Activation of *Escherichia coli* F_1-ATPase by Lauryldimethylamine Oxide and Ethylene Glycol: Relationship of ATPase Activity to the Interaction of the ϵ and β Subunits†

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Received August 3, 1989; Revised Manuscript Received October 13, 1989

ABSTRACT: The stimulation of the ATPase activity of *Escherichia coli* F_1-ATPase by the detergent lauryldimethylamine oxide (LDAO) and the relationship of this activation to removal of the inhibitory ϵ subunit were studied. The detergent caused a dramatic decrease in the affinity of ϵ-depleted enzyme for ϵ subunit, suggesting that release of ϵ is involved in LDAO activation. However, even in the absence of any ϵ subunit, the detergent caused a 140% increase in activity, indicating activation by effects independent of ϵ. In contrast, the addition of 30% ethylene glycol to the reaction buffer caused a modest inhibition of the ATPase activity of ϵ-depleted F_1-ATPase but rendered the enzyme insensitive to inhibition by ϵ subunit. This solvent prevented the cross-linking of ϵ to β by a water-soluble carbodiimide, although ϵ remained linkable to both β and γ by dithiobis(succinimidyl propionate). Thus, ϵ was not dissociated from F_1-ATPase, but its intimate interaction with the β subunit was altered. These results suggest that the inhibitory action of ϵ is expressed through its interaction with β. Kinetic analysis revealed that LDAO activated hydrolysis at both the high- and low-affinity promotional sites, with little change in K_m values. Ethylene glycol caused a substantial increase in K_m at the low-affinity promotional site and made the enzyme resistant to inhibition by aurovertin D.

The membrane-bound F_1F_0-ATPase[1] of *Escherichia coli* couples the vectorial movement of protons across the plasma membrane to the synthesis or hydrolysis of ATP. The peripheral portion of this complex, F_1-ATPase, has a subunit composition of $\alpha_3\beta_3\gamma\delta\epsilon$. Both α and β subunits bind adenine nucleotides; some nucleotide binding sites are likely at α/β interfaces. No purified subunit hydrolyzes ATP with substantial efficiency, but much evidence suggests that β contains the catalytic site. The γ subunit is required to organize the α and β subunits into the active complex. The δ and ϵ subunits play roles in coupling the *E. coli* F_1-ATPase to F_0, the integral membrane component of the complex [for reviews, see Futai et al. (1987, 1989) and Senior (1988)].

The ϵ subunit of the *E. coli* enzyme is of substantial interest because it inhibits the ATP hydrolytic activity of soluble F_1-ATPase by about 90% (Smith & Sternweis, 1977). ϵ binds to the enzyme largely through interactions with γ (Dunn, 1982) but can also be cross-linked to one of the β subunits (Bragg & Hou, 1980; Lötscher et al., 1984a). We have shown that the linkage to β occurs within the carboxyl-terminal region of that subunit (Tozer & Dunn, 1987).

The K_d for the interaction of ϵ with the remainder of F_1-ATPase is in the nanomolar range (Sternweis & Smith, 1980; Dunn, 1982). Thus, the degree of association of ϵ in standard F_1-ATPase varies with enzyme concentration, ranging from virtually nil under dilute ATPase assay conditions to essentially complete at the higher concentrations used for most other types of studies (Laget & Smith, 1979). In order to simplify our studies of the effects of ϵ on F_1-ATPase and the mechanism of ϵ inhibition, we have used ϵ-depleted enzyme, supplementing with saturating concentrations of purified ϵ to obtain an ϵ-replete state (Dunn et al., 1987). Under unisite conditions where the reaction proceeds very slowly, ϵ slows product release about 15-fold, in agreement with the ^{18}O exchange studies of Wood and co-workers (Wood et al., 1987). We also characterized the effects of ϵ on the multisite kinetic parameters of ATP hydrolysis and found that the nucleotide-induced alterations of aurovertin fluoescence are largely dependent on the presence of the ϵ subunit.

Either the effects of ϵ could be expressed directly at the β subunit that it touches, and thus be of a relatively local nature, or they could be transmitted through strong interactions with γ to the entire enzyme. The studies reported here use two reagents that reduce the inhibition of ATPase activity by ϵ. The first, lauryldimethylamine oxide (LDAO), has been used by earlier workers with differing results. Lötscher and coworkers (Lötscher et al., 1984b) initially described the stimulation of the ATPase activity of *E. coli* F_1 by this detergent and suggested a relationship to the displacement of ϵ from its site of contact with the β subunit. Bragg and Hou (1986), however, reported that LDAO did not cause release of ϵ. More recently, Gavilanes-Ruiz et al. (1988) reported that LDAO activates F_1-ATPase which has been treated with trypsin, leading to the apparent removal of the δ and ϵ subunits, with limited cleavage of the α, β, and γ subunits. The studies reported here clarify the relationship of LDAO and ϵ and probe the mechanism of LDAO stimulation. In addition, we describe the use of ethylene glycol, present at 30% concentration, to cause a subtle modification of the interaction of ϵ with ATPase, as demonstrated through cross-linking experiments.

† These studies were supported by Grant MA-7906 from the Medical Research Council of Canada.

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[1] Abbreviations: F_1, portion of the proton-translocating ATPase complex which is peripheral to the membrane; F_0, portion of the proton-translocating ATPase which is an integral membrane component; EDC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide; DSP, dithiobis(succinimidylpropionate); LDAO, lauryldimethylamine oxide.

0006-2960/90/0429-4335$02.50/0

Experimental Procedures

Preparations. F_1-ATPase (Dunn et al., 1985) and ϵ subunit (Dunn, 1986a) were prepared from *E. coli* strain AN1460 by methods described previously. F_1-ATPase was depleted of ϵ subunit by ϵ-4 monoclonal antibody affinity chromatography as described (Dunn, 1986a), except that a 4-mL column containing 24 mg of ϵ-4 monoclonal antibody was used and 20 mg of F_1-ATPase was treated in each batch. The ϵ-depleted enzyme was chromatographed on a column of Sephacryl S-400 (85 × 1.5 cm) in 50 mM Tris-HCl, pH 7.4, 10% glycerol, 1 mM EDTA, and 1 mM ATP to remove any dissociated material, and stored at −80 °C in this buffer. The preparation was judged to be more than 95% depleted of ϵ subunit, based on scans of SDS–polyacrylamide gels stained with Coomassie blue R-250.

Isolation of the α, β, and γ subunits and reconstitution and purification of the $\alpha_3\beta_3\gamma$ complex were performed as described previously (Dunn & Futai, 1980). The specific activity of the preparation using the assay method of Futai et al. (1974) was 131 units/mg.

The monoclonal antibodies to F_1-ATPase subunits used in this study have been described previously (Dunn et al., 1985).

Aurovertin D was prepared from the culture medium of *Calcarisporium arbuscula* as described previously (Dunn et al., 1987).

Other Materials. LDAO was obtained from Calbiochem-Behring Diagnostics. Ethylene glycol was from J. T. Baker Chemical Co. 1-Ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC) and dithiobis(succinimidyl propionate) (DSP) were from Pierce Chemical Co.

Chemical Cross-Linking. Chemical cross-linking of F_1-ATPase was carried out at room temperature in buffer containing 20 mM Mops–NaOH, pH 7.5, 10 mM ADP, 5 mM $MgCl_2$, and ethylene glycol or LDAO as indicated. Cross-linking by EDC was started by adding an aqueous solution of EDC to achieve a final concentration of 4 mM. Samples were removed at various time intervals, and the reaction was quenched by the addition of sodium acetate and ethanolamine hydrochloride, pH 9.0, to final concentrations of 50 mM. A zero-time point consisted of F_1-ATPase to which the quenching agents were added before the EDC. Cross-linking with DSP was started by adding the reagent dissolved in dimethyl sulfoxide to obtain a final DSP concentration of 0.1 mM. Samples were removed at various time intervals, and the reaction was quenched by the addition of Tris-HCl, pH 7.8, to a final concentration of 0.5 M. The zero-time point consisted of a sample withdrawn before addition of DSP.

Other Methods. Multisite ATPase assays and kinetic studies were performed in the presence of an ATP-regenerating system and analyzed as described previously (Dunn et al., 1987). In a few experiments, in which ATP was in excess over Mg^{2+}, the assay method of Futai et al. (1974) was used. SDS–polyacrylamide gel electrophoresis was performed as described by Laemmli (1970). Western blots were performed as described previously (Dunn, 1986b) except that the blocking step was performed for 1 h at room temperature. Protein was determined by the method of Bradford (1976), except for ϵ, which was determined by the method of Lowry et al. (1951). Bovine serum albumin was used as the standard for both methods.

Results

Stimulation of ATPase Activity by LDAO. To assess the involvement of the ϵ subunit in the activation of ATPase activity by LDAO, ϵ-depleted F_1-ATPase was prepared by anti-ϵ monoclonal antibody affinity chromatography (Dunn, 1986a).

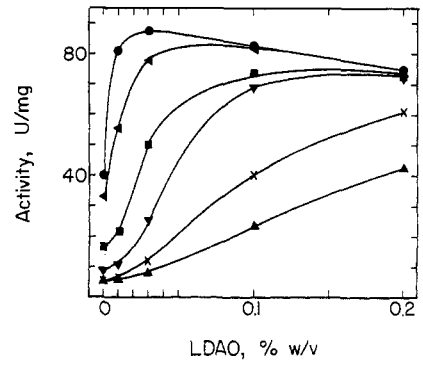

FIGURE 1: Effect of ϵ concentration on the stimulation of ATPase activity by LDAO. The ATPase activity of ϵ-depleted F_1 was assayed at 30 °C using an ATP-regenerating system in the presence of various concentrations of ϵ and LDAO. The concentration of enzyme in the assays was 1.6 nM. The concentration of ϵ present in the assays was 0 (●), 1 (◀), 3 (■), 10 (▼), 30 (×), 100 nM (▲).

The effect of various concentrations of LDAO and ϵ subunit on the ATPase activity of ϵ-depleted enzyme is shown in Figure 1. The first point to note is that even in the absence of any ϵ subunit (●), LDAO caused a large stimulation. A maximal activation of 140% was obtained at 0.03% LDAO. As ϵ-depleted enzyme is activated so strongly, activation must involve effects other than release of ϵ, or alteration of the interaction of ϵ with F_1-ATPase.

LDAO has been shown to cause dissociation of the δ subunit (Lötscher et al., 1985b). The remote possibility that this subunit dissociation could be related to the activation was examined by using the reconstituted $\alpha_3\beta_3\gamma$ complex (Dunn & Futai, 1980) which lacks δ, unlike the ϵ-depleted F_1-ATPase used in the other work described here. The specific activity of the $\alpha_3\beta_3\gamma$ complex under the assay conditions used for Figure 1 was 36 units/mg. Addition of 0.03% LDAO raised the specific activity to 72 units/mg, confirming that release of the δ subunit is not responsible for activation.

The assay conditions used in most of this work included Mg^{2+} present in 6 mM excess over ATP. This condition reduces the activity of F_1-ATPase substantially compared to that expressed when ATP is in excess over Mg^{2+}. The activation by LDAO could be essentially a matter of overcoming or preventing this inhibition. The effect of LDAO on the ATPase activity of the preparations was therefore determined in the presence of excess ATP by using the assay system of Futai et al. (1974). Under these conditions, the specific activity of the ϵ-depleted enzyme was 80 units/mg, and this basal level was stimulated 56% to 125 units/mg by inclusion of 0.1% LDAO. Thus relief from inhibition by excess divalent cations may account for part of the activating effect of LDAO, but the stimulation involves other effects as well.

Figure 1 also shows the stimulation of ATPase by LDAO in the presence of ϵ subunit added to final concentrations ranging from 1 nM (◀) to 100 nM (▲). The concentration of ϵ-depleted F_1-ATPase was 1.5 nM. In the absence of LDAO, half-maximal inhibition occurred at about 2 nM ϵ. LDAO notably activated the enzyme at all ϵ concentrations, but higher LDAO concentrations were required to activate as the ϵ concentration was raised, suggesting a competition between LDAO and ϵ. This apparent competition can also be seen by examining the effect of LDAO on the concentration of ϵ giving half-maximal inhibition, which is raised to 25–30 nM in the presence of 0.1% LDAO. It is also notable that the activation curves became notably sigmoidal at the higher ϵ concentrations, suggesting the cooperative binding of more than one LDAO molecule.

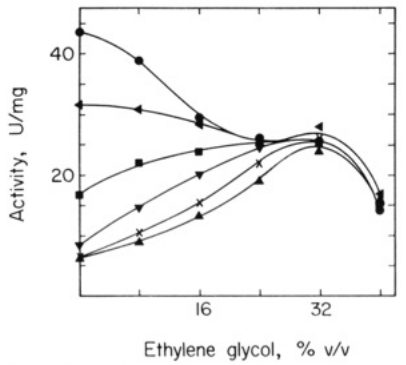

FIGURE 2: Effect of ε and ethylene glycol on the ATPase activity of F_1. The ATPase activity of ε-depleted F_1-ATPase was determined at 30 °C using an ATP-regenerating system in the presence of various concentrations of ε and ethylene glycol. The concentration of enzyme was 3.2 nM. The concentration of ε present in the assays was 0 (●), 1 (◀), 3 (■), 10 (▼), 30 (×), or 100 nM (▲).

We have confirmed the report by Bragg and Hou (1986) that LDAO did not prevent the linkage of β and ε in standard F_1-ATPase containing the normal complement of ε by 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC) in the presence of MgADP (data not shown). It should be borne in mind, however, that the cross-linking experiments are typically carried out at protein concentrations in the range of 1 mg/mL, or 2.6 μM, F_1-ATPase, so that even with the lower affinity for ε which is induced by LDAO, most ε would remain bound to the enzyme at this concentration.

Reversal of ε Inhibition by Ethylene Glycol. Studies similar to those shown in Figure 1, but with ethylene glycol in place of LDAO, are shown in Figure 2. The ATPase activity of ε-depleted enzyme (●) was mildly inhibited by moderate concentrations of ethylene glycol. This inhibition may be due to a combination of viscosity, perturbation of protein conformation, and other solvent effects. It is notable, however, that the activity in the presence of high concentrations of ε (▲) was stimulated rather than inhibited by increasing amounts of ethylene glycol. At ethylene glycol concentrations above 30%, the presence of ε had no effect on activity, suggesting that under these conditions either ε is bound in a noninhibitory mode or else it is not bound at all.

Evidence supporting the first possibility was obtained by determining the effect of ethylene glycol on the relationship of the ε subunit to other subunits of F_1-ATPase through chemical cross-linking studies. The cross-linking agents used were EDC, a water-soluble carbodiimide which forms a highly specific zero-length link between ε and a residue in the carboxyl-terminal region of β (Lötscher et al., 1984a; Tozer & Dunn, 1987), and DSP, a more generally reactive agent which cross-links lysyl residues that may be as much as 1.1 nm apart. F_1-ATPase was cross-linked at a concentration of 1 mg/mL.

The SDS–polyacrylamide gel in Figure 3 shows the time course of cross-linking by EDC in buffer lacking ethylene glycol (panel A), or buffer containing 30% ethylene glycol (panel B), or buffer containing 40% ethylene glycol (panel C). The β–ε product migrates in about the same position as BSA, with an apparent molecular weight of 68 000. Note in panel A that formation of β–ε is apparent within 5 min (lane 2) and continues with a concomitant reduction in the intensity of ε. However, in the presence of 30% ethylene glycol, the formation of β–ε was very much slower, and in 40% ethylene glycol, it was eliminated entirely. One might imagine that ethylene glycol, despite being a poor nucleophile, could interfere chemically with the cross-linking reaction because of its high concentration. If this was the case, one would expect the formation of all cross-linked products to be reduced equally. As can be seen from Figure 3, however, the formation of the larger cross-linked products, with molecular weights from 75 000 to 130 000, was not inhibited by ethylene glycol; formation of only the β–ε product was blocked. Other experiments (not shown) revealed that 30% glycerol did not reduce the formation of any of the products. In contrast, 25 mM ethanolamine, a good nucleophile, reduced the formation of all products by about 50%, and was equally effective in plain aqueous buffer or in 30% ethylene glycol or in 30% glycerol. These results indicate that ethylene glycol, or impurities in it, did not interfere chemically with the carbodiimide cross-linking reaction.

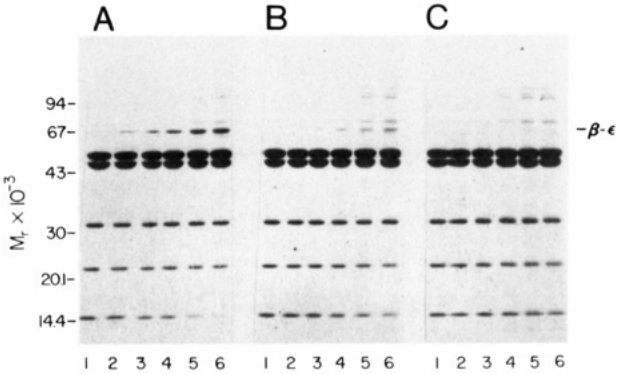

FIGURE 3: Cross-linking of F_1 by EDC in the presence of various concentrations of ethylene glycol. F_1-ATPase (1 mg/mL) was cross-linked with EDC in the presence of 0% (panel A), 30% (panel B), or 40% (panel C) ethylene glycol as described under Experimental Procedures. Samples were prepared for electrophoresis using SDS sample buffer containing dithiothreitol and then run on a 10–20% SDS–polyacrylamide gel. The gel stained with Coomassie brilliant blue R-250 is presented. The times of exposure to EDC were as follows: lane 1, 0 min; lane 2, 5 min; lane 3, 10 min; lane 4, 20 min; lane 5, 40 min; lane 6, 60 min.

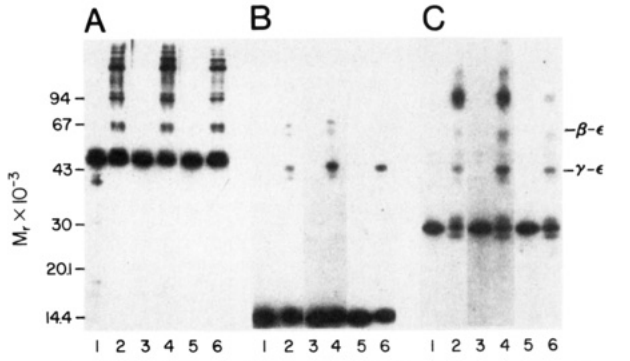

FIGURE 4: Cross-linking of F_1 by DSP in the presence of various concentrations of ethylene glycol. F_1-ATPase (1 mg/mL) was cross-linked with DSP for 2 min in the presence of 0%, 30%, or 40% ethylene glycol as described under Experimental Procedures. Samples were prepared for electrophoresis using SDS sample buffer containing 10 mM *N*-ethylmaleimide in place of reducing agent. Samples were run on a 10–20% SDS–polyacrylamide gradient gel and then electroblotted onto nitrocellulose. Blots were probed with ^{125}I-β-2 (panel A), ^{125}I-ε-1 plus ^{125}I-ε-4 (panel B), or ^{125}I-γ-1 (panel C). Lanes 1, 3, and 5 contain non-cross-linked zero-time controls. Lanes 2, 4, and 6 contain cross-linked samples. Lanes 1 and 2, 0% ethylene glycol; lanes 3 and 4, 30% ethylene glycol; lanes 5 and 6, 40% ethylene glycol.

Evidence that dissociation of ε from the enzyme was not occurring was obtained by cross-linking F_1-ATPase with DSP under the same conditions (Figure 4). This reagent induces many cross-links including β–ε (M_r 68 000) and γ–ε (M_r 47 000) (Bragg & Hou, 1980). A low concentration of DSP (0.1 mM) was used to avoid linking the subunits into large aggregates. Samples containing control or cross-linked enzyme

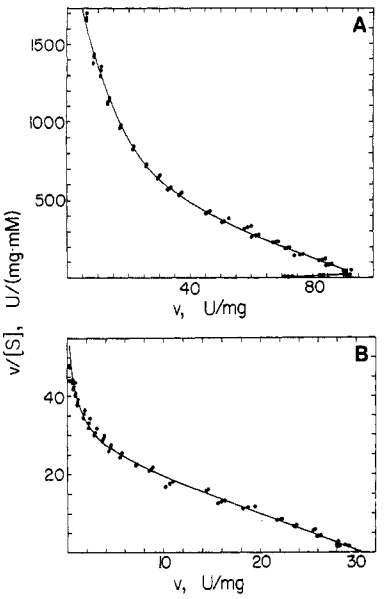

FIGURE 5: Kinetic analysis of ATP hydrolysis by ϵ-depleted F_1-ATPase in buffer containing 0.1% LDAO (panel A) or 30% ethylene glycol (panel B). The rate of ATP hydrolysis by ϵ-depleted F_1-ATPase was measured at ATP concentrations ranging from 4 μM to 20 mM as described under Experimental Procedures. The data are presented as Eadie–Hofstee plots. The lines represent the best fit to three (panel A) or two (panel B) kinetic components as described in the text.

were run on SDS–polyacrylamide gels, blotted onto nitrocellulose, and probed with radioiodinated monoclonal antibodies to β (panel A), ϵ (panel B), or γ (panel C). The samples in lanes 2, 4, and 6 were cross-linked in the presence of 0%, 30%, and 40% ethylene glycol, respectively, while lanes 1, 3, and 5 contain non-cross-linked control samples. Because of the small amount of DSP used, and the inefficient nature of the process, only a small proportion of ϵ was linked to other subunits, even in the aqueous control where all the ϵ is bound to F_1 (panel B, lane 2). Note that addition of 30% ethylene glycol did not reduce the efficiency of ϵ linkage to either γ or β (compare lanes 2 and 4 and panel B). The identities of the two products of interest, and their formation in the presence of ethylene glycol, are confirmed in panels A and C. These results indicate that 30% ethylene glycol did not cause dissociation of ϵ from F_1-ATPase, or migration of ϵ to a substantially different site. In 40% ethylene glycol, however (lane 6), ϵ was not cross-linked to β although it remained cross-linkable to γ. Taken together, the experiments shown in Figures 3 and 4 indicate that ethylene glycol alters the exact juxtaposition of the β and ϵ subunits in F_1-ATPase, without causing dissociation of ϵ.

Effects of LDAO and Ethylene Glycol on Kinetic Parameters for Multisite ATP Hydrolysis. As LDAO activated ATP hydrolysis by the $\alpha_3\beta_3\gamma$ enzyme by some mechanism other than dissociation of the minor subunits, it was of interest to determine the kinetic behavior in the presence of the detergent. The rate of ATP hydrolysis by the ϵ-depleted F_1-ATPase in the presence of 0.1% LDAO was measured at ATP concentrations ranging from 4 μM to 20 mM, and the results are presented as an Eadie–Hofstee plot in Figure 5A. The data were fitted to a multiple component analysis using the SAS NLIN program as described previously (Dunn et al., 1987), and derived parameters are shown in Table I. The best fit was obtained for a high-affinity component with a K_m of 9.0 μM and a maximal velocity of 14 units/mg, a major lower affinity component had a K_m of 180 μM and a maximal velocity of 87 units/mg, and a poorly defined low-affinity inhibitory component (note the reversal of the curve seen in the lower right-hand corner of the plot).

Table I: Effects of LDAO and Ethylene Glycol on Kinetic Parameters of Multisite ATP Hydrolysis

	additions to assay medium		
parameter	none	0.1% LDAO	30% ethylene glycol
V_1 (units/mg)	0.74[a]	14	0.44
K_{m1} (μM)	18[a]	9	10
V_2 (units/mg)	25[a]	87	30
K_{m2} (μM)	250[a]	180	1100

[a] As reported previously (Dunn et al., 1987).

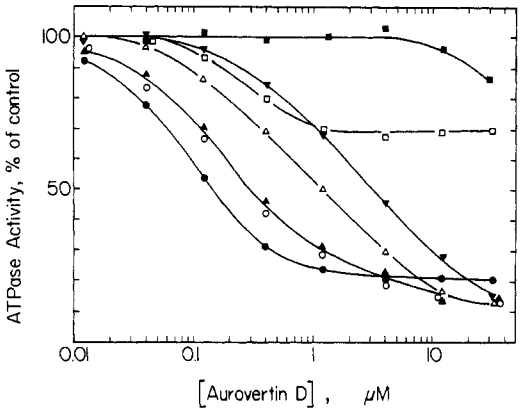

FIGURE 6: Effects of LDAO and ethylene glycol on aurovertin inhibition of ϵ-depleted F_1-ATPase. ATPase activity was measured by using an ATP-regenerating system under standard conditions (●) or in buffer containing 0.01% LDAO (○), 0.03% LDAO (▲), 0.1% LDAO (△), 0.3% LDAO (▼), 15% ethylene glycol (□), or 30% ethylene glycol (■). In each case, activity is expressed as percent of control under the same conditions but receiving no aurovertin.

A similar kinetic analysis performed in the presence of 30% ethylene glycol is shown in Figure 5B. The most notable effect of the cosolvent was to increase the K_m of the major component to a value of 1100 μM, with a maximal velocity of 30 units/mg. The higher affinity component, with a K_m value of 10 μM, contributed a maximal velocity of 0.44 unit/mg. The effect of 100 nM ϵ on hydrolysis in 30% ethylene glycol at a few ATP concentrations was also tested, although a complete kinetic analysis was not done. The effect was small, ranging from a 10% stimulation at 30 μM ATP to a 5–10% inhibition at 10 mM ATP (data not shown).

Effects of LDAO and Ethylene Glycol on Aurovertin Inhibition. The effects of LDAO and ethylene glycol on the inhibition of ATPase activity of ϵ-depleted F.ATPase by aurovertin D are shown in Figure 6. In this figure, each activity point is plotted as a percentage of the control activity under the same conditions, but lacking any aurovertin. Thus, the control points for assays containing LDAO had about twice the activity of those in the unsupplemented buffer. In standard reaction buffer (●), aurovertin reduced activity by 80% with half-maximal inhibition occurring at about 0.1 μM, in agreement with earlier work (Dunn et al., 1987). In the presence of LDAO at 0.01% (○) or 0.03% (▲), the concentration of aurovertin required for half-maximal inhibition increased by a factor of about 2. It is also notable that the maximal inhibition in the presence of LDAO was greater. This effectively canceled the higher control rate obtained with LDAO, so that the specific activity of ATPase at high aurovertin concentrations was hardly affected by the detergent. This suggests that LDAO and aurovertin may compete for a hydrophobic site on β. Higher LDAO concentrations displaced the curve further to the right (△, ▼), but this is likely due to partitioning of aurovertin into detergent micelles.[2]

Ethylene glycol at concentrations of 15% (□) and 30% (■) notably reduced the affinity of ATPase for aurovertin, and the extent of maximal inhibition as well.

Discussion

The activation of ATPase activity by LDAO clearly involves at least two effects. First, ε-depleted enzyme was activated by 140%, with essentially complete activation occurring at 0.01%, or 0.44 mM, LDAO. This indicates that monomeric detergent is the activating species, as the critical micelle concentration is around 0.05% (Helenius et al., 1979), and that in the absence of ε the detergent is bound with reasonably high affinity. Other hydrophobic compounds, such as aurovertin (Dunn & Futai, 1980), bind to the β subunit, and dicyclohexylcarbodiimide (Vignais et al., 1979) and 3-(trifluoromethyl)-3-(*m*-iodophenyl)diazirine (Hoppe et al., 1984) both label β, although there is no strong evidence that the same site is involved in binding any two of these ligands. Our finding that high concentrations of aurovertin overcame the LDAO activation is consistent with the possibility that both bind to the same site on the β subunits.

The binding of LDAO alters the behavior of the enzyme in at least two aspects. First, the affinity for ε is reduced by at least an order of magnitude. Second, a large stimulation in multisite ATP hydrolysis rates, with little change in apparent K_m values, indicates that cooperativity is much more efficient with the detergent bound. In contrast, bound ε decreased cooperativity (Dunn et al., 1987). Thus, it seems that ε and LDAO favor antipodal conformations in some region(s) of the enzyme involved in the transmission of cooperativity, while the substrate binding sites are not much affected.

Our results are similar to those reported for the activation of chloroplast F_1-ATPase by alkyl β-D-glucopyranoside detergents (Pick & Bassilian, 1982; Yu & McCarty, 1985) in that the detergents cause both the dissociation of ε and the activation of ε-depleted enzyme. In contrast, LDAO does not stimulate the purified mitochondrial F_1-ATPase, unless it has been reconstituted with the mitochondrial ATPase inhibitor protein (Vasquez-Laslop & Dreyfus, 1986), which is not homologous in sequence to the ε subunit of *E. coli* or chloroplast F_1-ATPase.

Ethylene glycol has previously been shown to promote the synthesis of F_1-bound ATP from ADP and orthophosphate (Gômez-Puyou et al., 1986; Yohda et al., 1986). We found that ethylene glycol relieved the inhibition of ATPase activity by both ε subunit and aurovertin. The experiments presented in Figures 3 and 4 indicate that ethylene glycol caused a subtle rearrangement which prevented the linkage of ε to β, while the linkage to γ was not inhibited, indicating that ε remained bound to the enzyme. The nature of the rearrangement could be the movement of ε to a slightly different site, a change in the conformation of β or ε, or a small change in the entire enzyme. Although it cannot be decided from our data which of these possibilities is most likely correct, a change in the conformation of β is suggested by the effect of ethylene glycol on both the affinity for aurovertin D and the maximal inhibition obtained. Recently, the amino acid change conferring aurovertin resistance in a mutant *E. coli* F_1-ATPase has been identified as arginine-398 to histidine in the β subunit (Lee et al., 1989), implying that the aurovertin binding site is in the C-terminal section of β, as is the site of ε interaction (Tozer & Dunn, 1987). Thus, on the basis of both the aurovertin inhibition study and the ε cross-linking work, we suggest that ethylene glycol induces conformational changes in the C-terminal region of β.

The major kinetic effect of ethylene glycol was to increase the K_m for the low-affinity component about 5-fold, while the high-affinity component was hardly altered, suggesting that the cosolvent may affect one particular β subunit in the complex. In the alternating-sites mechanism of ATPase activity (Cross et al., 1984), this would be the second promotional site. Regardless of the exact nature of the structural change, however, the correlation between reversal of ε inhibition with this change suggests that the inhibitory effect of ε is mediated through specific interactions with one of the β subunits.

[2] The critical micelle concentration of LDAO is 2.2 mM or 0.05% (Helenius et al., 1979). At higher concentrations of LDAO, the fluorescence of aurovertin solutions increased notably, suggesting that it was partitioning into the more viscous micelles.

References

Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.

Bragg, P. D., & Hou, C. (1980) *Eur. J. Biochem. 106*, 495–503.

Bragg, P. D., & Hou, C. (1986) *Biochim. Biophys. Acta 851*, 385–394.

Cross, R. L., Cunningham, D., & Tamura, J. K. (1984) *Curr. Top. Cell. Regul. 24*, 335–344.

Dunn, S. D. (1982) *J. Biol. Chem. 257*, 7354–7359.

Dunn, S. D. (1986a) *Anal. Biochem. 159*, 35–42.

Dunn, S. D. (1986b) *Anal. Biochem. 157*, 144–153.

Dunn, S. D., & Futai, M. (1980) *J. Biol. Chem. 255*, 113–118.

Dunn, S. D., Tozer, R. G., Antczak, D. F., & Heppel, L. A. (1985) *J. Biol. Chem. 260*, 10418–10425.

Dunn, S. D., Zadorozny, V. D., Tozer, R. G., & Orr, L. E. (1987) *Biochemistry 26*, 4488–4493.

Futai, M., Sternweis, P. C., & Heppel, L. A. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 2725–2729.

Futai, M., Noumi, T., Miki, J., & Maeda, M. (1987) in *Bioenergetics: Structure and Function of Energy Transducing Systems* (Ozawa, T., & Papa, S., Eds.) pp 153–165, Japan Scientific Society Press, Tokyo.

Futai, M., Noumi, T., & Maeda, M. (1989) *Annu. Rev. Biochem. 58*, 111–136.

Gavilanes-Ruiz, M., Tommasino, M., & Capaldi, R. A. (1988) *Biochemistry 27*, 603–609.

Gômez Puyou, A., Tuena de Gômez Puyou, M., & de Meis, L. (1986) *Eur. J. Biochem. 159*, 133–140.

Helenius, A., McCaslin, D. R., Fries, E., & Tanford, C. (1979) *Methods Enzymol. 56*, 734–749.

Hoppe, J., Brunner, J., & Jorgensen, B. B. (1984) *Biochemistry 23*, 5610–5616.

Laemmli, U. K. (1970) *Nature 227*, 680–685.

Laget, P. P., & Smith, J. B. (1979) *Arch. Biochem. Biophys. 197*, 83–89.

Lee, R. S.-F., Pagan, J., Satre, M., Vignais, P. V., & Senior, A. E. (1989) *FEBS Lett. 253*, 269–272.

Lötscher, H. R., deJong, C., & Capaldi, R. A. (1984a) *Biochemistry 23*, 4134–4140.

Lötscher, H. R., deJong, C., & Capaldi, R. A. (1984b) *Biochemistry 23*, 4140–4143.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Pick, U., & Bassilian, S. (1982) *Biochemistry 21*, 6144–6152.

Satre, M., Lunardi, J., Pougeois, R., & Vignais, P. V. (1979) *Biochemistry 18*, 3134–3140.

Senior, A. E. (1988) *Physiol. Rev. 68*, 177–231.

Smith, J. B., & Sternweis, P. C. (1977) *Biochemistry 16*, 306–311.

Sternweis, P. C., & Smith, J. B. (1980) *Biochemistry 19*, 526–531.

Tozer, R. G., & Dunn, S. D. (1987) *J. Biol. Chem. 262*, 10706–10711.
Vasquez-Laslop, N., & Dreyfus, G. (1986) *J. Biol. Chem. 261*, 7807–7810.
Wood, J. M., Wise, J. G., Senior, A. E., Futai, M., & Boyer, P. D. (1987) *J. Biol. Chem. 262*, 2180–2186.
Yohda, M., Kagawa, Y., & Yoshida, M. (1986) *Biochim. Biophys. Acta 850*, 429–485.
Yu, F., & McCarty, R. E. (1985) *Arch. Biochem. Biophys. 238*, 61–68.

Red Shift of Absorption Maxima in Chlorobiineae through Enzymic Methylation of Their Antenna Bacteriochlorophylls†

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Received October 26, 1989; Revised Manuscript Received January 11, 1990

ABSTRACT: The bacteriochlorophyll *d* producing photosynthetic green sulfur bacteria *Chlorobium vibrioforme* forma *thiosulfatophilum* strain NCIB 8327 and *C. vibrioforme* strain Bl-20 respond to reduced light conditions in culture by performing methylations at the 4- and 5-substituents, for example, converting the 4-Et into 4-*n*-Pr, 4-*i*-Bu, and even 4-neoPn. During this process, the absorption maximum in living cells of *C. vibrioforme* strain Bl-20 red shifts from 714 to about 728 nm. Eventually, the *C. vibrioforme* forma *thiosulfatophilum* strain NCIB 8327 culture carries out a δ-methylation to produce the bacteriochlorophylls *c* (λ_{max} ca. 750 nm); the new UC Davis bacteriochlorophyll *c* culture is named *C. vibrioforme* forma *thiosulfatophilum* strain D. It is possible that the homologation process increases hydrophobic interactions between individual BChl molecules, giving rise to larger aggregates in the antenna system. Alternatively, the additional methyl units attached to the 4-position shift the absolute configuration of the 2-(1-hydroxyethyl) group from pure *R* in the case of 4-Et to pure *S* in the case of 4-neoPn, which in turn might determine the size of the in vivo aggregates due to the intrinsic nature of the pigment protein system. It is suggested that the bacteriochlorophylls *c* from *Chloroflexus aurantiacus* strain J-10-fl and the bacteriochlorophylls *e* from *Chlorobium phaeovibrioides* might have undergone similar meso methylation as a response to external environmental pressure such as low light intensity.

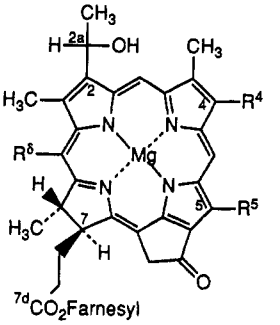
The structural assignments of the bacteriochlorophylls *d* (BChl-d)[1] were made by Holt in the 1960s after classical oxidative degradation (Holt & Hughes, 1961; Hughes & Holt, 1962; Holt & Purdie, 1965). Over the years some alterations to Holt's assignments have been made, mostly with regard to stereochemistry; the currently accepted structures (Smith & Goff, 1985) for the BChl-d homologous mixture are shown in **1** (Table I). The structures of the BChl-c were the subject of considerably more discussion, but their finalized structures (Smith et al., 1983a) are depicted in **2** (Table I).

The most novel features in the structures of the BChl-c and BChl-d are the extra methylation attached to the 4 and 5 side chains and the δ-methyl substituent (BChl-c only). Soon after the gross structural assignments were made (Holt & Purdie, 1965; Kenner et al., 1976), efforts were concentrated on establishing the origin of the extra methyl units. Carbon-13 labeling experiments showed that the extra methyl groups in the BChl-c from *Prosthecochloris aestuarii* (formerly *Chloropseudomonas ethylica*) [i.e., the δ-methyl, the terminal carbons of the isobutyl (*i*-Bu) and *n*-propyl (*n*-Pr) at position 4 and of the ethyl at position 5] were all derived from L-methionine (Kenner et al., 1976, 1978).

Experimental Procedures

General. Proton NMR spectra were obtained in $CDCl_3$ at 360 MHz (Nicolet NT360) with chemical shifts reported in parts per million relative to internal chloroform (7.258 ppm, 300 MHz). *Chlb* strain D was cultured and the Bmphs were

Table I: Structural Assignments for the BChl-d (**1**) and BChl-c (**2**)



	cmpd	Holt's band no.	R^4	R^5	R^8	configuration at C-2a
BChl-d	**1a**	6	Et	Me	H	*R*
	1b	4	Et	Et	H	*R*
	1c	5	*n*-Pr	Me	H	*R*
	1d	2	*n*-Pr	Et	H	*R*
	1e	3	*i*-Bu	Me	H	*S*
	1f	1	*i*-Bu	Et	H	*S*
	1g		neoPn	Me	H	*S*
	1h		neoPn	Et	H	*S*
BChl-c	**2a**	6	Et	Me	Me	*R*
	2b	5	Et	Et	Me	*R*
	2c	4	*n*-Pr	Et	Me	*R*
	2d	3	*n*-Pr	Et	Me	*S*
	2e	2	*i*-Bu	Et	Me	*R*
	2f	1	*i*-Bu	Et	Me	*S*

† This research was supported by a grant from the National Science Foundation (CHE-86-19034).
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0006-2960/90/0429-4340$02.50/0