Esch, F. S., Böhlen, P., Otsuka, A. S., Yoshida, M., & Allison, W. S. (1981) *J. Biol. Chem. 256*, 9084-9089.
Fillingame, R. H. (1980) *Annu. Rev. Biochem. 49*, 1079-1113.
Foster, D. L., & Fillingame, R. H. (1979) *J. Biol. Chem. 254*, 8230-8236.
Foster, D. L., Mosher, M. E., Futai, M., & Fillingame, R. H. (1980) *J. Biol. Chem. 255*, 12037-12041.
Futai, M., & Kanazawa, H. (1983) *Microbiol. Rev. 47*, 285-312.
Futai, M., Sternweis, P. C., & Heppel, L. A. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 2725-2729.
Gay, N. J., & Walker, J. E. (1981a) *Nucleic Acids Res. 9*, 2187-2194.
Gay, N. J., & Walker, J. E. (1981b) *Nucleic Acids Res. 9*, 3919-3926.
Hoare, D. G., & Koshland, D. E. (1967) *J. Biol. Chem. 242*, 2447-2453.
Kanazawa, H., Mabuchi, M., Kayano, T., Noumi, T., Sekiya, T., & Futai, M. (1981) *Biochem. Biophys. Res. Commun. 103*, 613-620.
Kanazawa, H., Kayano, T., Kiyasu, T., & Futai, M. (1982) *Biochem. Biophys. Res. Commun. 105*, 1257-1264.
Kiehl, R., & Hatefi, Y. (1980) *Biochemistry 19*, 541-548.
Laemmli, U. K. (1970) *Nature (London) 227*, 680-685.
Laget, P. P., & Smith, J. B. (1979) *Arch. Biochem. Biophys. 197*, 83-89.
Lowry, O. H., Rosebrough, N. J., Fasr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265-275.
Millett, F., deJong, C., Paulson, L., & Capaldi, R. A. (1983) *Biochemistry 22*, 546-552.
Pougeois, R. (1983) *FEBS Lett. 154*, 47-50.
Pougeois, R., Satre, M., & Vignais, P. V. (1978) *Biochemistry 17*, 3018-3023.
Pougeois, R., Satre, M., & Vignais, P. V. (1979) *Biochemistry 18*, 1408-1413.
Pullman, M. E., & Monroy, G. C. (1963) *J. Biol. Chem. 238*, 3762-3769.
Runswick, M. J., & Walker, J. E. (1983) *J. Biol. Chem. 258*, 3081-3089.
Satre, M., Lunardi, J., Pougeois, R., & Vignais, P. V. (1979) *Biochemistry 18*, 3134-3140.
Senior, A. E. (1981) *J. Biol. Chem. 256*, 4763-4767.
Senior, A. E., & Wise, J. G. (1983) *J. Membr. Biol. 73*, 105-124.
Senior, A. E., Richardson, L. V., Baker, K., & Wise, J. G. (1980) *J. Biol. Chem. 255*, 7211-7217.
Sheenan, J. C., Cruickshank, P. A., & Boshart, G. L. (1961) *J. Org. Chem. 26*, 2525-2528.
Shoshan, V., & Selman, B. R. (1980) *J. Biol. Chem. 255*, 384-389.
Sternweis, P. C., & Smith, J. B. (1980) *Biochemistry 19*, 526-531.
Towbin, H., Staehelin, T., & Gorden, J. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4350-4354.
Walker, J. E., Eberle, N. J., Gray, M. J., Runswick, M. J., & Saraste, M. (1982a) *Biochem. Soc. Trans. 10*, 203-206.
Walker, J. E., Runswick, M. J., & Saraste, M. (1982b) *FEBS Lett. 146*, 393-396.
Yoshida, M., Poses, J. W., Allison, W. S., & Esch, F. S. (1981) *J. Biol. Chem. 256*, 148-253.
Yoshida, M., Allison, W. S., Esch, F. S., & Futai, M. (1982) *J. Biol. Chem. 257*, 10033-10037.

Interconversion of High and Low Adenosinetriphosphatase Activity Forms of *Escherichia coli* F_1 by the Detergent Lauryldimethylamine Oxide†

Hans-Ruedi Lötscher, Catharina deJong, and Roderick A. Capaldi*

ABSTRACT: The amphipathic detergent lauryldimethylamine oxide (LDAO) stimulated ATP hydrolytic activity of *Escherichia coli* membranes and isolated ECF_1 and ECF_1-F_0 ATPase complexes in a concentration-dependent manner. The enzyme was maximally activated 3-fold in membranes and 5-6-fold for isolated ECF_1 or the ECF_1-F_0 complex. The maximal specific activity of activated ECF_1 was 140-160 μmol of ATP hydrolyzed min^{-1} mg^{-1}. The activation by LDAO was reversible. LDAO specifically released subunit δ from ECF_1, generating a four subunit enzyme (α, β, γ, and ε subunits). The removal of subunit δ was not responsible for the stimulation of ATPase activity as evidenced by the full activation of the four subunit enzyme by LDAO. Treatment of ECF_1 with 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide generated a β-ε cross-link in high yield [Lötscher, H. R., DeJong, C., & Capaldi, R. A. (1984) *Biochemistry* (accompanying paper in this issue)]. The formation of this cross-link was greatly reduced in the presence of LDAO, indicating that the detergent perturbated the interaction between ε and β subunits although ε was not removed from the ECF_1 complex. The results suggest that the interconversion of ECF_1 from a low to a high ATPase activity form by LDAO is in major part due to a release of the inhibitory action of subunit ε on subunit β.

The oligomeric proton translocating ATPases[1] from bacteria, mitochondria, and chloroplasts can exist in forms with low ATPase activity and forms with high ATP hydrolytic activity [for recent reviews, see Futai & Kanazawa (1983), Senior & Wise (1983), and Hammes (1983)]. In mitochondria and chloroplasts, the modulation of ATPase activity is due in large part to an endogenous inhibitor protein (Pullman & Monroy, 1963; Pederson et al., 1981). Binding of this small polypeptide converts the F_1 from a high activity to a low activity ATPase,

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[1] Abbreviations: LDAO, lauryldimethylamine oxide; ECF_1-F_0, complete H^+-translocating ATPase complex of *E. coli*; ECF_1, water-soluble, extrinsic ATPase sector of the F_1-F_0 complex of *E. coli*; EDC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide; $NaDodSO_4$, sodium dodecyl sulfate; ATPase, adenosinetriphosphatase; EDTA, ethylenediaminetetraacetic acid; TPCK, tosylphenylalanine chloromethyl ketone.

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the results being to switch the enzyme from an ATPase to an ATP synthase. Bacteria such as *Escherichia coli* do not contain this inhibitor protein, but a similar role has been assigned to the ϵ subunit in the F_1 complex (Laget & Smith, 1979; Sternweis & Smith, 1980; Dunn & Heppel, 1981). As part of our structural study of ECF_1–F_0, we have examined many different conditions for releasing ECF_1 from F_0. Here we show that the amphipathic detergent lauryldimethylamine oxide (LDAO) liberates a highly active ECF_1 both from purified ECF_1–F_0 and from *E. coli* membranes directly. This detergent also effects the conversion of ECF_1 from the low ATPase activity form to a high ATP hydrolytic activity form, making it possible to compare structurally these different conformations of the enzyme.

Experimental Procedures

Materials. LDAO (Ammonyx LO) was obtained as a 30% aqueous solution from Onyx Chemical Co., Jersey City, NJ. TPCK-treated trypsin (Worthington) was purchased from Millipore Corp., Freehold, NJ.

Source of Cells and Preparation of Membranes, ECF_1–F_0 and ECF_1. *E. coli* strain KY 7485 containing a λ-transducing phage which carries the genes of the unc operon was kindly provided by Dr. R. H. Fillingame, University of Wisconsin. Cells were grown on minimal medium with glucose as energy source. The λ-phage production was induced by raising the temperature to 42 °C for 30 min (Foster et al., 1980). Membranes, ECF_1–F_0, and ECF_1 were prepared by using the procedures of Foster et al. (1980) and Foster & Fillingame (1979). Protein was determined according to Lowry et al. (1951).

ATPase Activity Assay. ATPase activity of membranes, ECF_1–F_0, and ECF_1 was assayed in 1 mL of a solution containing 25 mM tris(hydroxymethyl)aminomethane, pH 7.5, 25 mM KCl, 5 mM $MgCl_2$, 5 mM KCN, 2 mM phosphoenolpyruvate, 2 mM ATP, 0.5 mM NADH, 30 units of L-lactic dehydrogenase, and 30 units of pyruvate kinase at 37 °C. When the activity of ECF_1–F_0 was measured, the assay buffer also contained 0.2% Tween 80. This medium was supplemented with LDAO as indicated in the figures. The linear absorbance change at 340 nm was followed in a Beckman DU7 spectrophotometer. The specific activity is expressed as units per milligram. One unit corresponds to 1 μmol of ATP hydrolyzed/min.

$NaDodSO_4$ Gel Electrophoresis. Samples were dissolved in 6% $NaDodSO_4$, 10% mercaptoethanol, 20% glycerol, and 0.125 M tris(hydroxymethyl)aminomethane, pH 6.2. One-millimeter-thick slab gels were run as described by Lëmmli (1970) with a 5% polyacrylamide stacking gel and a 12–18% polyacrylamide separating gel (linear gradient), both containing 0.2% $NaDodSO_4$. Staining with Coomassie brilliant blue R and destaining were carried out according to Downer et al. (1976).

Sucrose Gradient of ECF_1. A 3-mg sample of ECF_1 was applied on top of a 10–40% linear sucrose gradient in 50 mM tris(hydroxymethyl)aminoethane, pH 7.4, 50 mM KCl, 1 mM $MgCl_2$, 1 mM dithiothreitol, and ±0.2% LDAO. The total volume was 40 mL. The gradient was run in a Beckman vertical rotor (VTI 50) at 45 000 rpm for 14 h. A total of 29 fractions of 1.38 mL was collected following puncture of the tube bottom and assayed for ATPase activity.

Results

Table I lists the specific ATPase activities of membranes, ECF_1–F_0, and ECF_1 purified from *E. coli* (strain KY 7485 infected with a λ-transducing phage containing the unc operon) in our laboratory over the last 18 months by using the methods of Foster et al. (1980). The specific activity of ECF_1–F_0 is typical of that reported for the intact ATP synthase by other laboratories (Foster et al., 1980; Friedl et al., 1979). The activity of ECF_1 is at the low end of the range of reported activities for such a preparation, i.e., 20–150 μmol of ATP hydrolyzed min^{-1} (mg of protein)$^{-1}$ (Dunn & Futai, 1980; Nelson et al., 1974; Bragg & Hou, 1975; Satre et al., 1979). $NaDodSO_4$–polyacrylamide gels of the ECF_1 and ECF_1–F_0 preparations have shown the expected five and eight subunit enzymes.

Table I: ATPase Specific Activity ± SD[a]

	μmol of ATP hydrolyzed min^{-1} (mg of protein)$^{-1}$
membranes	2.9 ± 0.7 (n = 18)
ECF_1–F_0	15.1 ± 6.0 (n = 21)
ECF_1	23.1 ± 6.2 (n = 5)

[a] Membranes, ECF_1–F_0, and ECF_1 were prepared, and ATPase activity was determined in the absence of LDAO as described under Experimental Procedures.

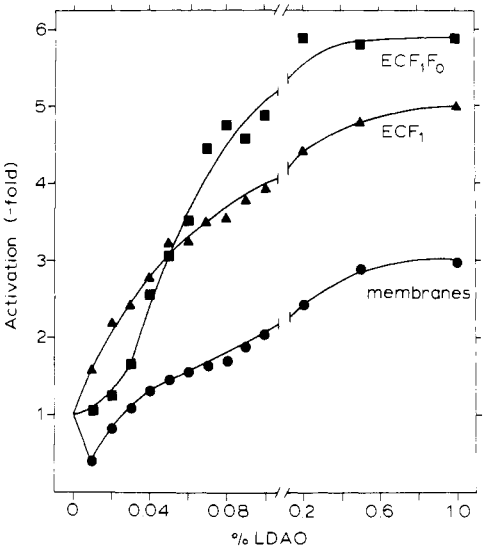

FIGURE 1: Stimulation of ATPase activity by LDAO. ATPase activity of membranes (circles), ECF_1 (triangles), and ECF_1–F_0 (squares) was assayed in the presence of increasing concentration of LDAO; 5 μg of membrane protein, 0.8 μg of ECF_1, and 3 μg of ECF_1–F_0 were used per assay.

The effects of increasing concentrations of LDAO on the ATPase activity of ECF_1, ECF_1–F_0, and membrane are shown in Figure 1. The presence of this detergent in the assay mixture increased the ATPase activity of both ECF_1 and ECF_1–F_0 by at least 5-fold, with the activity of ECF_1 being 140–160 μmol of ATP hydrolyzed min^{-1} mg^{-1}, the highest reported under the standard conditions of assays (pH, temperature). This activation did not convert the enzyme into a Ca^{2+}-ATPase as occurs during octyl glucoside activation of the chloroplast F_1 (Pick & Bassilian, 1982) (octyl glucoside inhibits ECF_1 rather than activating the enzyme). The Ca^{2+}-ATPase activity of ECF_1 in 0.5% LDAO was 2% of the Mg^{2+}-ATPase activity of the complex, a value similar to that of control untreated enzyme. The lag in activation of ECF_1–F_0 seen in Figure 1 probably represents equilibrium partitioning of LDAO into micelles of Tween 80 at the lower concentrations of the activating detergent. The activity of ECF_1 in *E. coli* membranes was maximally increased about 3-fold by the LDAO treatment (Figure 1). Low concentration of LDAO (0.01%) reproducibly inhibited the ATPase activity of membrane preparations for reasons not clear at present.

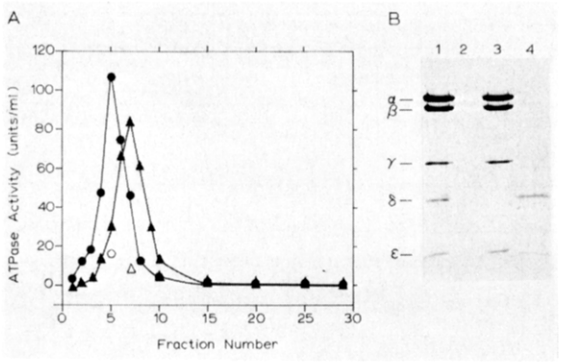

FIGURE 2: Separation of subunit δ from ECF_1 by sucrose density gradient centrifugation. (A) ECF_1 was sedimented on a 10–40% linear sucrose gradient in the absence (circles) and presence of 0.2% LDAO (triangles). The collected fractions were assayed for ATPase activity in the presence of 0.5% LDAO. Open symbols indicate ATPase activity of the peak fractions assayed in the absence of LDAO. (B) The subunit composition of ECF_1 after sucrose density gradient centrifugation in the absence (control) and presence of LDAO is shown; 20-μL aliquots were dissolved in $NaDodSO_4$ denaturing buffer and subjected to $NaDodSO_4$ gel electrophoresis. (Lane 1) Fraction 5 of control gradient; (lane 2) fraction 29 of control gradient; (lane 3) fraction 7 of gradient in LDAO; (lane 4) fraction 29 of gradient in LDAO.

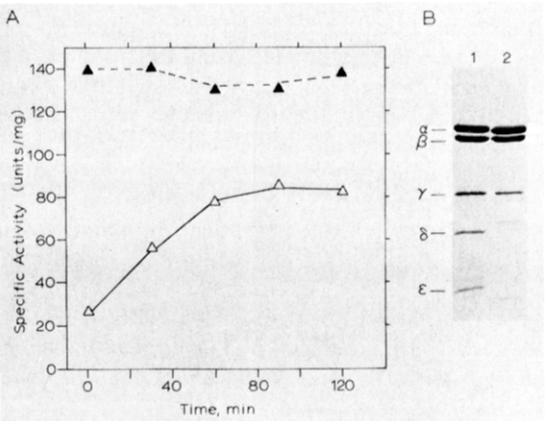

FIGURE 3: Activation of ECF_1 ATPase activity with trypsin. (A) ECF_1 (1 mg/mL) was incubated in 50 mM tris(hydroxymethyl)-aminomethane, pH 8.0, 10% sucrose, 10% glycerol, 2 mM EDTA, and 1 mM ATP with trypsin (1:1000 w/w) at 25 °C (Dunn et al., 1980). At the indicated times 2-μL aliquots were withdrawn and immediately assayed for ATPase activity in the absence (open symbols) and presence of 0.5% LDAO (closed symbols). (B) After 120 min, the proteolysis was stopped by addition of phenylmethanesulfonyl fluoride (100-fold excess by weight over trypsin), and the sample was passed over a Sephadex G-150 column in 25 mM, pH 7.0, and 100 mM KCl. The subunit composition was analyzed by $NaDodSO_4$ gel electrophoresis. (Lane 1) ECF_1 control sample, 15 μg; (lane 2) ECF_1 trypsin treated, 15 μg.

Figure 2 (part A) shows a comparison of the sedimentation of ECF_1 in a sucrose gradient in the presence and absence of LDAO (at a concentration giving optimum ATPase activity). The enzyme sedimented as a sharp band in both cases but as a four-subunit enzyme in LDAO (α, β, γ, and ϵ) and as a five-subunit enzyme with LDAO absent as shown by $NaDodSO_4$–polyacrylamide gels of peak fractions (Figure 2, part B). The δ subunit released from ECF_1 by LDAO treatment ran at the top of the sucrose gradient.

ATPase activity was measured both with (solid symbols) and without (open symbols) LDAO in the assay mixture. Enzyme from which δ had been removed by centrifugation in the gradient containing LDAO had high activity when assayed with LDAO in the assay buffer but low activity without the detergent in the assay. Thus, the activation by LDAO is reversible, with the high-affinity form depending on the presence of LDAO in the assay buffer. Moreover, generation of the high-activity form is not a consequence of removing the δ subunit, as enzyme missing this subunit is still switched from high- to low-activity form depending on the presence or absence of the detergent.

Several lines of evidence indicate that the ϵ subunit of ECF_1 modulates the ATPase activity of the enzyme (Smith & Sternweis, 1977; Sternweis & Smith, 1980). In this connection, it has been shown that protease digestion of ECF_1 activates the enzyme concomitantly with the removal of the δ and ϵ subunits and partial cleavage of the γ subunit (Bragg & Hou, 1975; Smith & Sternweis, 1982). Figure 3A shows a time course of trypsin cleavage of ECF_1, with the ATPase activity assayed in the absence (solid symbols) and in the presence (open symbols) of LDAO.

After 2 h of incubation with trypsin, both the δ and ϵ subunits were missing from the $NaDodSO_4$–polyacrylamide gel polypeptide profile (Figure 3B). The ATPase activity of the preparation had increased about 3-fold. The activity of the trypsin-treated preparation could be further enhanced when assayed in LDAO (Figure 3) to give activities identical with those of the enzyme assayed in detergent without prior protease digestion.

We have previously shown that the water-soluble carbodiimide EDC effects the cross-linking of the ϵ to the β subunit in ECF_1 and ECF_1–F_0 (Lötscher et al., 1984a). When this cross-linking reaction was done in the presence of LDAO, there was 40–60% less of the cross-linked product (five different experiments using densitometry to determine the amount of the β–ϵ product) than in controls without the detergent. This result cannot be explained by competition between the detergent and protein for the carbodiimide, and the implication is that LDAO alters the binding of ϵ to the β subunit. The detergent does not release the ϵ subunit completely as evident from the sucrose gradient experiments but must cause relocation of the small polypeptide to a second possibly overlapping site on the ECF_1.

Discussion

Results presented here show that the addition of LDAO to ATPase preparations from *E. coli* causes an enhancement of ATP hydrolytic activity whether membranes or nonmembranous preparations are assayed. Isolated ECF_1 and ECF_1–F_0 are activated 5–6-fold by LDAO; the activation of the enzyme in *E. coli* membranes is 3-fold. The maximal activity of isolated ECF_1 under standard assay conditions is in the range 140–160 μmol of ATP hydrolyzed min^{-1} (mg of protein)$^{-1}$ with $\geq$0.4% LDAO in the assay buffer. This is the highest turnover rate reported for the enzyme. LDAO releases ECF_1 from the F_0 part of the ATP synthase as a four-subunit enzyme (α, β, γ, and ϵ subunits) at the concentration of detergent used in activation.

The use of LDAO in the assay mixture for ATPase activity is appropriate not only for maximizing activity but also where the site of action of an inhibitor is being explored. A good example is our recent study of the effect of the water-soluble carbodiimide EDC on the ATP synthase (Lötscher et al., 1984b). This reagent at low concentrations was found to inhibit the ATPase activity of the *E. coli* membrane. By assaying of aliquots of the EDC-treated membranes in the absence of LDAO where inhibition of ATPase activity was essentially complete and in the presence of LDAO where inhibition was negligible, it was possible to conclude that the site of EDC reaction was in the F_0 portion of the complex.

This was fully borne out by peptide analysis which established that the site of EDC modification was in subunit c.

It is important to note that the specific activity of ECF_1 isolated in this laboratory is essentially the same as that of ECF_1–F_0 when account is taken of the extra protein associated with the intact ATP synthase complex (ECF_1, M_r 382K; ECF_0, M_r 100K–150K; Senior & Wise, 1983). This indicates that it is not the physical separation of ECF_1 from F_0 alone that increases ATPase activity but rather some structural rearrangement of ECF_1. LDAO is able to reversibly alter isolated ECF_1 from a low ATPase activity to a high ATPase activity form. This activation is not due to release of the δ subunit which is one clear consequence of the LDAO treatment. Enzyme from which the δ subunit has been removed by sucrose density gradient centrifugation in the presence of LDAO can be converted from the high-activity to low-activity form by diluting out the detergent.

Our working hypothesis is that the ϵ subunit modulates the ATPase activity of ECF_1. Several lines of evidence support this proposition. It has been found that the ATPase activity of ECF_1 is concentration dependent, with the highest ATP hydrolytic activities being obtained when the enzyme is diluted to concentrations below the K_m for binding of the ϵ subunit (Laget & Smith, 1979; Sternweis & Smith, 1980). Antibodies to the ϵ subunit have been shown to stimulate ATPase activity as would be expected if they remove loosely bound ϵ from the ECF_1 complex (Laget & Smith, 1979). Finally, it has been shown that the ATPase activity of a reconstituted ECF_1, prepared by mixing purified α, β, and γ subunits, is inhibited by adding purified ϵ subunit (Laget & Smith, 1979; Sternweis & Smith, 1980). This inhibition is about 5-fold and thus in the same range as the activation obtained by LDAO in this study. The ϵ subunit remains bound to ECF_1 in the presence of LDAO as it is not removed by sedimentation in a sucrose gradient. However, it must equilibrate between two or more (possibly overlapping) sites on the ECF_1 when LDAO is present, in order to explain the decrease in cross-linking between β and ϵ in this detergent.

It has been found that trypsin cleavage of ECF_1 enhances ATPase activity (Bragg & Hou, 1975; Smith & Sternweis, 1982), and this is confirmed in the present study. The activation by proteolysis has been attributed to the removal of ϵ from the ECF_1 complex. LDAO is able to activate trypsin-cleaved ECF_1 almost 2-fold. It could be that trypsin cleavage leaves fragments of γ or ϵ bound to the core of the complex and that these affect activity but are removed by LDAO treatment. Alternatively, LDAO may activate ECF_1 through interactions beside the removal of the ϵ subunit.

Registry No. LDAO, 1643-20-5; ATPase, 9000-83-3.

References

Bragg, P. D., & Hou, C. (1975) *Arch. Biochem. Biophys. 167*, 311–321.

Downer, N. W., Robinson, N. C., & Capaldi, R. A. (1976) *Biochemistry 15*, 2930–2936.

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

Dunn, S. D., & Heppel, L. A. (1981) *Arch. Biochem. Biophys. 210*, 421–436.

Foster, D. L., & Fillingame, R. H. (1979) *J. Biol. Chem. 254*, 8230–8236.

Foster, D. L., Mosher, M. E., Futai, M., & Fillingame, R. H. (1980) *J. Biol. Chem. 255*, 12037–12041.

Friedl, P., Friedl, C., & Schairer, H. U. (1979) *Eur. J. Biochem. 100*, 175–180.

Futai, M., & Kanazawa, H. (1983) *Microbiol. Rev. 47*, 285–312.

Hammes, G. G. (1983) *Trends Biochem. Sci. (Pers. Ed.) 8*, 131–134.

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

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

Lötscher, H. R., DeJong, C., & Capaldi, R. A. (1984a) *Biochemistry* (accompanying paper in this issue).

Lötscher, H. R., DeJong, C., & Capaldi, R. A. (1984b) *Biochemistry* (accompanying paper in this issue).

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

Nelson, N., Kanner, B. I., & Gutnick, D. L. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 2720–2724.

Pederson, P. L., Schwerzmann, K., & Cintrŏn, N. (1981) *Curr. Top. Bioenerg. 11*, 149–199.

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

Pullman, M. E., & Monroy, G. C. (1963) *J. Biol. Chem. 238*, 3762–3769.

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

Senior, A. E., & Wise, J. G. (1983) *J. Membr. Biol. 73*, 105–124.

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

Smith, J. B., & Sternweis, P. C. (1982) *Arch. Biochem. Biophys. 217*, 376–387.

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