene *atpF* from three mycoplasms will not be considered, because of their atypical hydropathy profile and size: they possess two putative spans instead of one and are with more than 200 amino acids significantly larger than other eubacterial b-type subunits (Rasmussen *et al.*, 1992).

The equivalence of the F_o subunits denoted proteolipid, chloroplast subunit III and subunit c from nonphotosynthetic bacteria like *E. coli* as well as from photosynthetic eubacteria, is demonstrated (Sebald and Hoppe, 1981; Cozens and Walker, 1987); also the equivalence of chloroplast subunit IV and subunit a from eubacteria is clear from gene locations, molecular weights and hydropathy profiles, in spite of low sequence identities (Cozens *et al.*, 1986; Hennig and Herrmann, 1986).

From primary structure analysis, it is obvious that chloroplast subunits I and II are equivalent to subunits b and b' from photosynthetic eubacteria, respectively (Cozens and Walker, 1987; Berzborn *et al.*, 1990; Pancic *et al.*, 1992; Herrmann *et al.*, 1993). Subunits b and b' from photosynthetic eubacteria are proposed to result from duplication of gene *atpF* encoding subunit b from eubacteria (Cozens and Walker, 1987). Subunit b from nonphotosynthetic eubacteria and subunits b and b' from photosynthetic eubacteria as well as chloroplast subunits I and II therefore are homologous subunits. But are they also equivalent?

While in *E. coli* only one kind of b-type subunits – present in two copies (Foster and Fillingame, 1982) – is sufficient for function, ATP synthases from chloroplasts and photosynthetic eubacteria possess two nonidentical b-type subunits. Irrespective of the precise function(s) of the b-type subunits, which is still unknown, two nonidentical b-type subunits may be merely two divergent forms of *E. coli* subunit b, but equivalent to each other, or one of them may correspond to the essential and sufficient subunit b from *E. coli* and the other may be an additional and nonequivalent one, possibly with an additional function.

From gene location, hydropathy profile and, in addition, from the predicted secondary structure, chloroplast subunit I has been identified as equivalent to subunit b from *E. coli* (Bird *et al.*, 1985), although the identity in primary structure is low, only 19% according to Hudson *et al.* (1987). Both subunits I and b contain one hydrophobic stretch at the N-terminus which is thought to span the respective membrane; the remainder of the proteins is hydrophilic with a predicted α-helical secondary structure, facing the same side of the membrane as the peripheral F_1 (Walker *et al.*, 1982; Bird *et al.*, 1985).

At that time, no amino acid sequence of any subunit II was available. After publication of the complete primary structure of subunit II from spinach (Herrmann *et al.*, 1993) and several algae (Pancic *et al.*, 1992; Kostrzewa and Zetsche, 1992, 1993; Reith and Munholland, 1995), a more detailed analysis is possible. The characteristics which have identified chloroplast subunit I as equivalent to *E. coli* subunit b hold for chloroplast subunit II as well. However, this subunit is still considered to be unique to photosynthetic ATP synthases and to have no counterpart in *E. coli* (Herrmann *et al.*, 1993; van Walraven and Bakels, 1996).

In this publication, a new assignment of ATP synthase subunits b is deduced which is in contrast to the above consideration. Based on the analysis of 51 amino acid sequences of b-type subunits concerning similarities in primary structure, isoelectric point and a discovered discontinuous structural feature, our data provide evidence that chloroplast subunit II (subunit b' of photosynthetic eubacteria) and not chloroplast subunit I (subunit b of photosynthetic eubacteria) is the equivalent of subunit b of nonphotosynthetic eubacteria, and therefore does have a counterpart in e.g. *E. coli*. In consequence, structural features essential for function should be looked for on subunit II (b') and possibly additional functions should be looked for on subunit I (b).

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                                                                  >   >   *   >
Spinacia oleracea          -II EEIEKASLFDFNLTLPIIMAEFLFLMFALDKIYYTP-LGDFMDKRDASI  48
Odontella sinensis         -II EVNGPGGLFDINATLPLVAIQFLLLMVLLNVILYSP-LLTIIEERKEYI  59
Antithamnion sp.           -II STEVQGGLFDFNATLPLMALQFLALTIILNLIYYKP-LGKILDERDEYI  61
Galdieria sulphuraria      -II SLLAAGGLFDFDATLSFIALEFLLLTSVLNLIYYQP-ISKVIDSREDYI  57
Ochrosphaera neapolitana   -II LSEGEGGLFDFNATLPLMALQFILLTVILTFVFYKP-IGNLLEEREAYI  69
Porphyra purpurea          -II AEEIEGGLFDFNGTLPLMALQFLILMLLLNTIFYKP-VTKILDERDEYI  56
Escherichia coli           -b          MNLNATILGQAIAFVLFVLFCMKYVWPP-LMAAIEKRQKEI  40
Spinacia oleracea          -I        GSFGFNTDILATNLINLSVVLGVLIFFGKGVLSDLLDNRKQRI  43
Odontella sinensis         -I        EGIGLNTDILETGIINIAALVGILIYAGRDFLGSFLEQRKTSI  57
Antithamnion sp.           -I        HSIGLNSDFLEANVLNIMLLLFGLIYVLKQFLGSLLTIRQEKV  62
Galdieria sulphuraria      -I        SSIKINLDLLETNIINIFILIIILIYLGRKFLGNILINRQNRV  55
Ochrosphaera neapolitana   -I        GGVSFNPDIFETNVVNLAILTGGIFYLGSNALSESLVERQQKI  60
Porphyra purpurea          -I        HTFGFNSDIFEANVINILLLLFGLIYVLKQSLGSTLNERQLKV  63

Spi-II     KEQLSGVKDTSSEVKQLEEQANAVMRAARAEISAALNKMKKETQLEVEAKLAEGRKKIEVELQEALGS 116
Odo-II     LNKLAKASEILSQANELTAQYEQELNTVRKEAQLEITNSQKIHKEILEIELNISQKYIDNLLENITDD 127
Ant-II     ANSLTAASAALSKANDLTKRYEQDLAESRKKAQDIIKNAQQDAQNIVSSKIKEAQKDADQLMSNTYDQ 129
Gal-II     RENLNKASLYLDQANELTKKYELELITARKEAIKMVTTSQTEAQEFVNAQISQAQKEAQQLIQSSMMQ 125
Och-II     NGNLSDASAKLLQADELCKQYEEQLKDAKADAQSCIADAETEAKQVVALELAQARKDAASLVEQVNKE 129
Por-II     RTTLTTASSMLVKADELAAKYEEDLSKARRDAQAKIAASQKDAQSIVSEDIKKAQMNAEKLITEASKQ 124
Eco-b      ADGLASAERAHKDLDLAKASATDQLKKAKAEAQVIIEQANKRRSQILDEAKAEAEQERTKIVAQAQAE 108
Spi-I      LNTIRNSEELRGKAIEQLEKARARLKKVEMDADQFRVNGYSEIEREKMNLINSTYKTLEQFENYKNET 111
Odo-I      VQGVQDAEGRLEEANRRLSEAQKQLSQAHIVISEIKNETISAKKVLLESDAYQAKKDLTTRFSRALAT 125
Ant-I      IFAISECEERLQQANNRLLESEKQLEQTQLVITQVLNDAEITAQKVRQSILDKGKIDVEKLIGASKAS 130
Gal-I      ITSIRESEERLEKSTIRLNEAKNQLSSAQIIINQIKQEAKNTAANVKESILKQGKTDIERLLLNTKNY 123
Och-I      LGAIQEAEERLEQATERLKESKTQLEQAQLVIASIKEDAETTAKQVKSAILTEGKNEIERLTSAAKSQ 128
Por-I      LAAIQESEERLEQASSRLSESEKQLAQTQIIINQIKKEAQLTAEKVRSSILAQGQIDIERLAITGKSN 131

           <   +   <   <   <   <   <   +<
Spi-II     LEQQKEDTIKSLDSQISALSDDIVKKVLPVS                                147   (24/15%)
Odo-II     LLEKKNTALNSLDTIVQSLCVQIENRLSI                                  156   (30/19%)
Ant-II     LNIQKEQALQNLEKQVDILSNQIQIKLLGN                                 159   (30/19%)
Gal-II     FEKEKNKAIYSLFKQVEQLSEQIKNKLISIYI                               157   (27/17%)
Och-II     LEAQKELALKQLEAQIDELSQLIKEKLLGKQAIL                             163   (37/24%)
Por-II     LNIQKEEALKTLEDQVDTLSDQIKTKLLSSQSLK                             165   (30/19%)
Eco-b      IEAERKRAREELRKQVAILAVAGAEKIIERSVDEAANSDIVDKLVAEL               156
Spi-I      IQFEQQKAINQVRQRVFQQALQGALGTLNSCLNNELHLRTINANIGMFGAMNEITD       167   (22/14%)
Odo-I      FRSKERQIFLEVKEQIILLVLKRTVARAQQTFGPKERATALITETINKLEGDLL         179   (26/17%)
Ant-I      IVVAENQINQQIKQKITALAIQKVSSQLKAQVDTTMQAKIIDSSIIKLRGDI          182   (24/15%)
Gal-I      IYNTELQIKKQIKQQIAALALQKVQSKLKDELDNNIQQKIIDQSLAMLTIRNK         176   (24/15%)
Och-I      IVTIEAQVRKQISDYVVSLALQRVTLQLEGKLSDAAQQQILDRNISKLKD            178   (30/19%)
Por-I      IETAEKQIRRQIQQQIAFLALKKVTLQLENQMSSDIQLRIIDNNIAKLGDQL          183   (29/19%)
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Fig. 1. Alignment of subunits I and II from chloroplasts with subunit b from *E. coli*. First, the subunits I as well as the subunits II were aligned corresponding to their putative span regions with no gaps allowed. Then these two alignments were aligned with subunit b from *E. coli* corresponding to its putative span region. For proper alignment of subunits I with subunits II and b, one gap (indicated by hyphens) has to be introduced into the sequences of subunits II and b. Sequences were taken from the National Center for Biotechnology Information databank. Sequences of subunits I and II from spinach are those of the mature proteins as determined by chemical sequencing (Bird *et al.*, 1985; Berzborn *et al.*, 1990). The N-terminus of the other subunits is not yet determined; therefore, the beginning in the alignment is choosen as if these subunits were processed at homologous positions compared with their corresponding subunits from spinach. Residues in subunits I and II identical with residues in subunit b are highlighted by bold letters. The strictly conserved amino acid arginine is marked by (*), a strictly conserved pattern of hydrophobic residues by (>). A conserved discontinuous structural feature of subunits II is indicated by (+) for positively charged residues and by (<) for nonpolar residues. The number/relative amount (calculated from the number of amino acids of *E. coli* subunit b) of identical amino acids is given in parenthesis at the end of each sequence.

Results of the analysis of protein sequences of b-type subunits

Comparison of primary structures

Only one complete amino acid sequence of a chloroplast subunit II of higher plants has been published (Herrmann *et al.*, 1993); the computer program used indicated 29% sequence identity between subunit II and *E. coli* subunit b, but none (i.e. below 25%) between chloroplast subunit I and subunit b. However, no new conclusions concerning subunit equivalence were drawn. An alignment of the sequences of subunits I and II, including sequences of published b-type subunits from five alga, with e.g. *E. coli* subunit b was used by us to recalculate the number and percentage of identical residues in respective positions, taking 156, the number of residues of *E. coli* subunit b, as 100% (Fig. 1). Despite a smaller number af aligned residues, subunits II from all six species display more identities with subunit b in these binary comparisons than respective subunits I do; furthermore, for proper alignment of subunits I and b, a gap has to be introduced which is not necessary for alignment of subunits II and b. More identities between subunits II and b are also found, if subunits I and II are compared with subunits b from other nonphotosynthetic eubacteria: in 81% of all cases (Fig. 2, right side).

The currently stated relationship between b-type subunits from photosynthetic ATP synthases and subunits b from nonphotosynthetic sources was suggested by sequence comparison of the *atp* operons from cyanobacterium *Synechococcus* 6301 (Sy1) and *E. coli* (Eco) (Cozens and Walker, 1987); indeed, there are more identities between subunit Sy1-b and Eco-b than between Sy1-b' and Eco-b (Fig. 2). But only in 49% of all binary comparisons of subunits b from nonphotosynthetic eubacteria with subunits b versus b' from photosynthetic eubacteria there are more identical residues between subunits b than between subunits b and b' (Fig. 2, left side).

Comparison of isoelectric points

When looking at the total charges of the subunits I and II of spinach chloroplasts a remarkable difference in the calculated isoelectric points (pIs) of these subunits was noticed. A comparison of

	Rru b/b'	Rca b/b'	Sy1 b/b'	Ana b/b'	Sy3 b/b'	Sy6 b/b'	Cpa b/b'	Och I/II	Por I/II	Gal I/II	Ant I/II	Odo I/II	Spi I/II
Eco-b	**32/34**	**26/34**	36/31	**36/38**	40/31	43/33	38/23	**30/37**	**29/30**	**24/27**	**24/30**	**26/30**	**22/24**
Val-b	30/29	**25/27**	36/32	**37/40**	42/35	39/34	*32/32*	**34/37**	**33/36**	**28/31**	**26/36**	**27/34**	**19/24**
Tfe-b	**27/28**	**27/29**	38/34	33/32	38/33	39/31	32/31	**31/32**	**23/34**	**25/31**	**25/35**	**24/29**	**21/27**
Bme-b	32/24	31/26	**32/36**	**39/43**	*38/38*	**38/39**	**32/33**	40/34	**36/37**	**30/31**	**29/33**	**22/29**	**27/33**
Bfi-b	30/28	*27/27*	28/29	*35/35*	36/30	**28/30**	27/26	**27/36**	**28/32**	**28/29**	**25/36**	**22/27**	**27/30**
Bsu-b	**27/29**	**24/28**	44/43	**38/39**	41/40	36/35	31/27	37/34	**30/37**	34/33	**29/35**	**25/32**	**30/31**
Bca-b	25/24	28/24	**26/34**	**36/43**	**31/37**	**30/38**	**27/31**	**30/38**	**27/39**	**26/31**	**26/36**	**24/30**	**27/33**
Sli-b	**27/39**	**22/28**	**29/33**	**31/32**	**26/40**	32/31	33/27	32/28	**27/28**	**24/26**	**23/30**	**23/25**	*22/22*
Ehi-b	**28/29**	36/29	33/32	37/32	**36/40**	*32/32*	**31/32**	34/29	**30/35**	*29/29*	**25/29**	**18/34**	38/26
Mtu-b	**23/28**	27/24	28/25	**18/24**	**25/29**	**26/27**	**22/32**	**20/28**	**17/30**	**15/22**	**14/27**	**14/23**	**19/24**
Mle-b	**22/29**	28/23	**28/29**	**19/24**	**24/30**	27/25	**20/29**	**16/23**	**18/26**	**16/22**	**17/24**	**14/20**	**21/24**
PS3-b	27/23	27/24	**27/35**	**34/42**	**32/38**	**30/38**	29/28	**28/39**	**26/39**	**25/32**	**23/36**	**22/31**	**26/33**
Pmo-b	**29/33**	32/30	30/27	34/32	32/31	37/31	**29/37**	35/31	**30/34**	*31/31*	**29/30**	**26/28**	**25/29**
Hin-b	**27/35**	**26/32**	35/28	36/25	36/28	38/28	29/25	**24/35**	*25/25*	**23/27**	**25/31**	**18/30**	**16/21**
Smu-b	38/23	36/26	36/27	38/34	38/32	41/32	**26/30**	38/33	*33/33*	31/28	32/31	**22/32**	26/20
Spn-b	44/28	37/29	**31/36**	**34/44**	*38/38*	**35/40**	34/27	*36/36*	**33/35**	**31/33**	**31/36**	**21/36**	27/18
Mth-b	43/33	39/32	**39/43**	**38/45**	**42/47**	41/40	**28/38**	**33/41**	**32/39**	**30/36**	**27/37**	**20/32**	33/30

Fig. 2. Comparison matrix showing numbers of identical amino acids between subunits b of nonphotosynthetic eubacteria and subunits b/b' and I/II of photosynthetic eubacteria and chloroplasts, respectively. Sequences were taken from the National Center for Biotechnology Information databank. Better agreement between subunit b of nonphotosynthetic eubacteria and subunits b' and II of photosynthetic eubacteria and chloroplasts, respectively, is emphasized by dark boxes. Equal amounts of identical amino acids are presented in italics.
Abbrevations: Spi (*Spinacia oleracea*), Nic (*Nicotiana tabacum*), Pis (*Pisum sativum*), Ory (*Oryza sativa*), Tri (*Triticum aestivum*), Zea (*Zea mays*), Mar (*Marchantia polymorpha*), Eug (*Euglena gracilis*), Chl (*Chlorella vulgaris*), Odo (*Odontella sinensis*), Ant (*Antithamnion* sp.), Gal (*Galdieria sulphuraria*), Por (*Porphyra purpurea*), Och (*Ochrosphaera neapolitana*), Cpa (*Cyanophora paradoxa*), Sy6 (*Synechococcus* sp. PCC 6716), Ana (*Anabaena* sp. PCC 7120), Sy1 (*Synechococcus* sp. PCC 6301), Sy3 (*Synechocystis* sp. PCC 6803), Rru (*Rhodospirillum rubrum*), Rca (*Rhodobacter capsulatus*). In cases of nonphotosynthetic eubacteria, the abbrevation represents the first letter of the genus and the initial two letters of the species (cp. Fig. 3).

the calculated pIs of 51 b-type subunit sequences (Fig. 3) reveals that the pIs of all chloroplast subunits II and all subunits b' of photosynthetic eubacteria (including the cyanelle from *C. paradoxa*) are in the acidic pH range as are the pIs of 16 out of 17 subunits b of nonphotosynthetic eubacteria. In cyanobacteria, also the pIs of subunits b are in the acidic range; but the pIs of 12 out of 14 chloroplast subunits I are in the alkaline pH range as are the pIs of the subunits b from the two purple bacteria and the cyanelle from *C. paradoxa.*

Analysis of conserved structure elements in aligned b-type subunits

In the alignment of 51 b-type subunits of H^+-translocating ATP synthases, a discontinuous structural feature was discovered extending from Leu_{117} to Val_{143} of spinach subunit II (Fig. 4): seven nonpolar and two positively charged residues are found in a distinct pattern (4–4–3–4–3–4–3/4). Since for this region an α-helix is predicted as the secondary structure (data not shown), the seven nonpolar and the two positively charged residues would form one helix face. As emphasized in Fig. 4, this discontinuous structural feature is conserved among sequences of subunits II and b' of chloroplasts and photosynthetic eubacteria, respectively; the recognized feature is also present in the sequences of subunits b of nonphotosynthetic eubacteria (in seven cases it is less pronounced). But it cannot be found in the aligned sequences of the corresponding subunits I and b of photosynthetic organisms. In particular, the first positively charged residue is found only four times

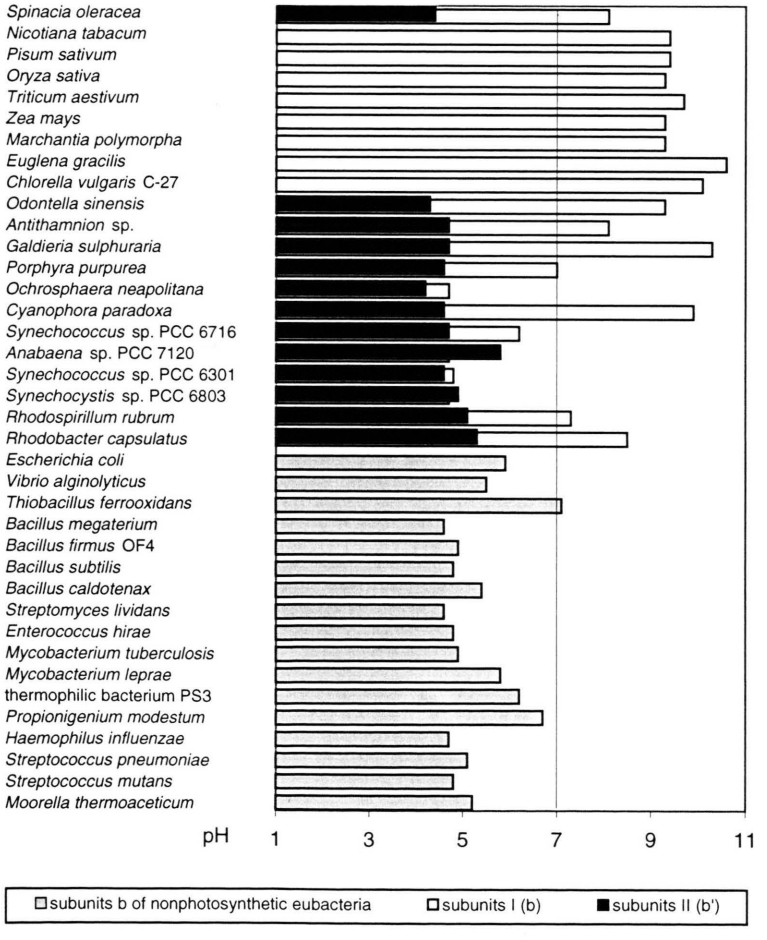

Fig. 3. Isoelectric points of subunits I and II from chloroplasts and subunits b and b' from eubacteria calculated from the amino acid composition. Sequences were taken from the National Center for Biotechnology Information databank. In cases of chloroplast subunits, only those amino acids were taken into account that are included in the mature protein; subunits from which the N-terminus of the mature protein is unknown were treated as if they were processed at homologous positions compared with their corresponding subunits from spinach (cp. Fig. 1).

		< + < < < < < +<
Spinacia oleracea	-II	...**L**EQQ**K**EDT**I**K**S**L**D**SQ**I**SA**L**SDD**I**VK**KV**...
Odontella sinensis	-II	...**L**LEK**K**NTA**L**N**S**L**D**TI**V**QS**L**CVQ**I**EN**RL**...
Antithamnion sp.	-II	...**L**NIQ**K**EQA**L**QN**L**EKQ**V**DI**L**SNQ**I**QI**KL**...
Galdieria sulphuraria	-II	...**F**EKE**K**NKA**I**YS**L**EKQ**V**EQ**L**SEQ**I**KN**KL**...
Porphyra purpurea	-II	...**L**NIQ**K**EEA**L**KT**L**EDQ**V**DI**L**SDQ**I**KT**KL**...
Ochrosphaera neapolitana	-II	...**L**EAQ**K**ELA**L**KQ**L**EAQ**I**DE**L**SQ**L**IKE**KL**...
Cyanophora paradoxa	-b'	...**L**ELQ**K**DEA**L**K**S**L**E**SE**V**QT**L**ST**K**IE**KL**...
Synechococcus sp. PCC 6716	-b'	...**I**DQQ**K**QAT**L**QA**L**EGQ**V**SS**L**SEQ**L**LA**KL**...
Anabaena sp. PCC 7120	-b'	...**I**EQQ**K**QQA**L**AS**L**EQQ**V**DA**L**SRQ**I**LE**KL**...
Synechococcus sp. PCC 6301	-b'	...**I**DQQ**K**AVA**L**QA**L**DQQ**V**DA**L**SHQ**I**LD**KL**...
Synechocystis sp. PCC 6803	-b'	...**I**EAQ**R**QSA**L**SS**L**EQE**V**AA**L**SNQ**I**LH**KL**...
Rhodospirillum rubrum	-b'	...**I**VQA**R**DEA**L**TH**V**REV**A**GA**V**ASD**I**VG**KL**...
Rhodobacter capsulatus	-b'	...**I**AEI**R**AGA**L**EA**V**QIV**A**TDTATA**I**VTA**L**...
Escherichia coli	-b	...**I**EAE**R**KRAREE**L**RKQ**V**AI**L**AVAGAE**KI**...
Vibrio alginolyticus	-b	...**L**EAE**R**NRARDD**L**RKQ**V**AT**L**AVAGAE**KI**...
Thiobacillus ferrooxidans	-b	...**I**DVETNRAREV**L**RGQ**V**VE**L**VVNGTQ**RI**...
Bacillus megaterium	-b	...**I**EQQ**K**DQA**V**AA**L**REQ**V**AS**L**SVL**I**AS**KV**...
Bacillus firmus OF4	-b	...**I**HRE**K**EQA**V**SA**L**REQ**V**AG**L**SVL**I**AT**KV**...
Bacillus subtilis	-b	...**I**VKE**K**EQA**V**SA**L**REQ**V**AS**L**SVM**I**AS**KV**...
Bacillus caldotenax	-b	...**I**ERE**K**EQA**M**AA**L**REQ**V**AS**L**SVV**I**AS**KV**...
Streptomyces lividans	-b	...**I**QAD**R**KAA**A**SA**L**RQD**V**GK**L**ATE**L**AG**KL**...
Enterococcus hirae	-b	...**I**MLE**R**DTA**L**NS**V**KDD**V**AD**L**SLQ**I**AA**KI**...
Mycobacterium tuberculosis	-b	...**L**KRE**R**DAVELD**L**RAH**V**GT**M**SAT**L**AS**RI**...
Mycobacterium leprae	-b	...**I**KRE**R**DAVELD**L**RAK**A**GA**M**SLI**L**AS**RI**...
thermophilic bacterium PS3	-b	...**I**ERE**K**EQA**M**AA**L**REQ**V**AS**L**SVL**I**AS**KV**...
Propionigenium modestum	-b	...**I**EKM**K**EQARKE**L**QLE**V**TD**L**AVK**L**AE**KM**...
Haemophilus influenzae	-b	...**V**EAE**R**KRVQEE**L**RLK**V**AS**L**AVAGAE**KI**...
Streptococcus mutans	-b	...**I**ATS**K**AEA**L**SS**V**KAD**V**AD**L**SVL**L**AE**KI**...
Streptococcus pneumoniae	-b	...**I**AQN**K**AEA**L**QS**V**KGE**V**AD**L**TVS**L**AG**KI**...
Moorella thermoacetica	-b	...**I**EGE**K**SKA**L**AA**I**RSE**A**AS**L**AIL**A**AG**KV**...
Spinacia oleracea	-I	...**I**QFEQQKA**I**NQ**V**RQR**V**FQQALQGALGT...
Nicotiana tabacum	-I	...**I**QFEQQRA**I**NQ**V**RQR**V**FQQALRGALGT...
Pisum sativum	-I	...**I**HFEQQRA**I**NQ**V**QQS**V**LQQALQGALGT...
Oryza sativa	-I	...**L**YFE**K**QRA**M**NQ**V**RQR**V**FQQAVQGALGT...
Triticum aestivum	-I	...**L**YFE**K**QRA**M**NQ**V**RQR**V**FQQAVQGALGT...
Zea mays	-I	...**L**YFE**K**QRA**M**NQ**V**RQQGFQQAVQGALGT...
Marchantia polymorpha	-I	...**I**RFE**K**QRA**I**EQ**V**RQQ**V**SR**L**ALERALET...
Euglena gracilis	-I	...**L**RTEDKKS**V**RE**I**FKN**L**YSQACQKAKAT...
Chlorella vulgaris C-27	-I	...**L**KFEQQKAQNE**L**AEK**L**VK**L**ALQQVREK...
Odontella sinensis	-I	...**F**RSKERQI**F**LE**V**KEQ**I**IL**L**VLKRTVAR...
Antithamnion sp.	-I	...**I**VVAENQINQQ**I**KQK**I**TA**L**AIQKVSSQ...
Galdieria sulphuraria	-I	...**I**YNTELQIKKQ**I**KQQ**I**AA**L**ALQKVQSK...
Porphyra purpurea	-I	...**I**ETAEKQIRRQ**I**QQQ**I**AF**L**ALKKVTLQ...
Ochrosphaera neapolitana	-I	...**I**VTIEAQVRKQ**I**SDY**V**VS**L**ALQRVTLQ...
Cyanophora paradoxa	-b	...**I**NTQQLSV**I**TY**L**RQQ**V**ALLALRRVVSQ...
Synechococcus sp. PCC 6716	-b	...TSAATERA**I**AE**I**RER**I**TA**L**ALAQAEQQ...
Anabaena sp. PCC 7120	-b	...**I**NAELDRA**I**AQ**L**RQR**V**VA**L**ALQKVESE...
Synechococcus sp. PCC 6301	-b	...**V**STEQQRV**L**DE**L**RRY**A**VAQALSRVETQ...
Synechocystis sp. PCC 6803	-b	...**L**GAEQERV**I**AE**L**KRR**I**AEQAVAKAEAD...
Rhodospirillum rubrum	-b	...**I**AQAEAQA**L**AQ**V**RNE**A**VD**V**AVS**A**ARS**L**...
Rhodobacter capsulatus	-b	...**I**ASAEAAA**L**KD**V**KDR**A**VQ**V**AVA**A**AAE**V**...

Fig. 4. Alignment of amino acid sequences of ATP synthase subunits I, II, b and b'. Sequences were taken from the National Center for Biotechnology Information databank. Shown are the regions of the conserved discontinuous structural feature of subunits II and corresponding regions of subunits I, b and b' (cp. Fig. 1). The feature is indicated by (+) for positively charged residues and (<) for nonpolar residues. Residues which match the feature are emphasized by bold letters.

and the second is always missing, and the two last nonpolar residues – except for subunits b of purple bacteria – are also missing.

Conclusion and Discussion

Until now subunit I (subunit b of photosynthetic eubacteria) is reviewed to be equivalent to subunit b of nonphotosynthetic eubacteria, whereas subunit II (subunit b' of photosynthetic eubacteria) is suggested to be an additional subunit having no counterpart in e.g. *E. coli* (Herrmann *et al.*, 1993; van Walraven and Bakels, 1996). This view originated from some sequence homology between subunit I and *E. coli* subunit b, similarities in predicted secondary structures and hydropathy profiles (Bird *et al.*, 1985) and was justified as long as no sequence of chloroplast subunit II was available. It was supported by the conclusion that chloroplast subunit I can rescue an *E. coli* subunit b deletion mutant (Schmidt *et al.*, 1990), and the claim that subunit II is not able to do this (Schmidt, 1992). However, in the first case, the conclusion has been withdrawn by the authors (Schmidt *et al.*, 1994), and in the latter case, the claim was not supported by the results, since no expression of subunit II in the deletion mutant had been shown at all.

With the increasing number of published amino acid sequences of chloroplast subunits II it became obvious that the characteristics which argue for an equivalence of chloroplast subunit I and subunit b from *E. coli* are also true of subunit II. Moreover, the comparision of primary structures shows more similarities between subunit II from spinach and subunit b from *E. coli* than between spinach subunit I and the latter (Herrmann *et al.*, 1993). Inclusion of additional 40 amino acid sequences of b-type subunits into the binary comparison reveals that subunits b from nonphotosynthetic eubacteria (i) only in about half of the cases have more sequence identities with subunits b from photosynthetic eubacteria than they have with subunits b' (Fig. 2, left side), and (ii) in more than 80% of all cases have more sequence identities with subunits II from chloroplasts than they have with subunits I (Fig. 2, right side). In most cases, the differences in the number of sequence identities are low and may not be significant. Therefore, additional criteria are needed to clearify the relationship of subunits I (b) and II (b') from photosynthetic organisms with subunit b from nonphotosynthetic eubacteria.

The calculated isoelectric points (pIs) reflect different overall amino acid composition of the b-type subunits. The pIs of all chloroplast subunits II and of all subunits b' of photosynthetic eubacteria are calculated to be in the acidic range as are the pIs of most subunits b of nonphotosynthetic eubacteria (Fig. 3). However, most chloroplast subunits I and subunits b from both purple bacteria and the cyanelle *C. paradoxa* possess an alkaline pI. From this criterion, chloroplast subunit II (subunit b' of photosynthetic eubacteria) rather than chloroplast subunit I (subunit b of photosynthetic eubacteria) is suggested to be the equivalent of subunit b of nonphotosynthetic eubacteria.

Searching for conserved structural features led to the discovery of a discontinuous pattern of residues that, resembling a heptade, would reflect an amphipathic helix of seven windings including two positive charges (Fig. 4). This feature is characteristic for subunits II and subunits b' of photosynthetic eubacteria and is also found to a high degree in subunits b of nonphotosynthetic eubacteria, but subunits I and subunits b of photosynthetic eubacteria do not show it. Since it is conserved in the former cases, it seems to be important, but it may not be essential, because a loss of the feature by truncation of the gene encoding subunit b' in *Synechocystis* 6803 was found not to be lethal for photoautotrophic growth on Bg11 medium (Lill *et al.*, 1994); the truncation led to instability of the ATP synthase upon illumination of isolated thylakoids, however (H. S. van Walraven, 1996, Botanical Congress Düsseldorf). This feature, together with the other findings, strongly argues that chloroplast subunit II (subunit b' of photosynthetic eubacteria) and not chloroplast subunit I (subunit b of photosynthetic eubacteria) is the equivalent of subunit b of nonphotosynthetic eubacteria.

Our assignment of b-type subunits implies that subunits II (b') and I (b) are not equivalent subunits which have merely diverged in a different way. If they were equivalent, one would expect that the deletion of one of them should be compensated by the other. But subunit b' cannot be replaced by the homologous subunit b even of the same species, since deletion of the gene encoding subunit b' in cyanobacterium *Synechocystis* 6803 is

lethal for photoautotrophic growth (Lill *et al.*, 1994). This result also agrees with our conclusion that subunit b' and not subunit b of cyanobacteria is equivalent to the essential subunit b of nonphotosynthetic eubacteria, e.g. *E. coli;* a putative heterodimer b',b cannot be replaced by a putative homodimer b,b. The deletion of the gene coding for subunit b of any cyanobacterium has not been decribed.

From studies of *in situ* topography, it was concluded that chloroplast subunits I and II together could perform the same function(s) as the two copies of subunit b in *E. coli* (Otto and Berzborn, 1989; Berzborn *et al.*, 1990). This functional relationship of chloroplast subunits I and II with the two identical subunits b of nonphotosynthetic eubacteria is supported by hybrid reconstitution experiments: the combination of spinach CF_o and F_1 from thermophilic bacterium PS3 reconstitutes ATP-driven proton translocation (Galmiche *et al.*, 1994). Previously, it has been suggested that *E. coli* subunit b contributes to the formation of the central stalk (Walker *et al.*, 1982; Cox *et al.*, 1984) connecting subcomplexes F_o and F_1. This has been supported by the result that an isolated, N-terminal truncated and soluble form of *E. coli* subunit b forming a dimer directly binds to an isolated *E. coli* F_1 subcomplex (Dunn, 1992). However, it has been demonstrated by cryoelectron microscopy that the binding does not occur to the central cavity, but to a peripheral region of the F_1 subcomplex, therefore leaving room for the central α-helical domain of the γ subunit to rotate relative to the α,β-hexamer (Wilkens *et al.*, 1994; Abrahams *et al.*, 1994). Recently, it was speculated that, in addition to the central stalk consisting of F_1 subunits γ and ε, a second stalk may exist, localized at the F_1 periphery and functioning as a stator, and that this stalk would be formed by subunits b and by F_1 subunit δ (Wilkens *et al.*, 1997). This working model is partially based on the identification of a zero-length crosslinking product of chloroplast subunits I and δ (Beckers *et al.*, 1992). However, the structural role of a stator does not exclude an additional functional role of b-type subunits in coupling of ATP synthesis to proton translocation.

In summary, the precise function(s) of b-type subunits still remains unknown, in particular, which part each individual subunit contributes to the collective function. Even for the two copies of *E. coli* subunit b, it has been suggested that they – although identical in primary structure – differ in that they bind to different F_1 subunits: one to subunit δ and the other to subunit ε (Cox *et al.*, 1984). In *E. coli* as in other nonphotosynthetic eubacteria, partial functions cannot be experimentally associated with individual subunits b, since the two identical copies encoded by the same gene are indistinguishable by means of biochemical, immunochemical and genetic methods. In photosynthetic ATP synthases, however, it is possible to distinguish between the two nonidentical b-type subunits and to investigate them independently from each other. For example, it can be analyzed whether residue arginine (position 44 of spinach subunit II, Fig. 1), is needed twice as suggested by its strict conservation in all 51 b-type subunits from chloroplasts and eubacteria.

Since two nonidentical b-type subunits occur in chloroplasts and photosynthetic eubacteria only, it is not unreasonable to assume that one of them may have gained additional functions in connection with photophosphorylation, for instance, with regulation of photosynthetic ATP synthases (Berzborn *et al.*, 1990; Lill *et al.*, 1994). This b-type subunit may be regarded as an 'additional' subunit when compared with *E. coli* subunit b. According to our new assignment, subunit I (b of photosynthetic eubacteria) would be the additional subunit, whereas subunbit II (b' of photosynthetic eubacteria) is the equivalent to subunit b of nonphotosynthetic eubacteria, e.g. *E. coli.*

The designation of ATP synthase b-type subunits of photosynthetic eubacteria as b and b' hitherto used is misleading. To correct, but not to confuse the nomenclature, we propose to change the name of ATP synthase subunit b from photosynthetic eubacteria to subunit I and the name of subunit b' to subunit II, irrespective of gene designation.

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