ATP Synthase

CONDITIONS UNDER WHICH ALL CATALYTIC SITES OF THE F₁ MOIETY ARE KINETICALLY EQUIVALENT IN HYDROLYZING ATP*

(Received for publication, February 1, 1996, and in revised form, August 15, 1996)

Baltazar D. Reynafarje‡ and Peter L. Pedersen

From the Department of Biological Chemistry, The Johns Hopkins University, School of Medicine, Baltimore, Maryland 21205-2185

Conditions have been reported under which the F₁ moiety of bovine heart ATP synthase catalyzes the hydrolysis of ATP by an apparently cooperative mechanism in which the slow rate of hydrolysis at a single catalytic site (unisite catalysis) is enhanced more than 10⁶-fold when ATP is added in excess to occupy one or both of the other two catalytic sites (multisite catalysis) (Cross, R. L., Grubmeyer, C., and Peneisky, H. S. (1982) J. Biol. Chem. 257, 12101–12105). In the novel studies reported here, and in contrast to the earlier report, we have (a) monitored the kinetics of ATP hydrolysis of F₁, by using nucleotide-depleted preparations and a highly sensitive chemiluminescent assay; (b) followed the reaction immediately upon addition of F₁ to ATP, rather than after prior incubation with ATP; and (c) used a reaction medium with Pₐ as the only buffer. The following observations were noted. First, regardless of the source of enzyme, bovine or rat, and catalytic conditions (unisite or multisite), the rates of hydrolysis depend on ATP concentration to the first power. Second, the first order rate constant for ATP hydrolysis remains relatively constant under both unisite and multisite conditions declining only slightly at high ATP concentration. Third, the initial rates of ATP hydrolysis exhibit Michaelis-Menten kinetic behavior with a single Vₘₐₙₐₓ exceeding 100 μmol of ATP hydrolyzed per min/mg of F₁ (turnover number = 635 s⁻¹) and a single Kₘₐₙₐ for ATP of about 57 μM. Finally, the reaction is inhibited markedly by low concentrations of ADP. It is concluded that, under the conditions described here, all catalytic sites that participate in the hydrolysis of ATP within the F₁ moiety of mitochondrial ATP synthase function in a kinetically equivalent manner.

ATP synthase (FₐF₁-ATPase), the enzyme that synthesizes and hydrolyzes the γ-phosphate bond of ATP, is crucial for the life of aerobic organisms. The enzyme resides in the inner mitochondrial membrane of animals, plants, yeast, and Neurospora; in the cytoplasmic membrane of bacteria and in the thylakoid membrane of chloroplasts in plants (see Refs. 1–6 for reviews). In accordance with the chemiosmotic hypothesis (7) the electrical energy of respiration is first conserved as a proton motive force (ΔρHᵗ), which via the F₁ moiety (8) of the synthase delivers the accumulated energy to the β-subunits of the F₁ moiety (α₃β₃γ₆ε), presumably through conformational changes in the stalk. There is a wealth of experimental evidence supporting the view that the step with the largest demand for energy is the one involved in the release of ATP from the catalytic sites of the enzyme. According to basic postulates of the “binding change” mechanism (9–11), there is an equivalent participation of the three β-subunits in the synthesis of ATP as they proceed through a cycle of “open,” “loose,” and “tight” states. Thus, at any one time, all three catalytic sites are in different conformations, but all pass sequentially through the same conformations (9–11). The alternating participation of catalytic sites was shown, for the first time by Adolfsen and Moundrianakis (12) in hydrolytic reactions catalyzed by bacterial F₁-ATPase.

Although the equivalent, alternating participation of catalytic sites within ATP synthases does not require positive cooperativity among them, many investigators believe that such cooperativity does in fact occur. The genesis of this long held view derives primarily from studies (13, 14) in which the F₁ moiety of the bovine heart enzyme was compared under unisite and multisite catalytic conditions, i.e. conditions in which the ATP/F₁ ratio was adjusted so that either one or all sites were operating. The resultant multisite/unisite rate enhancement ratio of 10⁶ was interpreted as reflecting strong positive cooperativity among catalytic sites (13). The bovine heart F₁ preparation used in these experiments contained bound nucleotide (2.8–3.5 mol/mol of F₁) and was prior incubated with ATP (15).

In studies reported here, and for the first time, the kinetics of ATP hydrolysis catalyzed by nucleotide-depleted F₁ have been determined by measuring the entire time course of individual reactions from the moment ATP (in the range from 1 μM to ~20 μM) enters in contact with the catalytic sites until it is totally hydrolyzed. Thus, catalytic sites are open when the experiment is commenced, and data are collected throughout the entire range from unisite through multisite reaction conditions. This study demonstrates that regardless of the ATP/F₁ molar ratio and enzyme source, bovine or rat, that all catalytic sites participating in ATP hydrolysis within F₁ can function in a kinetically equivalent manner.

EXPERIMENTAL PROCEDURES

Sources of Enzymes, Chemicals, and Materials—Bovine heart F₁ was obtained from Drs. William Allison and J. M. Jault (University of California, San Diego). The enzyme was prepared by a modification (15) of the procedure of Knowles and Peneisky (16) and depleted of nucleotides as described by Garrett and Peneisky (17). It was stored prior to use in the refrigerator at 4 °C in 100 mM Tris-Cl, pH 8.0, 4 mM EDTA, and 50% glycerol (v/v). Rat liver F₁ was purified by a modification (18) of the procedure of Catterall and Pedersen (19). The purified enzyme, in 250 mM KP, and 5.0 mM EDTA, was divided into 100-μl aliquots and lyophilized to dryness and stored at −20 °C until use. Immediately before use the enzyme was redissolved in 100 μl of water and precipi-

---

*This work was supported by National Institutes of Health Grant CA 10951 (to P. L. P.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡To whom correspondence should be addressed: Dept. of Biological Chemistry, The Johns Hopkins University, School of Medicine, 725 North Wolfe St., Baltimore, MD 21205-2185. Tel.: 410-955-3827; Fax: 410-614-1944.
tated twice with ammonium sulfate. The bovine heart and rat liver F₁ preparations obtained, respectively, <0.04 (11 determinations) and 0.9 (7 determinations) tightly bound nucleotide/mol enzyme when determined after denaturation by the highly sensitive chemiluminescent assay described below. As it is known that such preparations can bind a total of 5–6 mol of nucleotide/mol of F₁ (20, 21), these preparations are appropriately defined as “nucleotide-depleted.” In fact, the bovine heart F₁ preparation employed here is essentially nucleotide free.

ATPase was a product of Sigma and the 1243–200 ATP monitoring reagent, a mixture of luciferin and luciferase was a product of BioOrbit. The luminometer used in these studies to monitor ATP disappearance was a product of LKB (Wallac model 125), and the attached recorder was a product of Soltac (model 330). [γ-³²P]ATP was purchased from DuPont NEN and its radioactivity assessed in a Beckman LS600IC liquid scintillation counter using Budget solve complete counting mixture (Research Products International Corp.). Activated charcoal (number C4386), prewashed with HCl, was a product of Sigma, and the filtration device (Centricon 100, molecular weight cutoff = 100,000) used to separate F₁ and charcoal-bound [γ-³²P]ATP from ³²P was obtained from Amicon.

Chemiluminescent Method for Monitoring ATP Hydrolysis—The standard reaction medium, in 1 ml final volume at 24 °C, consisted of 200 mM sucrose, 50 mM KCl, 10 mM Na₂P, pH 7.05, 2 mM MgSO₄, and 50 μl of a 5-ml solution in distilled water of the ATP monitoring reagent. ATP in amounts indicated in the legends to the tables and figures was then added to the stirred reaction medium to elicit the chemiluminescent response, followed by the addition of F₁ to initiate the ATPase reaction. The reverse (adding ATP to suspensions of F₁) was also possible without affecting the steady-state kinetics of the reaction. Changes in ATP concentration were monitored with an LKB Wallac model 125 luminometer. The electrical signal elicited by light emission was suitably amplified up to 10,000 times by changes in the current from 10⁻⁶ to 10⁻³ A and the voltage (from 10 V to 1 mV), and recorded using a Soltac model 330 multichannel recorder, usually run at a chart speed of 120 cm/min. The contents of the cell were stirred with a magnetic bar rotating at speeds of near 1000 rpm by means of an electrical device placed on the side of the reaction chamber. The concentration of ATP, in different standard solutions, was spectrophotometrically determined from the absorbance at 259 nm using a millimolar extinction coefficient of 15.4.

As the observed initial rates of decrease of the chemiluminescent signal were always first order with respect to the concentration of ATP (−d[ATP]/dt = k (ATP)), the rate constant “k” was calculated from the integrated form of the equation ln[ATP] = ln[ATP]₀ − kt (Fig. 1) by plotting ln[ATP] versus t at 400-ms intervals. Only steady-state segments (>70% of the reaction) were considered in these calculations (Fig. 2).

Results and Discussion

Response Time and Accuracy of the Luciferin-Luciferase Chemiluminescent Assay in Detecting ATP—In kinetic studies described below, ATP hydrolysis was monitored by following the disappearance of the chemiluminescent signal induced by adding ATP to an assay medium containing luciferin and luciferase. Prior to commencing these experiments, it was important to define the response time of the system for detecting ATP and to establish whether concentrations of ATP could be accurately detected at ratios of ATP/F₁, in the assay ranging from less than 1 to much greater than 1 (i.e. from unisite to multisite conditions). Confirming previous studies of Bovina et al. (7, 24), the results depicted in Fig. 1A (traces a to c) show that, regardless of the amount of ATP (1–20,000 pmol), the response time of the luciferin-luciferase system is close to 500 ms, much faster than the time course of the ATPase reaction at concentrations of ATP and F₁ used in this study. The accuracy of the technique depicted in Fig. 1B, the data of which were derived from over 70 different experiments, shows that the correlation coefficient between light emission and ATP concentration is better than 0.999 at ATP concentrations of ≤15–17 μM ATP. As very low F₁ concentrations are used in the studies described below, the technique allows for accurate ATP measurements to be made with assays in which the ATP/F₁ ratio ranges from −0.1 to −1900. Above 15–17 μM ATP, the extent of the chemiluminescent signal rapidly decreases as the concentration of ATP is increased (Fig. 1), due most likely to the accumulation of dehydroxyluciferin (23). For this reason, and for the reason indicated below, the hydrolytic reaction was initiated by injecting F₁ into the reaction cell already containing ATP.

Time Course of ATP Hydrolysis Catalyzed by either Nucleotide-depleted Bovine Heart or Rat Liver F₁—Three conditions were adhered to in the performance of all experiments. First, nucleotide-depleted preparations of F₁ (bovine heart or rat liver) were used to avoid any possible allosteric effects resulting from nucleotides bound to noncatalytic sites, i.e. sites located predominantly on α-subunits (25). Second, the reaction was initiated by adding F₁, to the reaction mixture already containing ATP to allow the nucleotide free catalytic sites to bind and hydrolyze ATP immediately upon contacting ATP. Third, and in contrast to the earlier studies (13, 14), prior incubation of F₁ with ATP (“aging”) was avoided both to allow for detection of the actual initial rates of ATP hydrolysis and to avoid product (ADP) inhibition that might give rise to “apparent” cooperative kinetics upon addition of excess ATP.

Fig. 2 shows that under the above conditions the initial rates of ATP hydrolysis depend on ATP concentration to the first power as precisely defined by the first order rate equation −d[ATP]/dt = k (ATP). This is true regardless of the source of enzyme (bovine heart or rat liver) or whether the reaction takes place under “unisite” (ATP/F₁ = 0.02, Fig. 2A) or “multisite” (ATP/F₁ = 48.5, Fig. 2B) catalytic conditions. Plots (insets in Fig. 2) of the integrated form of the first order rate equation ln[ATP] = ln[ATP]₀ − kt were used to calculate the first order
rate constant, \( k \), which in turn was used to calculate the turnover number. For example, for the hydrolysis of 1 pmol of ATP by bovine heart F1 (45.9 pmol) the first order rate constant, \( k \), obtained from the plot \( \ln(\text{ATP}) \) versus \( t \) (Fig. 2A, inset) was 0.475 s\(^{-1}\), and the calculated turnover number (1 pmol of ATP \( \times 0.475\) s\(^{-1}\)45.9 pmol of F1) was 0.010 s\(^{-1}\).

**Initial Rates of ATP Hydrolysis by F1 in the Substrate Concentration Range Spanning 1 pmol to 17.1 \mu M ATP**—It has been reported that the F1, moiety of bovine heart F1, catalyzes the hydrolysis of ATP by a mechanism in which the slow rate of hydrolysis at a single catalytic site is enhanced by \(-10^6\)-fold when the ATP concentration is increased by more than \( 3 \times 10^3\)-fold, a process interpreted to result in strong cooperative interactions between catalytic sites (13). The important data leading to this conclusion is actually derived from two separate experiments. The first was conducted under unisite conditions (see Fig. 5 in Ref. 14) in which a turnover number of \( 3.6 \times 10^4 \) s\(^{-1}\) was determined by monitoring the dissociation of ADP at the multisite/unisite ratio of 300 (or 600 s\(^{-1}\)) as described under “Experimental Procedures.” The first order rate constant \( k \) of the reaction was calculated from the slope of the line that results from plotting the integrated form of the first order rate equation: \(-d\text{ADP}/dt = k[\text{ATP}]\) at 0.4-s intervals. A molecular mass for F1, of 371,000 was used in calculating turnover numbers. Values represent averages of duplicate determinations.

| ATP added pmol | First order rate constant \( k \) s\(^{-1}\) | Turnover number | Specific activity ATP hydrolyzed nmol/min/mg protein |
|----------------|------------------------------------------|-----------------|-----------------------------------------------|
| 1              | 0.103                                    | 0.011           | 1.81                                          |
| 2              | 0.103                                    | 0.022           | 3.62                                          |
| 5              | 0.103                                    | 0.056           | 9.07                                          |
| 10             | 0.102                                   | 0.111           | 17.92                                         |
| 20             | 0.103                                   | 0.225           | 37.01                                         |
| 50             | 0.102                                   | 0.560           | 90.03                                         |
| 100            | 0.102                                  | 1.111           | 179.69                                        |
| 200            | 0.102                                  | 2.223           | 359.52                                        |
| 500            | 0.102                                  | 5.559           | 899.07                                        |
| 1000           | 0.101                                  | 11.009          | 1780.42                                       |
| 1710           | 0.100                                  | 18.639          | 3014.34                                       |
| 4275           | 0.096                                  | 44.903          | 7237.75                                       |
| 8550           | 0.090                                  | 83.875          | 13,505.76                                     |
| 17,100         | 0.080                                  | 149.11          | 24,109.96                                     |

In experiments reported here (Tables I and II, Fig. 3), and in contrast to those described above, nucleotide-depleted F1 preparations (bovine and rat) were employed; a single method (chemiluminescent assay) was used to measure both unisite and multisite turnover rates; the entire time course of the reaction was monitored at each ATP concentration as indicated in Fig. 2; and ATP concentration was varied over a wide range. Table I and Table II summarize kinetic data obtained for typical experiments with bovine and rat liver F1, respectively. The first order rate constant for ATP hydrolysis remains relatively constant under both unisite and multisite conditions declining only slightly at high concentrations of ATP. Turnover numbers under unisite conditions range from 0.01 to 0.06 s\(^{-1}\) at the lowest ATP/F1 assay ratios, i.e. from 0.11 to 0.55. Fig. 3, A and B, summarize \( V \) versus ATP and \( 1/V \) versus 1/ATP (Lineweaver-Burk) plots of the data presented in Table I for bovine heart F1. Significantly in neither plot, which spans the range from unisite condition, with an ATP/F1 assay ratio as small as 0.11, to multisite conditions, with an ATP/F1 ratio as high as 1900, is there any indication of sigmoidicity characteristic of cooperative behavior. Rather, it is clear that bovine heart F1 assayed under these conditions exhibits strictly Michaelis-Menten kinetic behavior consistent with the view that all participating catalytic sites are kinetically equivalent. The extrapolated single \( K_m \) is 57 \mu M, and the extrapolated \( V_{max} \) is 103 \mu mol of ATP hydrolyzed per min/mg of F1. The turnover number of 635 s\(^{-1}\) is very close to the value of 600 s\(^{-1}\) obtained under multisite conditions in the earlier study (Ref. 13, see discussion above). Similar results were obtained with rat liver F1 (Table II, Fig. 3B), which exhibits a \( V_{max} \) nearly identical to bovine heart F1 and a slightly higher \( K_m \) of 79 \mu M.

**Comparison of the Chemiluminescent Assay for Monitoring ATP Hydrolysis with an Assay That Monitors Release of 32Pi**—Although the turnover number of 635 s\(^{-1}\) for multisite catalytic conditions reported here for the bovine heart F1 is nearly identical to that reported in the earlier study (13), the multisite turnover numbers at the lowest ATP/F1 ratios are much higher, in the range of 0.01–0.06 s\(^{-1}\) (Tables I and II), rather than near 10\(^{-4}\) s\(^{-1}\) (13, 14). Consequently, data obtained here reflect multihistite/unisite ratios near 10\(^4\) rather than near...
Equivalent Catalytic Sites in ATP Synthase

Reactions were initiated by injecting 29.6 pmol of nucleotide-depleted F1 into a 1-ml chemiluminescent reaction system containing the indicated amount of ATP. Assays were carried out exactly as described under "Experimental Procedures." The first order rate constant (k) of the reaction was calculated from the slope of the lines that result from plotting the integrated form of the first order rate equation \(-\Delta T/P = k\Delta T\) at 0.4-s intervals. Values represent averages of duplicate determinations.

| ATP added (pmol) | First order rate constant (s\(^{-1}\)) | Turnover number | Specific activity ATP hydrolyzed (nmol/min/mg protein) |
|------------------|----------------------------------------|-----------------|------------------------------------------------------|
| 2                | 0.237                                  | 0.016           | 2.532                                                |
| 5                | 0.237                                  | 0.040           | 6.319                                                |
| 7.5              | 0.237                                  | 0.080           | 9.487                                                |
| 10               | 0.237                                  | 0.080           | 12.628                                               |
| 15               | 0.237                                  | 0.120           | 18.974                                               |
| 20               | 0.237                                  | 0.160           | 25.277                                               |
| 25               | 0.237                                  | 0.200           | 31.610                                               |
| 50               | 0.237                                  | 0.400           | 63.193                                               |
| 100              | 0.237                                  | 0.798           | 126.01                                               |
| 250              | 0.237                                  | 1.997           | 315.30                                               |
| 500              | 0.236                                  | 3.976           | 627.67                                               |
| 1000             | 0.234                                  | 7.855           | 1244.72                                              |
| 2500             | 0.230                                  | 19.377          | 3058.99                                              |
| 5000             | 0.223                                  | 37.575          | 5931.79                                              |
| 10000            | 0.211                                  | 71.107          | 11,225.41                                            |

10\(^6\) as reported earlier. For this reason, it might be argued that the chemiluminescent assay, which monitors ATP disappearance, may not accurately report ATP hydrolytic rates at low ATP/F1 assay ratios (unisite conditions). To address this question, we compared the chemiluminescent assay for monitoring ATP hydrolysis under unisite conditions with that of an assay that monitors \(^{32}\)P release from \(\gamma^{32}\)ATP. Two experimental conditions were chosen, one in which the ATP/F1 assay ratio was only 0.11 (lowest data point in Table I), and one in which the ratio was 1.1. The assay medium was identical to that used for monitoring ATP disappearance except that luciferin and luciferase were omitted. Significantly, the specific activities of 2.9 ± 0.21 and 14.5 ± 2.9 nmol of P1 released per min/mg of protein obtained using the \(^{32}\)P release assay (Table III) compare favorably with those of 1.8 and 18 nmol of ATP disappeared per min/mg of protein obtained using the chemiluminescent assay (Table I), for ATP/F1 ratios, respectively, of 0.11 and 1.1. Therefore, it seems clear that the chemiluminescent assay is a reliable indicator of the ATP hydrolytic rate under unisite as well as multisite conditions.

Summary and Mechanistic Implications—In studies described here, the bovine heart F1 preparation was essentially nucleotide-free, and the rat liver F1 preparation contained less than 1 mol/mmol of F1. Both enzymes exhibited strictly Michaelis-Menten kinetic behavior with maximal turnover numbers of 635 s\(^{-1}\) and multisite/unisite rate enhancement ratios near 10\(^6\), consistent with the view that F1-ATPases can catalyze ATP hydrolysis by a mechanism in which all participating catalytic sites are kinetically equivalent. In earlier studies (13, 14), the bovine heart F1 preparations used contained 2.8–3.5 mol of bound nucleotide/mmol of F1. These preparations exhibited maximal turnover numbers of 600 s\(^{-1}\) and multisite/unisite rate enhancement ratios of 10\(^6\), consistent with the view that F1-ATPases can catalyze ATP hydrolysis by a mechanism in which positive cooperativity occurs between catalytic sites. Perhaps the simplest interpretations of the two studies is that, depending on nucleotide content and its subunit distribution, F1_ATPases can exist in different conformational states, one in which all participating catalytic sites are kinetically equivalent and one in which they are kinetically nonequivalent. In support of this view are two different x-ray structures of F1 (25, 26), one in which the catalytic \(\beta\)-subunits appear more structurally equivalent (25) than in the other (26).

Alternative interpretations are possible and require further investigation. One possibility is that F1-ATPases normally function during ATP hydrolysis as simple Michaelis-Menten enzymes and that deviations from this behavior (i.e. sigmoid kinetic behavior), rather than reflecting positive catalytic co-

| ATP added (pmol) | ATP/F1 in assay | Number of experiments | Specific activity P1/min/mg protein |
|------------------|-----------------|----------------------|-----------------------------------|
| 1                | 0.11            | 4                    | 2.9 ± 0.21                        |
| 10               | 1.1             | 10                   | 14.5 ± 2.9                        |
The chemiluminescent assay conditions are exactly as described under “Experimental Procedures” except that the reaction was initiated by adding ATP to a medium containing 9.16 pmol of F$_1$ and an amount of ADP equal to that of the added ATP.

Specifically, as it applies to the earlier studies (13, 14) leading to the view that bovine heart F$_1$ exhibits positive catalytic cooperativity, it is interesting to note that the unisite turnover number of 10$^{-4}$ s$^{-1}$ was based neither on the release of P$_i$ nor ADP following ATP hydrolysis per se, but rather on the dissociation of ADP remaining bound to F$_1$ following hydrolysis (Fig.5 in Ref. 14). Thus, the possibility exists that the unisite turnover number of 10$^{-4}$ s$^{-1}$ obtained from this earlier analysis is not an accurate reflection of the actual value and corresponds to release of ADP from a noncatalytic site. It is important to note that the same investigators in a separate study (Fig. 1 in Ref. 13) find that incubation of [γ-32P]ATP (0.3 μM in 2.5 ml) under unisite conditions with F$_1$ (3 μM in 2.5 ml) for 2 s prior to adding excess ATP results in 20% hydrolysis of the total ATP present. Had these investigators calculated the unisite turnover number under these conditions, they would have obtained a value of 0.01 s$^{-1}$ (i.e. 0.15 μM ATP × 0.20/1.5 μM F$_1$ × 2 s). This value is almost identical to the unisite turnover number of 0.011 s$^{-1}$ reported here as the first entry in Table I and consistent with Michaelis-Menten rather than cooperative kinetic behavior.

Finally, consistent with the studies reported here, recent work on nucleotide-depleted Escherichia coli F$_1$ (27) show that in the ATP concentration range of 1 μM to 1 mM a single $K_m^{\text{uni}}$ value of 38 μM is sufficient for an adequate description of the ATP hydrolytic behavior of the enzyme. However, these authors believe that all catalytic sites (presumably three) must be occupied to achieve significant rates of ATP hydrolysis. Clearly, this is not the case with the nucleotide-depleted F$_1$ preparations from the animal systems examined here, as significant rates of ATP hydrolysis are readily detected at ATP/F$_1$ assay ratios as low as 0.02 (Fig. 2A). Moreover, at 17 μM ATP, well below the $K_m$ of 57 μM, the catalytic turnover number is already 149 s$^{-1}$ (~24 μmol of ATP hydrolyzed per min/mg of F$_1$), as shown in Table I. Thus, nucleotide-depleted animal F$_1$ preparations, in accordance with simple Michaelis-Menten kinetic behavior, show significant rates of ATP hydrolysis at all concentrations of ATP tested.

**Acknowledgments**—We are grateful to Drs. William S. Allison and J. M. Jault, Department of Chemistry, University of California at San Diego for providing nucleotide-free bovine heart F$_1$-ATPase for these studies. We also thank Drs. Albert Mildvan and L. Mario Amzel for many helpful discussions and Dr. Young Hee Ko for critically reading the manuscript prior to its submission. Joanne Hullihen is acknowledged for expert technical assistance.

**REFERENCES**

1. Capaldi, R. A., Agerer, R., Turina, P., and Wilkins, S. (1994) Trends Biochem. Sci. 12, 186–189
2. Pedersen, P. L., and Amzel, L. M. (1993) J. Biol. Chem. 268, 9937–9940
3. Allison, W. S., Jault, J. M., Zhuo, S., and Park, S. R. (1992) J. Bioenerg. Biomembr. 24, 469–477
4. Penefsky, H. S., and Cross, R. L. (1991) Adv. Enzymol. Related Areas Mol. Biol. 64, 173–214
5. Fillingame, R. J. (1990) in The Bacteria (Krubelch, T. A., ed.) Vol. 12 pp. 345–391, Academic Press, New York
6. Senior, A. E. (1988) Physiol. Rev. 68, 177–231
7. Mitchell, P. (1961) Nature 191, 144–148
8. Fillingame, R. H. (1992) J. Bioenerg. Biomembr. 24, 485–491
9. Boyer, P. D., Cross, R. L., and Momeni, W. (1973) Proc. Natl. Acad. Sci. U.S.A. 70, 2837–2839
10. Boyer, P. D. (1979) in Membrane Bioenergetics (Lee, C. P., Schatz, G., and Ernst, L., eds) pp. 451–479, Addison-Wesley Publishing Co., Reading, MA
11. Boyer, P. D. (1989) J. Biol. Chem. 234, 2164–2178
12. Adolfsen, R., and Moudrianakis, E. N. (1976) Arch. Biochem. Biophys. 172, 425–433
13. Cross, R. L., Grubmeyer, C., and Penefsky, H. S. (1982) J. Biol. Chem. 257, 12101–12105
14. Grubmeyer, C., Cross, R. L., and Penefsky, H. S. (1982) J. Biol. Chem. 257, 12092–12102
15. Esch, F. S., and Allison, W. S. (1978) J. Biol. Chem. 253, 6100–6106
16. Knowles, A. F., and Penefsky, H. S. (1972) J. Biol. Chem. 247, 6617–6623
17. Garrett, N. E., and Penefsky, H. S. (1975) J. Biol. Chem. 250, 6640–6647
18. Pedersen, P. L., Hullihen, J., and Wehrle, J. P. (1981) J. Biol. Chem. 256, 1362–1369
19. Catterall, W. A., and Pedersen, P. L. (1971) J. Biol. Chem. 246, 4987–4994
20. Pedersen, P. L., Hullihen, J., Bianchet, M., Amzel, L. M., and Lebowitz, M. S. (1995) J. Biol. Chem. 270, 1775–1784
21. Cross, R. L., and Nalin, C. M. (1982) J. Biol. Chem. 257, 2874–2881
22. Lewry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
23. DeLuca, M., and McElroy, W. D. (1974) Biochemistry 13, 921–925
24. Affalo, C., and DeLuca, M. (1988) J. Biol. Chem. 263, 337–342
25. Bianchet, M., Ysern, X., Hullihen, J., Pedersen, P. L., and Amzel, L. M. (1990) J. Biol. Chem. 266, 2197–2201
26. Abrahams, J. P., Leslie, A. G. W., Lutter, R., and Walker, J. E. (1994) Nature 370, 621–628
27. Weber, J., Wilke-Mounta, S., Lee, R. S.-F., Grell, E., and Senior, A. E. (1993) J. Biol. Chem. 268, 20126–20133