Specific Heterodimer Formation by the Cytoplasmic Domains of the *b* and *b′* Subunits of Cyanobacterial ATP Synthase†

Stanley D. Dunn,*,‡ Eva Kellner,§ and Holger Lill§,||

Department of Biochemistry, University of Western Ontario, London, Ontario, Canada N6A 5C1, and Abteilung Biophysik, Universität Osnabrück, D-49069, Osnabrück, Germany

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ABSTRACT: The soluble domains of the *b* and *b′* subunits of the ATP synthase of the cyanobacterium *Synechocystis* PCC 6803 were expressed with His tags attached to their N-termini. Following purification, the polypeptides were characterized by chemical cross-linking, analytical ultracentrifugation, and circular dichroism spectroscopy. Treatment of a mixture of the soluble *b* and *b′* domains with a chemical cross-linking agent led to substantial formation of cross-linked dimers, whereas similar treatment of either domain by itself resulted in only trace formation of cross-linked species. The molecular weights of the domains of *b* and *b′* in solution at 20 °C, measured by sedimentation equilibrium, were 17 800 ± 700 and 16 300 ± 400, respectively, compared to calculated polypeptide molecular weights of 16 635 and 15 422, whereas a mixture of *b* and *b′* gave a molecular weight of 29 800 ± 800. The sedimentation coefficient of an equimolar mixture was 1.73 ± 0.03. The circular dichroism spectra of the individual polypeptides indicated helical contents in the range of 40−50%; the spectrum of the mixture revealed changes indicative of coiled-coil formation and a helical content of 60%. The results indicate that the cytosolic domains of the *b* and *b′* subunits exist individually as monomers but form a highly extended heterodimer when they are mixed together.

Oxidative phosphorylation and photophosphorylation are catalyzed by similar members of the ATP synthase family. These membrane-bound enzymes consist of a peripheral F_1 sector that catalyzes phosphoanhydride bond formation and an integral F_0 sector that conducts protons across the membrane. These sectors are linked by two stalks which are required for coupling the endergonic synthesis of ATP with the exergonic movement of H^+ ions down their electrochemical gradient as they cross the membrane. One of the stalks occupies a central position and has been shown to rotate during the hydrolysis of ATP by F_1, while the other stalk is located at the periphery of the complex and extends from the membrane to the top of F_1. This peripheral or second stalk is believed to maintain static interactions between F_1 and F_0 while the central stalk rotates during catalysis. A number of reviews of F_1 structure and mechanism have appeared recently (*1*−*4*).

All ATP synthases contain multiple subunits in both the F_1 and F_0 sectors. The prototypical bacterial enzymes, exemplified by that from *Escherichia coli*, consist of eight types of subunits, five being present in F_1 in a stoichiometry of $\alpha_3\beta_3\gamma\delta\epsilon$ and three combining in a stoichiometry of ab_2c_{10-12} to form F_0. The peripheral stalk of the complex is composed of the two *b* subunits of F_0 and the δ subunit of F_1 (*5*). Though the sequence of *b* is poorly conserved in evolution, all *b* sequences contain hydrophobic, membrane-spanning domains near their N-termini, while the balance of the polypeptides are predominantly polar and are expected to extend outward from the membrane to interact with δ and other parts of F_1 (*6*, *7*). Typically, *b* sequences are between 130 and 180 amino acid residues in length.

The peripheral stalk clearly functions in maintaining the relationship between F_1 and F_0 (*5*), and it is possible that the structure may store energy temporarily during the catalytic cycle by undergoing elastic deformation. Junge and colleagues (*8*) have suggested that both the $b_2\delta$ and the $\gamma\epsilon$ stalks undergo stepwise elastic deformation as protons pass through F_0 and that the stored energy is expended in one step to drive the conformational change causing release of ATP from the catalytic site. Alternatively, Oster and Wang (*9*) have stressed the theoretical importance of *b* as a flexible, elastic link coupling the two sectors, so that torque may be delivered more smoothly between them, increasing the efficiency.

How the structure of *b* may provide the elasticity necessary to accommodate either of these functions is a major unresolved question. To date, the *b* subunit of *E. coli* has been examined in greatest detail. The isolated membrane region composed of residues 1−34 has been studied by nuclear magnetic resonance spectroscopy, revealing a helical structure with a bend at residues 23−26 just outside the membrane (*10*). The isolated soluble domain composed of residues 25−156 forms a highly extended dimer in solution and is capable of binding to F_1 (*11*). A number of studies

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* To whom correspondence should be addressed: telephone, 519-661-3055; fax, 519-661-3175; e-mail, sdunn@julian.uwo.ca.

‡ Department of Biochemistry, University of Western Ontario.

§ Abteilung Biophysik, Universität Osnabrück.

|| Present address: Department of Structural Biology, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands.

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indicate that much of the dimer exists as a pair of parallel helices, perhaps in a coiled-coil arrangement (*5*, *12*−*14*). However, no atomic structure of the isolated cytoplasmic domain of any *b* subunit has been reported. Furthermore, the recently published c_{10}−F_1 complex of yeast (*15*) lacked both the entire peripheral stalk and the *a* subunit. These portions of the enzyme thus remain the least understood from a structural standpoint.

In addition, the subunit compositions of the F_0 sector and the peripheral stalk exhibit substantial variations between species. The DNA sequences of gene clusters encoding the subunits of the ATP synthase complexes of cyanobacteria (*16*, *17*) and other photosynthetic eubacteria (*18*, *19*) reveal open reading frames, designated *uncF* and *uncX*, encoding two *b*-type subunits, called *b* and *b*′, respectively. The b_2 homodimer of *E. coli* may be replaced by a *bb*′ heterodimer in such prokaryotes, but it has also been suggested that the *b*′ may be supplemental to the b_2 dimer (*20*). While the ATP synthase complexes of a number of photosynthetic bacteria have been purified to various degrees (*20*−*24*), in no case has subunit stoichiometry been quantitatively measured. The chloroplast ATP synthase also contains two *b*-type subunits, called subunits I and II (*25*−*27*). The second stalks of mitochondrial enzymes are more complex, containing at least four subunits (*28*, *29*).

In the work presented here, we have analyzed the solution behavior of the expressed and purified soluble domains of *b* and *b*′ from *Synechocystis*. The quaternary state of these polypeptides, either alone or present as a mixture, has been examined by chemical cross-linking and sedimentation equilibrium ultracentrifugation, while their conformational state has been examined by sedimentation velocity ultracentrifugation and circular dichroism spectroscopy.

EXPERIMENTAL PROCEDURES

Plasmid Construction. DNA fragments corresponding to residues 49−179 of subunit *b* and residues 30−143 of subunit *b*′ were amplified by the polymerase chain reaction from genomic DNA of the cyanobacterium *Synechocystis* sp. PCC 6803 (*17*). The employed primers contained, in addition to parts of the coding sequences, short overhangs comprising recognition sequences for restriction nucleases *Nde*I and *Bam*HI in the case of the *b*′ primers or *Nco*I and *Bam*HI in the case of the *b* primers. The restricted polymerase chain reaction product encoding the *b*′ domain was cloned into the expression vector pET16b (Novagen, Madison, WI), yielding plasmid pETb'_{sol}, which expresses the proposed soluble region of *b*′ with an N-terminal His tag. The product encoding the proposed soluble region of *b* was cloned into pET-3d. The resulting plasmid was digested with *Nco*I and a pair of complementary oligonucleotides was ligated in, resulting in an encoded tag region identical to that of pET-16b, except for the presence of six, rather than ten, consecutive histidine residues. The final plasmid expressing the soluble domain of *b* with an N-terminal His tag was called pETb_{sol}.

Protein Expression and Purification. Strains BL21 (DE3) pLysS/pETb_{sol} and BL21 (DE3) pLysS/pETb'_{sol} were grown in LB medium at 37 °C. At a density that gave an A_{600} of 0.6, isopropyl β-thiogalactoside was added to a final concentration of 1 mM. After incubation overnight at 30 °C, cells were harvested and washed. The cells were suspended in 10 mL of buffer containing 50 mM Tris-HCl, pH 8.0, and 300 mM NaCl, then benzonase (Boehringer) was added to an activity of 100 units/mL, and the cells were incubated for 30 min at 4 °C. Cells were sonicated at 4 °C for three periods of 1 min, separated by intervals of 1 min. Purification was monitored by SDS−PAGE.[1] The cell lysate was cleared by centrifugation at 100000*g* for 30 min, and the protein was purified by fast protein liquid chromatography using an Äkta Explorer (Amersham Pharmacia). The supernatant was diluted with 50 mM Tris-HCl, pH 8.0, and 300 mM NaCl to about 5 mg of protein/mL and loaded onto a 2 mL column of Ni−NTA Superflow (Qiagen). After being washed with more of the same buffer, the column was washed more stringently with buffer containing 50 mM MES−NaOH, pH 6.0, 300 mM NaCl, and 10% (w/v) glycerol. The protein was eluted with a gradient of 0−1 M imidazole in the same buffer. The subunits were further purified by anion-exchange chromatography in 20 mM Tris-HCl, pH 8.0, on a MonoQ column, using a gradient of 0−250 mM NaCl to effect elution. The resulting polypeptides, which were essentially pure as judged by SDS−PAGE, were stored at −80 °C. Final yields of both polypeptides were 10−15 mg for each liter of L-broth culture (2−2.5 g of wet cells).

Quantitation of Protein. Protein concentrations were routinely measured by the Bradford (*30*) technique using BSA as a standard. Quantitative amino acid analysis was carried out on triplicate samples at the Alberta Peptide Institute, University of Alberta, Edmonton, AB, Canada. On the basis of this determination, the values obtained by the Bradford method were corrected by factors of 0.71 and 0.79 for b_{49-179} and b'_{30-143}, respectively.

Analytical Ultracentrifugation. Analytical ultracentrifugation of the *b* domains in 50 mM sodium phosphate, pH 7.0, was carried out in a Beckman XL-A instrument with a four-hole An-60Ti rotor. Sedimentation equilibrium runs utilized six-sector cells with Epon charcoal centerpieces. Absorbance measurements at 240 nm were taken in 0.002 cm radial steps and averaged over ten observations. Equilibrium was ascertained by comparing scans taken at 2 h intervals. Software supplied by Beckman was used for data processing and curve fitting. The partial specific volumes of b_{49-179} and b'_{30-143} were calculated using the method of Cohn and Edsall (*31*) to be 0.725 and 0.720, respectively.

Sedimentation velocity analyses were carried out in double-sector cells at a rotor speed of 60 000 rpm. Scans were taken at 240 nm at 10 min intervals. Sedimentation coefficients were calculated by the Svedberg program (*32*) using the modified Fujita−MacCosham model fitted for a single species and corrected to standard conditions in the usual manner.

Circular Dichroism Spectroscopy. CD spectra were collected using a Jasco J-810 spectropolarimeter with a jacketed cell of 1 mm path length. Proteins were dialyzed into 10 mM sodium phosphate buffer, pH 7.0, and diluted with the same buffer to obtain the desired concentrations. Samples

[1] Abbreviations: b_{49-179} and b'_{30-143}, the expressed polar domains of the *b* and *b*′ subunits of *Synechocystis* sp. PCC 6803 containing residues 49−179 and 30−143 of the sequences of the respective natural proteins expressed with N-terminal His-tag leaders; BS^3, bis(sulfosuccinimidyl)suberate; CD, circular dichroism; SDS−PAGE, polyacrylamide gel electrophoresis in the presence of 0.1% sodium dodecyl sulfate.

Table 1: Solution Molecular Weights Determined by Sedimentation Equilibrium Analytical Ultracentrifugation[a]

	av mol wt		
polypeptide(s)	5 °C	20 °C	40 °C
b_{49-179}	18 900 (18 400−19 500)	17 800 (17 100−18 500)	14 900 (14 200−15 600)
b'_{30-143}	16 700 (16 200−17 200)	16 300 (15 900−16 700)	15 200 (14 700−15 600)
$b_{49-179} + b'_{30-143}$	29 400 (28 500−30 300)	29 800 (29 000−30 600)	16 400 (15 500−17 300)

[a] Sedimentation equilibrium analytical ultracentrifugation was carried out as described under Experimental Procedures. The rotor speed was 30 000 rpm. Initial protein concentrations were 1.06 mg/mL for b_{49-179} alone and 0.87 mg/mL for b'_{30-143} alone, while the mixture contained 0.53 mg/mL b_{49-179} and 0.44 mg/mL b'_{30-143}. Samples were equilibrated consecutively at 5, 20, and 40 °C. Cells were scanned at 240 nm. For each determination, three data sets were simultaneously fitted to a single component model. Numbers in parentheses indicate the 95% confidence interval.

were maintained at 20 °C during equilibration and collection of spectra. The helical content was calculated by the method of Gans et al. (*33*).

Other Methods. Chemical cross-linking of proteins with bis(sulfosuccinimidyl)suberate (BS^3) was carried out as described previously (*14*) except that the buffer was 50 mM sodium phosphate, pH 7.0. SDS−PAGE analyses of proteins utilized the buffer system of Laemmli (*34*) on 15% polyacrylamide gels. Proteins were stained with Coomassie Brilliant Blue R-250.

RESULTS

Expression and Purification of the Polar Domains of b and b′. DNA sequences encoding the polar domains of *b* and *b′* were cloned into expression vectors so as to express forms of the polypeptides with N-terminal His tags. These constructs were denoted b_{49-179} and b'_{30-143}, where the subscripts indicate that the *b* and *b′* polypeptides include residues Leu-49 through Arg-179 and residues Met-30 through Lys-143, respectively, of the native subunit sequences. In each case the expressed region begins a few residues past the membrane-spanning domain and extends to the C-terminus. Assuming removal of the N-terminal methionine residues, the inferred molecular weights of b_{49-179} and b'_{30-143} are 16 635 and 15 422, respectively.

The expressed polar domains were purified by metal chelate affinity chromatography and ion-exchange chromatography as described under Experimental Procedures, monitoring purification by SDS−PAGE. These polypeptides were essentially pure, though traces of slightly smaller proteins, most likely proteolytic fragments, remained (see control lanes of Figure 1). Quantitative amino acid analysis was carried out to determine protein concentrations; the residue compositions showed excellent agreement with those expected (data not shown).

Chemical Cross-Linking of b Domains. In initial experiments, the quaternary structure of the *b* domains in solution was explored by chemical cross-linking. This method has been useful in analysis of the soluble domain of the *b* subunit of the *E. coli* enzyme and has the advantage that it permits the analysis of heterodimer formation between two species, each of which is capable of forming homodimers (*14*). Figure 1 presents results of a typical experiment, in which the *Synechocystis b* and *b′* domains were treated with 0.5 mM bis(sulfosuccinimidyl)suberate (BS^3), a homobifunctional reagent specific for amino groups. When the domains were treated individually, only traces of species migrating on SDS−PAGE with apparent molecular weights appropriate for dimers were observed. In contrast, treatment of a mixture of the polypeptides resulted in strong formation of a product

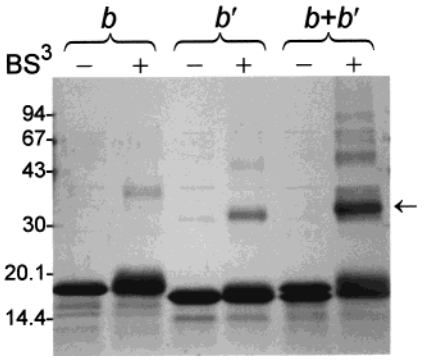

FIGURE 1: Chemical cross-linking of the soluble domains of *b*, *b′*, or a mixture was carried out in 50 mM sodium phosphate buffer, pH 7.0, using 0.5 mM BS^3 at 22 °C. The total protein concentration was 0.5 mg/mL; for the mixture, each polypeptide was present at 0.25 mg/mL. After 20 min, ethanolamine−HCl, pH 7.5, was added to a final concentration of 100 mM and allowed to react with residual BS^3 for 10 min. Samples were treated with SDS sample buffer at 100 °C and then products of the reaction were analyzed by SDS−PAGE on a 15% polyacrylamide gel and stained with Coomassie Brilliant Blue R-250. Each lane received 1.5 μg of protein. The arrow indicates the position of the cross-linked product of the *b* and *b′* domains.

that migrated at 32 000 (see arrow) and trace formation of higher order species. This experiment clearly demonstrates the preferential formation of heterodimers; determining the quaternary state of the individual domains and the extent of heterodimer formation in solution required application of an orthogonal method.

Analysis of b_{49-179} and b'_{30-143} by Sedimentation Equilibrium Analytical Ultracentrifugation. Sedimentation equilibrium in the analytical ultracentrifuge allows determination of the molecular weight of molecules in solution independent of their shapes and has been a technique of prime importance in studies of the *b* subunit of *E. coli* (*11*, *12*, *14*, *35*). This method was therefore used to ask if the individual polar domains of the *Synechocystis b* and *b′* were monomeric or dimeric and if evidence of interaction could be obtained when the two were mixed together.

As seen in Table 1, at 5 or 20 °C the individual domains gave molecular weights just slightly above the polypeptide molecular weights inferred from the DNA sequences (16 635 for b_{49-179} and 15 422 for b'_{30-143}), while at 40 °C the molecular weight of the *b* domain was slightly lower than monomer and that of the *b′* domain was very close to monomer. These results indicate that at the observed concentrations these polypeptides are largely monomeric, though a slight tendency to self-associate may be indicated at the lower temperatures, particularly for b_{49-179}.

A number of sedimentation equilibrium experiments were carried out with mixtures of the two subunits. The data

Table 2: Hydrodynamic Analysis of Cytoplasmic Domains of *Synechocystis b* and *b′* [a]

polypeptide(s)	M_r [b]	$s_{20,w}$ (S)	f/f_{min}
Synechocystis b_{49-179}	16 635	1.34 ± 0.01	1.75
Synechocystis b'_{30-143}	15 422	1.23 ± 0.01	1.83
Synechocystis $b_{49-179} + b'_{30-143}$	32 057[c]	1.72 ± 0.05	2.10[c]
E. coli $(b_{24-156})_2$	31 018	1.74 ± 0.01[d]	1.93

[a] Sedimentation velocity analytical ultracentrifugation was carried out at 60 000 rpm at a temperature of 20 °C, and results were analyzed as described under Experimental Procedures. Protein concentrations were 0.98 mg/mL for b_{49-179} alone and 0.80 mg/mL for b'_{30-143} alone, while the mixture contained 0.49 mg/mL b_{49-179} and 0.40 mg/mL b'_{30-143}. Values presented for $s_{20,w}$ represent the mean and standard error of three determinations. [b] Polypeptide molecular weight calculated from the sequence. [c] The calculated mass of the heterodimer and the measured sedimentation coefficient were used to calculate the frictional ratio of the complex without attempting to correct for unassociated subunits in the mixture. [d] Value reported previously (*14*).

presented in Table 1 illustrate the major results. When the subunits were present at near equimolar concentrations, the average molecular weight observed at 5 or 20 °C approached the sum of the polypeptide molecular weights, 31 018, indicating the interaction of the two species. When the temperature was raised to 40 °C, monomers were observed. After collection of the latter data, the samples were reequilibrated at 5 °C and the molecular weight rose to greater than 25 000 (data not shown), indicating that degradation of the polypeptides was not responsible for the lower molecular weights observed at 40 °C. This experiment showed that the complex of the polar domains of *b* and *b′* was easily melted at warm temperatures, as observed with the *E. coli b* subunit (*14*).

The results described above were most consistent with formation of a heterodimer. To explore the possible formation of a heterotrimer, which would have a molecular weight in excess of 46 000, the molar ratios of the two domains in mixtures analyzed by sedimentation equilibrium were varied from 2:1 to 1:2. However, in no case could the average molecular weight observed be raised above 30 000, again implying that the two domains interact to form heterodimers.

Hydrodynamic Analysis of the b and b′ Domains. The cytoplasmic domain of the *E. coli b* subunit has been characterized as a highly extended homodimer (*11*). To determine if the heterodimer formed by the polar domains of the *b* and *b′* subunits of the *Synechocystis* enzyme has a similar shape, these polypeptides were examined individually or as a mixture using the technique of sedimentation velocity ultracentrifugation. As seen in Table 2, the individual *b* and *b′* polypeptides sedimented at 1.34 and 1.23 S, respectively, while the heterodimeric complex sedimented at 1.72 S. The frictional ratios calculated from these sedimentation coefficients and the molecular weights fall in the range of 1.75−2.10. The large deviations of these values from 1.0 imply that all of the proteins have a highly elongated shape. The hydrodynamic properties of the heterodimer are very similar to those previously observed for the *E. coli* b_{24-156} homodimer (*14*).

CD Spectra of the b and b′ Domains. CD spectra of the individual *b* and *b′* domains, as well as an equimolar mixture, were collected (Figure 2). In all cases the minima in the ranges of 206 and 222 nm, characteristic of α-helices, are evident. From the strength of the signals at 222 nm, the

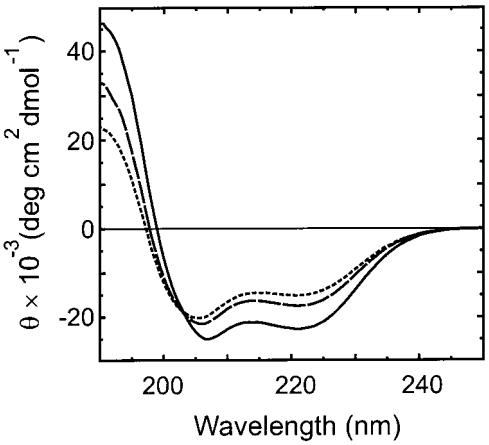

FIGURE 2: Circular dichroism spectra of the soluble domains of *Synechocystis b*, *b′*, and an equimolar mixture were taken in 10 mM sodium phosphate buffer, pH 7.0, at 20 °C using a jacketed cell with a path length of 1 mm. Key: long dashes, b_{49-179}, 0.33 mg/mL; short dashes, b'_{30-143}, 0.31 mg/mL; solid line, mixture of b_{49-179} and b'_{30-143} at concentrations of 0.16 and 0.15 mg/mL, respectively.

helical contents of the individual b_{49-179} and b'_{30-143} polypeptides were calculated to be 45% and 39%, respectively.

A clear increase in helical content was observed when the two polypeptides were mixed in an equimolar ratio (Figure 2, solid line). On the basis of the ellipticity at 222 nm, the mixture was calculated to be 58% helical. The minimum at 206 nm in the individual domain spectra shifted to 207 nm in the mixture, and the signal at 222 nm was intensified relative to that at 206−207 nm. The relative intensification of the minimum at 222 nm is generally considered to be indicative of the formation of coiled-coil structures (*36*).

DISCUSSION

The essential results of the current work are that the cytoplasmic domains of the *b* and *b′* subunits of *Synechocystis* exist individually as moderately helical monomers which, when mixed together, form a highly extended heterodimer. This finding supports the idea that the two *b*-type subunits in ATP synthases of photosynthetic organisms exist within the complex in a heterodimeric form, occupying positions and performing functions highly similar to those of the homodimeric *b* subunits of nonphotosynthetic eubacteria.

Other workers have reported that mutations at residues Ala-79 or Ala-128 of *E. coli b* disrupt dimerization in expressed soluble constructs and, if present in intact *b*, cause defective assembly of ATP synthase (*37*, *38*), suggesting by correlation that dimerization of *b* is critical to its function. More recently, however, Sorgen and co-workers (*39*) found that some internal multiresidue deletions extending into the region of *b* essential for dimerization (*14*) are without significant effects on enzyme function in vivo. Furthermore, work in one of our laboratories (*40*) has revealed that, in contradiction to the previous report that the A128D mutation disrupts dimer formation (*37*), this mutation has no effect on dimerization and instead specifically unfolds the C-terminal region of *b*, a region known to be essential for interactions with F_1. The significance of dimerization may therefore be questioned. The present demonstration of specific dimer formation between nonidentical subunits in a completely different system indicates that dimerization of

b-type subunits is a general phenomenon, rather than one limited to *E. coli*, reinforcing the position that it is functionally significant. The heterodimeric nature of the *Synechocystis* complex also allows examination of monomeric *b* subunits at concentrations not obtainable with homodimerizing polypeptides.

Secondary structure prediction programs imply *b* subunits to be highly helical, and analysis of the *E. coli b* subunit by CD spectroscopy has fulfilled this expectation (*11*, *41*, *42*). The present determinations of helical content of the *Synechocystis* subunits should probably be considered as minimal values for the actual *b* and *b′* domains, since the His tags attached to their N-termini are unlikely to contribute helicity. These studies show that the helicity of *b*-type subunits is not entirely dependent on quaternary structure, as substantial helical content was observed in the monomeric polar domains. The two most abundant residues in each of the sequences are alanine and glutamic acid, both of which favor helix formation. In addition, examination of the sequences of these polypeptides reveals many potential favorable intrapolypeptide electrostatic interactions between residues in positions *i* and *i* + 3 or *i* + 4, whereas potential unfavorable electrostatic interactions between residues in these relative positions are much less frequent. Both of these factors will contribute to the helicity of the monomers.

While the detailed structure of the dimer interface formed between *b* subunits is unknown, sequence analysis suggests the involvement of an α-helical coiled coil (*11*, *43*). An expressed polypeptide containing the region of *E. coli b* essential for dimerization, contained within residues 53−122 of that subunit sequence, has hydrodynamic (*14*) and spectral[2] properties consistent with a coiled coil. In the current work, the increase in helicity and the relative intensification at 222 nm coincident with dimer formation again suggest that the subunit interface forms a coiled coil. Electrostatic interactions, both favorable and unfavorable, are believed to play major roles in defining specificity of coiled-coil formation though the relative contributions of each type have been argued (*44*, *45*). Recent high-resolution structural analysis of coiled-coil domains has revealed that subunit−subunit interfaces may be unexpectedly complex and include favorable interactions between residues in heptad positions g−a′, d−e′, and d−a′, where the prime symbol indicates positions on the other helix, in addition to the more widely recognized interactions between residues in positions *g*−*e′* (*46*). For this reason, a good understanding of factors responsible for heterodimerization of the *b* and *b′* polypeptides will probably require high-resolution structural analysis of at least one *b*-type dimer.

Besides revealing the existence of the heterodimer of *Synechocystis b* and *b′* soluble domains, the current studies show the properties of that complex to be similar to those of the homodimer formed by the polar domain of the *b* subunit of *E. coli* in terms of helical content, shape, and ease of melting (*11*, *14*, *41*). These results imply that, despite the low sequence conservation of *b* subunits revealed by phylogenetic analysis (*6*, *7*), the b_2 dimer of *E. coli* provides a good model for those of other bacteria and photosynthetic organisms.

One critical role of *b* subunits is to hold F_1 and F_0 together. Binding studies and site-directed cross-linking experiments using the *E. coli* enzyme indicate that *b* subunits interact directly with the single δ subunit and that various parts of *b* are proximal to either the N-terminal domain of one α subunit or an αβ interface (*47*−*49*). Thus a homodimer of *b* must bind F_1 in an asymmetric manner, with the two subunits occupying completely nonequivalent positions. The development of *b′* in photosynthetic organisms may be an adaptation to this situation, selected because it allows the *b*-type subunit occupying each of these positions to evolve independently with resultant increases in functionality, such as efficiency or potential for regulation.

How, then, would the two identical *b* subunits of nonphotosynthetic organisms cope with the asymmetric environment they face? Clearly, those portions of the structure outside the dimerization domain could adopt different conformations. Additionally, however, it should be recognized that homodimeric coiled-coil structures may be intrinsically asymmetric, as was recently observed for the actin-bundling protein cortexillin I from *Dictyostelium discoideum* (*46*).

While the present study supports the idea that the *b* and *b′* subunits were designed by nature to form a heterodimer, one of us previously showed that photoautotrophic growth of *Synechocystis* was unimpaired after genetic deletion of the polar domain of *b′* (*24*). Exactly how this deletion is accommodated is not known, but given the slight tendency of the polar domain of *Synechocystis b* to form dimers, it might be possible that in the cell a functional but structurally aberrant enzyme containing two *b* subunits could have formed, allowing growth to proceed.

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