Biochemistry

 Volume 26, Number 13 June 30, 1987

Accelerated Publications

Chloroplast F_1 ATPase Has More Than Three Nucleotide Binding Sites, and 2-Azido-ADP or 2-Azido-ATP at both Catalytic and Noncatalytic Sites Labels the β Subunit†

Zhixiong Xue,‡ Jun-Mei Zhou,‡,§ Teri Melese,‡ Richard L. Cross,‖ and Paul D. Boyer*,‡

Department of Chemistry and Biochemistry and Molecular Biology Institute, University of California, Los Angeles, California 90024, and Department of Biochemistry and Molecular Biology, Health Science Center, State University of New York, Syracuse, New York 13210

Received February 26, 1987; Revised Manuscript Received April 2, 1987

ABSTRACT: The photolabeling of chloroplast F_1 ATPase, following exposure to Mg^{2+} and 2-azido-ATP and separation from medium nucleotides, results in derivatization of two separate peptide regions of the β subunit. Up to 3 mol of the analogue can be incorporated per mole of CF_1, with covalent binding of one moiety or two moieties per β subunit that can be either AMP, ADP, or ATP derivatives. These results, the demonstration of noncovalent tight binding of at least four [^{3}H]adenine nucleotides to the enzyme and the presence of three β subunits per enzyme, point to six potential adenine nucleotide binding sites per molecule. The tightly bound 2-azido nucleotides on CF_1, found after exposure of the heat-activated and EDTA-treated enzyme to Mg^{2+} and 2-azido-ATP, differ in their ease of replacement during subsequent hydrolysis of ATP. Some of the bound nucleotides are not readily replaced during catalytic turnover and covalently label one peptide region of the β subunit. They are on noncatalytic sites. Other tightly bound nucleotides are readily replaced during catalytic turnover and label another peptide region of the β subunit. They are at catalytic sites. No α-subunit labeling is detected upon photolysis of the bound 2-azido nucleotides. However, one or both of the sites could be at an α–β-subunit interface with the 2-azido region close to the β subunit, or both binding sites may be largely or entirely on the β subunit.

The isolated ATPase component of the chloroplast ATP synthase (CF_1)[1] has an $\alpha_3\beta_3\gamma\delta\epsilon$ subunit composition. Catalytic sites are on the β subunit or at an α–β-subunit interface. The enzyme has generally been regarded as having three binding sites for ADP and ATP [see reviews by Strottman and Bickel-Sandkotter (1984), Merchant and Selman (1985), and Galmiche et al. (1985)]. The most extensive studies of individual binding sites have been from Hammes' laboratory [see Leckband and Hammes (1987)]. Their results have shown a tight ADP-binding site, a tight MgATP-binding site, and a loose ADP- or ATP-binding site. Some indications of additional sites on CF_1 have appeared (Shoshan et al., 1978; Anthon & Jagendorf, 1984; Hisabori & Sakurai, 1985) and are suggested by the presence of six sites on MF_1 (Cross & Nalin, 1982) and ECF_1 (Wise et al., 1983).

The nucleotide binding sites on CF_1 have been widely studied, including the demonstration of a pronounced inhibition of ATPase activity by tightly bound ADP (see above reviews). The studies of nucleotide binding reported in this paper are based on the use of 2-azidoadenine nucleotides. Abbott et al. (1984) showed that 2-azido-ADP was a good substrate for

†Supported by U.S. Public Health Service Grants GM 11094 (P.D.B.) and GM 23152 (R.L.C.) and by Department of Energy Contract No. 76 ER70102.

*Author to whom correspondence should be addressed.

‡University of California, Los Angeles.

§Present address: Institute of Biophysics, Academia Sinica, Beijing, China.

‖State University of New York, Syracuse.

[1] Abbreviations: CF_1, MF_1, and ECF_1, the ATPase portion of the ATP synthase from chloroplasts, mitochondria, and *Escherichia coli*, respectively; Tricine, *N*-[tris(hydroxymethyl)methyl]glycine; P_i, inorganic phosphate.

0006-2960/87/0426-3749$01.50/0

photophosphorylation and that photolysis of either tightly bound 2-azido-ADP or tightly bound 2-azido-ATP, formed by photophosphorylation, resulted in labeling of the same large peptide from the partially digested β subunit. The possibility was left open that more than one site within the peptide was labeled.

In the present study, the efficient site labeling attainable by the use of 2-azido-ATP has revealed salient features of nucleotide binding by the CF_1 ATPase that have not been recognized previously. Our data show that the enzyme has more than three and likely six nucleotide binding sites, that tightly bound nucleotides can be present at both catalytic and noncatalytic sites, and that both types of binding sites are located on or are in close contact with different portions of the β subunit.

Experimental Procedures

Preparation of CF_1 and 2-azidoadenine nucleotides was as previously described (Melese & Boyer, 1985). Concentrations of CF_1 were based on an extinction coefficient of 0.483 at 277 nm for 1 mg/mL (Bruist & Hammes, 1981) and a M_r of 400 000 (Moroney et al., 1983). Incubations were made in the presence of 40 mM Tricine, pH 7.8, at room temperature. Measurements of bound nucleotides were made after a single passage through a centrifuge–Sephadex column (Penefsky, 1977). Under the conditions used, negligible amounts of radioactive nucleotide appeared in eluates when CF_1 was omitted. The type and number of residues attached to the β subunit were determined on two-dimensional polyacrylamide gels by measuring the position of electrophoretic migration and the amount of protein in the modified and unmodified spots (Melese & Boyer, 1985). The location of radioactivity was assessed by autoradiography. The reproducibility and definition of such gels allowed good evaluation of the extent and nature of the modifications. Other experimental details are given in the figures and tables.

Conditions for succinylation were essentially as described by Walker et al. (1980). After succinylation of 2–3 mg of CF_1, the protein was precipitated by the addition of $^1/_{10}$ volume of 0.15% (w/v) deoxycholate, followed 10 min later by $^1/_{10}$ volume of 72% (w/v) trichloroacetic acid. After collection by centrifugation, the precipitate was dissolved in 0.5 mL of 0.1 M NH_4HCO_3, pH 8.2, trypsin ($^1/_{40}$th the weight of ATPase) was added, and the sample was incubated at room temperature overnight.

HPLC separations were obtained with a Vydac C-4 protein column, using a Waters system kindly made available to us by Professor David Sigman. Peptides were eluted with a linear gradient of 0–54% (v/v) acetonitrile in 0.1% (w/v) trifluoroacetic acid in water. Fractions were separated manually for analyses.

Results

Extensive Derivatization of the β Subunit by 2-Azido Nucleotides. For most of our studies the covalent derivatization was confined to nucleotides that were sufficiently tightly bound to pass through a centrifuge–Sephadex column. Most of the tightly bound nucleotide becomes covalently attached during repeated or prolonged exposure to ultraviolet light; this gives time for the tetrazole forms to convert to the photoreactive azido form (Czarnecki, 1984). Our first evidence for the presence of more than three nucleotide binding sites come from measurements of the covalent derivatization of the enzyme following prolonged exposure to 2-azido-ATP. These showed that some β subunits were doubly derivatized, indicating a potential presence of up to six binding sites for ADP and ATP on the CF_1 ATPase.

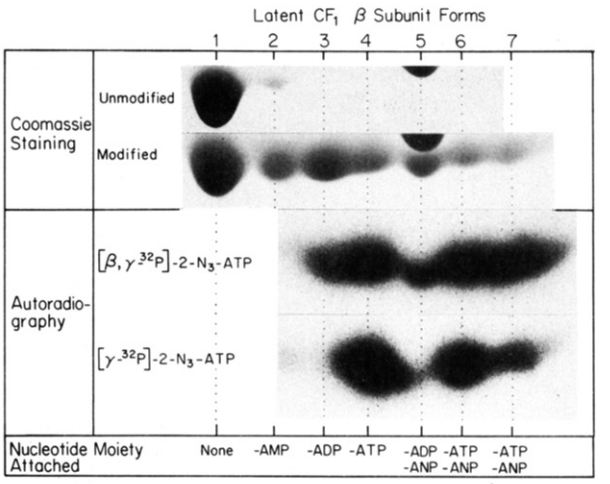

FIGURE 1: Two-dimensional gel patterns and autoradiographs for the β subunits of latent enzyme labeled by exposure to 2-azido-ATP. The top photograph shows the gel pattern for the unlabeled enzyme; the second photograph, the pattern for the latent enzyme exposed to 500 μM 2-azido-ATP and 1 mM Mg^{2+} for 16 h in the dark, followed by centrifuge–Sephadex column separation from unbound nucleotides and 50-min ultraviolet light exposure. In both of these photographs the dark tip of the main α-subunit spot is also evident (column 5). The third and fourth photographs show autoradiographs for similar experiments, but with labeling by [β,γ-^{32}P]- or [γ-^{32}P]-2-azido-ATP. The various forms of the β subunit separated by isoelectric focusing as shown in the second gel photograph are numbered from 1 to 7 in order of increasing migration toward the cathode. The basis for identification of the labeled spots is as follows: (1) Major portion of unmodified subunit. (2) Minor portion of unmodified subunit plus AMP modified; from migration position and presence of ^{3}H when [^{3}H]-2-azido-ATP was used but no ^{32}P when [β,γ-^{32}P]-2-azido-ATP was used. (3) ADP modified; from migration position and presence of ^{32}P when [β,γ-^{32}P]-2-azido-ATP was used and lack of ^{32}P when [γ-^{32}P]-2-azido-ATP was used. (4) ATP modified; from migration position and presence of ^{32}P when [γ-^{32}P]-2-azido-ATP was used. (5) ADP modified plus modification by another nucleotide moiety (ANP), possibly ADP; from migration position and lack of ^{32}P when [γ-^{32}P]-2-azido-ATP was used and the presence of ^{32}P when [β,γ-^{32}P]-2-azido-ATP was used (top dark portion in this region is the bottom of an α subunit spot). (6) ATP modified and likely AMP or possibly ADP modified (ANP); from ^{32}P labeling when [γ-^{32}P]-2-azido-ATP was used and from migration position. (7) ATP modified and likely ADP modified (ANP); from ^{32}P labeling when [γ-^{32}P]-2-azido-ATP was used and from increased ^{32}P labeling compared to spot 6 when [β,γ-^{32}P]-2-azido-ATP was used.

The β-subunit portions of representative two-dimensional gels illustrating these important results are shown in Figure 1. The numbered columns correspond to various forms of β subunits in order of increasing distance of migration toward the cathode. The top photograph shows a typical pattern for the unmodified latent enzyme (the enzyme as isolated without heat activation). In addition to the major component (column 1) a small amount of a species with a slightly lower isoelectric point is evident (column 2), as is the dark lower tip of the main component of the α subunit (column 5). The pattern for the heat-activated enzyme is identical. The second photograph shows the β-subunit region following prolonged exposure of the latent enzyme to 2-azido-ATP in the dark, followed by removal of the unbound reagent and photolysis. No change in migration of the spots for the α subunit was observed (data not shown). However, extensive derivatization of the β subunit is evident from the appearance of a number of new spots. Similar treatment of the heat-activated ATPase resulted in similar patterns but with less extensive derivatization (Figure 2). The bottom two photographs in Figure 1 show the autoradiographs of gels from the latent enzyme labeled in the same manner as the preceding gel but derivatized by either

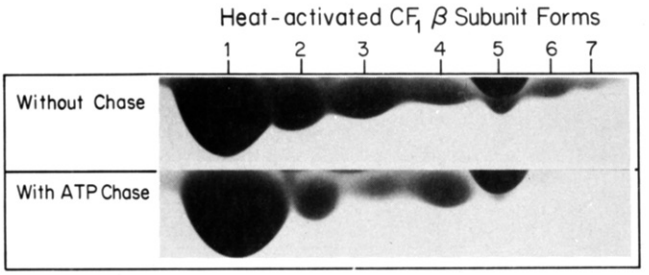

FIGURE 2: Two-dimensional gel separation of the β subunits of heat-activated enzyme labeled by exposure to 2-azido-ATP. The top photograph shows the β-subunit pattern for the enzyme exposed to 200 μM 2-azido-ATP and 1 mM Mg^{2+} for 2 min, followed by centrifuge–Sephadex column separation and 50-min exposure to ultraviolet light. The lower photograph is for a similar experiment, but with a 1-min chase with 5 mM ATP and 2 mM Mg^{2+} prior to the centrifuge–Sephadex column separation. For identification of modifications of the β subunit, see Figure 1.

[β,γ-^{32}P]-2-azido-ATP or [γ-^{32}P]-2-azido-ATP. Incorporation of radioactivity was limited to the β-subunit region of the gel. No ^{32}P was detected in the α-subunit region.

The positions of migration of the protein spots, together with location of the incorporated ^{3}H and ^{32}P from labeled 2-azido nucleotides as indicated in the figure Legend, allow identification of the various modified forms of the β subunit. Extensive covalent labeling results from attachment of AMP, ADP, and ATP moieties both singly and doubly to a β subunit. An important point is that a significant fraction of the β subunits is doubly derivatized. The amounts of the various forms can be quantitated by excision of the spots and dimethyl sulfoxide extraction of the Coomassie Blue stain (Melese & Boyer, 1985; Tal et al., 1985). Such measurements show that up to 25% of the β subunits are modified at two sites (columns 5–7) and up to 61% are modified at one or two sites (columns 2–7). Because modification is not 100% efficient, these are minimal values for the extent of modification. The observation that about one-third of the β subunits remain unmodified likely reflects the incomplete retention of tightly bound nucleotides and their incomplete covalent insertion upon exposure to ultraviolet light.

To ascertain which derivatives resulted from occupancy of catalytic and which from noncatalytic sites, labeling patterns were studied with the heat-activated enzyme after exposure to 2-azido-ATP in the dark with or without subsequent brief catalytic turnover with ATP to remove catalytic site nucleotides (Figure 2). The ATP chase almost eliminates the appearance of doubly labeled β subunits in the photolyzed samples (compare columns 5–7 before and after chase). The fact that the ATP chase decreases the singly labeled ADP spot (column 3) but increases the apparent amount of single ATP labeling (column 4) suggests that 2-azido-ADP binding predominates at catalytic sites and 2-azido-ATP binding at noncatalytic sites. The increase in single ATP labeling could result because 2-azido-ADP is displaced from doubly occupied β subunits (columns 5–7), leaving a β subunit singly occupied with 2-azido-ATP at a noncatalytic site at the time of photolysis. Although we do not detect an ATP moiety bound at catalytic sites, 2-azido-ATP must bind initially, followed by cleavage and release of P_i from the catalytic site before or after covalent modification to give rise to the labeling by an ADP moiety.

The appearance of β-subunit spots derivatized with an AMP moiety (column 2, and possibly column 6, Figures 1 and 2) is a result warranting further investigation. At this stage, whether the AMP moiety is at catalytic or noncatalytic sites or both is unknown, although it appears to be partially chased

Table I: Noncovalent and Covalent Binding of 2-Azido Nucleotides to CF_1

nucleotide moiety	nucleotides per CF_1[a]	
	noncovalent and tightly bound[b]	covalently bound[c]
AMP		0.61 ● 0.03
ADP	1.23 ± 0.05	1.04 ± 0.07
ATP	2.34 ± 0.07	0.93 ± 0.07
total	3.57 ● 0.04	2.58 ● 0.09

[a] Values are means of four or more determinations ± the average of the difference from the mean. [b] From ^{32}P bound as the ATP or ATP + ADP moieties after 16-h exposure of latent CF_1 ATPase to 200 μM [γ-^{32}P]- or [β,γ-^{32}P]-2-azido-ATP and 500 μM Mg^{2+}, followed by centrifuge–Sephadex column separation. [c] Measured after ultraviolet light exposure by two-dimensional gel separation and extraction of Coomassie Blue dyed spots.

with ATP (see Figure 2, columns 2 and 6). It could arise from an inherent adenylate kinase like reaction of CF_1 (Moudrianakis & Tiefert, 1976) or hydrolysis of the attched ADP or ATP moiety.

The extensive covalent labeling obtained indicated that under appropriate conditions more than three tightly but noncovalently bound nucleotides might be present on CF_1. The latent enzyme showed more ability to bind nucleotides tightly and was used for the experiments summarized in Table I. This table gives estimates of the noncovalent retention of the ^{32}P-labeled 2-azido-ADP and 2-azido-ATP after passage through a centrifuge–Sephadex column. Also shown are the amounts of covalently labeled species found after subsequent photolysis. The noncovalent binding of more than three azido nucleotides together with the conversion to a covalently bound form of about 70% of these tightly bound nucleotides, gives evidence for at least four tight nucleotide binding sites on the latent enzyme. These results and other considerations prompted further assessments of binding of ^{3}H-labeled ADP and ATP to the heat-activated enzyme following different types of exposure to [^{3}H]ATP.

More Than Three ^{3}H Nucleotides Can Be Noncovalently Bound to the Heat-Activated Enzyme. For these experiments, conditions were chosen that were considered likely to maximize the amount of tight, noncovalent binding of [^{3}H]ATP and [^{3}H]ADP to the heat-activated enzyme. To favor replacement of unlabeled endogenous nucleotides, heat activation of the latent enzyme was performed as usual in the presence of EDTA but with use of [^{3}H]ATP. Also, in view of data demonstrating a requirement of Mg^{2+} for the tight binding of an ATP to the heat-activated enzyme (Bruist & Hammes, 1981), it seemed plausible that additional retention of nucleotides might be favored by the presence of Mg^{2+}, not only during the exposure to [^{3}H]ATP but also in the centrifuge–Sephadex column during separation. Thus for the experiments reported in Table II, after exposure to [^{3}H]MgATP, unbound ligands were removed by passage through a centrifuge–Sephadex column containing 5 mM Mg^{2+}.

Following heat activation of CF_1 in the presence of [^{3}H]-ATP, the enzyme retains 3.5 tightly bound ^{3}H nucleotides per mole (Table II, experiment 1). Subsequent hydrolysis of ATP to displace nucleotides from catalytic sites reduces the amount bound to about 2 per mole (experiment 2). This suggests that the heat activation results in retention of at least 2 tightly bound nucleotides at noncatalytic sites and at least 1 at catalytic sites. When heat activation in the presence of [^{3}H]ATP is followed by hydrolysis of Mg[^{3}H]ATP, over 4 ^{3}H nucleotides are tightly bound per mole of enzyme (experiment 3) and this binding is reduced to 3 per mole (experiment 4) by hydrolysis

Table II: Nucleotide Binding by CF_1 following Exposure to [^{3}H]ATP during or after Heat Activation of CF_1 and Subsequent Catalytic Turnover[a]

expt no.	conditions	[^{3}H]ANP/F_1 (mol/mol)
1	CF_1 + [^{3}H]ATP + EDTA + heat ‖	3.46 ± 0.03
2	CF_1 + [^{3}H]ATP + EDTA + heat ‖ MgATP ‖	2.07 ± 0.07
3	CF_1 + [^{3}H]ATP + EDTA + heat ‖ Mg[^{3}H]ATP ‖	4.45 ± 0.05
4	CF_1 + [^{3}H]ATP + EDTA + heat ‖ Mg[^{3}H]ATP ‖ MgATP ‖	2.98 ± 0.17
5	CF_1 + ATP + EDTA + heat ‖ Mg[^{3}H]ATP ‖	1.94 ± 0.04
6	CF_1 + ATP + EDTA + heat ‖ Mg[^{3}H]ATP ‖ MgATP ‖	0.85 ± 0.12

[a] CF_1 was heat activated in the presence of 20 mM ATP or 20 mM [^{3}H]ATP and 2 mM EDTA as described by Lien and Racker (1971). Unbound ligand was removed on a centrifuge–Sephadex column (‖) equilibrated with 40 mM Tricine, pH 7.8. In subsequent steps, the enzyme was incubated with 200 μM [^{3}H]ATP and 500 μM $MgCl_2$ in 40 mM Tricine, pH 7.8, at 22 °C for 2 min (denoted as Mg[^{3}H]ATP) or with 5 mM ATP and 2 mM $MgCl_2$ in 40 mM Tricine, pH 7.8, at 22 °C for 2–10 min (denoted as MgATP). Following catalytic turnover, unbound ligand was removed on a centrifuge–Sephadex column equilibrated with 5 mM $MgCl_2$ and 40 mM Tricine, pH 7.8. Values are expressed as the mean of three or more analyses ± the average difference from the mean.

of ATP, as compared to 2 per mole without exposure to Mg[^{3}H]ATP (experiment 2). These results show that the exposure to Mg[^{3}H]ATP labeled an additional noncatalytic site on the enzyme. This site was vacant following heat activation in the presence of EDTA.

When the enzyme is heat activated in the presence of ATP and then exposed to Mg[^{3}H]ATP, two sites per mole are labeled (experiment 5). The ^{3}H nucleotide at one site is readily removed by a chase with MgATP (experiment 6). This shows the labeling of at least one catalytic and one noncatalytic site by [^{3}H]ATP. This is as expected for the tight binding of one Mg[^{3}H]ATP to a noncatalytic site (Bruist & Hammes, 1981) and replacement of one unlabeled nucleotide by a tightly bound ^{3}H nucleotide at a catalytic site.

The observation of more than 3 tightly bound ^{3}H nucleotides per mole after heat activation and more than 4 after exposure to Mg[^{3}H]ATP (experiments 1 and 3, Table II) could have several explanations. More than one catalytic site per mole could retain tightly bound nucleotides as measured by the centrifuge–Sephadex column method. This seems plausible, but does not harmonize well with the observation that slightly less than 2 (1 for a noncatalytic site and 1 for a catalytic site) and not 2.5 nucleotides per mole were retained when exposure to Mg[^{3}H]ATP followed heat activation in the absence of [^{3}H]ATP (experiment 5). Other possible explanations are as follows: (a) the latent enzyme may retain more than 1 tightly bound nucleotide per mole at catalytic sites, and conversion of latent to active enzyme may not be complete; (b) some denatured enzyme may retain higher amounts of poorly replaced nucleotides; (c) some nonspecific tight binding resulted during the heat activation; and (d) unrecognized systematic error(s) could account for the observed ~12% difference (4.5 vs. 4).

Catalytic and Noncatalytic Nucleotides Occupy Different Sites on the β Subunit. The labeling of more than one site on the β subunit by 2-azido-ATP (Figure 1) made experiments to localize the sites worthwhile. Figure 3 gives results of HPLC separation of peptides from tryptic digestion of CF_1 ATPase that had been labeled under different conditions. When the ATPase was covalently labeled following a 30-min exposure to 1 mM 2-azido-ATP, peptides migrating in two different regions were clearly discernible (upper panel, Figure 3). When the enzyme was then allowed to hydrolyze unlabeled ATP for 5 min prior to exposure to ultraviolet light, most of the ^{32}P label was lost from one of the regions (lower panel, Figure 3). These results show that peptide(s) arising from labeling of the noncatalytic sites appears (appear) when solvent B reaches about 18% and that peptide(s) from labeling of catalytic sites appears (appear) when solvent B reaches about 30%. They demonstrate that distinctly different sites are labeled and that little nucleotide bound at noncatalytic sites is replaced with a 5-min chase, while nearly all the nucleotide bound at catalytic sites is replaced.

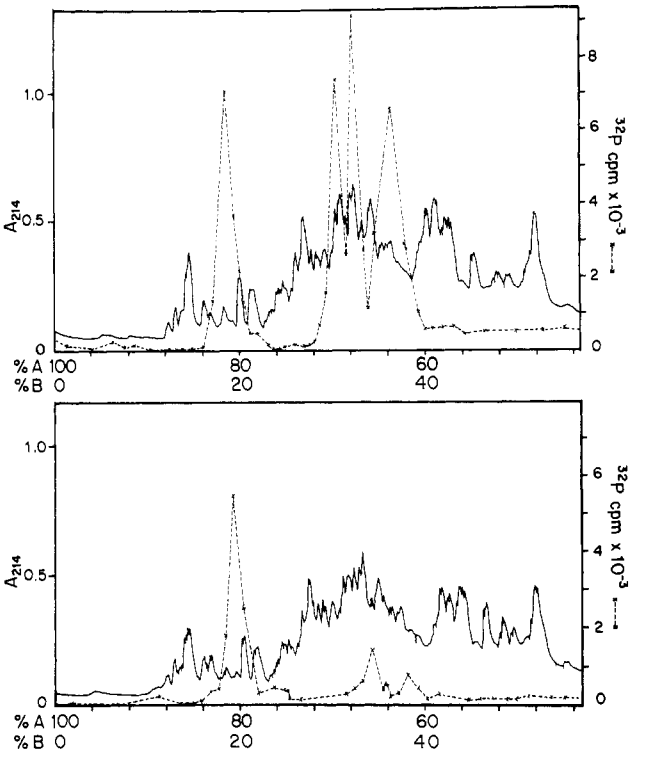

FIGURE 3: Separation by HPLC of peptides labeled by exposure of heat-activated CF_1 to 2-azido-ATP without and with subsequent catalytic turnover with ATP. For the upper panel, the CF_1 ATPase was exposed to 1 mM [β,γ-^{32}P]-2-azido-ATP and 0.8 mM Mg^{2+} for 30 min, followed by a centrifuge–Sephadex column separation. For the lower panel, part of the sample was then exposed to 5 mM ATP and 2 mM Mg^{2+} for 5 min, followed by a second centrifuge–Sephadex column separation. Samples were then exposed to ultraviolet light for 50 min. Conditions for the tryptic digestion and HPLC are given under Experimental Procedures. Solvent A was 0.1% (w/v) trifluoroacetic acid in water and solvent B was 0.1% (w/v) trifluoroacetic acid in 90% (v/v) acetonitrile–water.

Sequence studies on the labeled peptides are in progress. Their migration patterns on HPLC as reported in Figure 3 are closely similar to those observed for analogous derivatization of catalytic and noncatalytic sites of MF_1 (Cross et al., 1987). Their results and those of Garin et al. (1986) show that with the mitochondrial enzyme adjacent tryptic peptides containing tyrosine residues 345 and 368 are prominently labeled by the catalytic and noncatalytic bound nucleotides, respectively.

Discussion

The results establish that two distinct sites on the β subunit of CF_1 can be covalently labeled by exposure to 2-azido-ATP.

As observed for MF_1 (Kironde & Cross, 1987), one site is associated with a catalytic and the other with a noncatalytic function. Azido nucleotides that label the sites are tightly bound prior to photolysis, resulting in high yields of covalent labeling. The tightly bound nucleotides on noncatalytic sites are only slowly replaced during catalytic turnover.

The ability to assess whether tightly bound nucleotides are at catalytic or noncatalytic sites may help clarify a number of earlier observations. These include the lack of replacement of all of a tightly bound ADP fraction during ATP hydrolysis by CF_1 (Bruist & Hammes, 1981; Feldman & Boyer, 1985; Leckband & Hammes, 1987) and the rapid or slow labeling of tightly bound ATP during photophosphorylation (Rosen et al., 1979; Aflalo & Shavit, 1982).

Interpretation of other previous studies may be facilitated by the recognition that both catalytic and noncatalytic tight sites are present on CF_1 and that their occupancy can be modulated by the presence of Mg^{2+} and other conditions. For example, the labeling procedures used by Strotmann et al. (1979) and by Dunham and Selman (1981) involved adding unlabeled ADP and uncoupler to actively phosphorylating preparations just prior to centrifuging and washing of chloroplast thylakoids. Likely the tight binding to catalytic sites was largely missed because only a single turnover would be necessary to remove labeled nucleotide. The interpretation of studies from our laboratory stressing a transient tight binding at catalytic sites in contrast to data from other laboratories emphasizing a control function of tightly bound nucleotides is clarified by the recognition that up to six binding sites may be present, with three of them tight, slowly exchangeable noncatalytic sites.

The data of Table II show that the heat-activated enzyme in the presence of EDTA binds two nucleotides tightly at noncatalytic sites. In addition, in accord with the data of Bruist and Hammes (1981), the enzyme also has a noncatalytic site empty that can readily and tightly bind ATP in the presence of Mg^{2+}. The nucleotide bound at this noncatalytic site, in contrast to that at the tight catalytic site, is not readily replaced during catalytic turnover (Table II). This property is akin to that of nucleotide bound to a noncatalytic site of MF_1; such nucleotide is readily exchanged in the presence of EDTA but is tightly bound when excess Mg^{2+} is present (Kironde & Cross, 1986).

One or both of the sites on the β subunit that are labeled by the azido analogues could be at an interface of the α and β subunits with the 2-azido region in close proximity to the β subunit. That the location of one of the sites may be at an interface is also suggested by the demonstration that the 8-azido-ADP labels both the α and β subunits (Wagenvoord et al., 1981). On the other hand, both sites could be on the β subunit in analogy with the demonstration of Gromet-Elhanen and Khananshvili (1984) of two nucleotide binding sites on the isolated β subunit from *Rhodospirillum rubrum*.

That there are at least four sites on CF_1 that can bind ADP or ATP relatively tightly is shown by retention of up to 4.5 ^3H nucleotides per CF_1 under conditions that favor maximum bound nucleotide replacement and site filling (Table II) and by the extensive photolabeling that can be obtained (Figure 1, Table I). In addition to the direct binding data, the presence of two distinct sites on the β subunits that can be labeled and of three β subunits per enzyme points to the probability of six sites per mole. Our interpretation of these and other data is that there are three noncatalytic and three catalytic sites on the CF_1 and that tightly bound nucleotides can be bound to the three noncatalytic sites and to at least one catalytic site at any one time. This correlates well with the behavior of MF_1 (Cross & Nalin, 1982) and ECF_1 (Wise et al., 1983).

Registry No. 2-N_3-ATP, 72884-75-4; 2-N_3-ADP, 64020-53-7; ADP, 58-64-0; ATP, 56-65-5; ATPase, 9000-83-3.

References

Abbott, M. S., Czarnecki, J. J., & Selman, B. R. (1984) *J. Biol. Chem. 259*, 12271–12278.

Alflalo, C., & Shavit, N. (1982) *Eur. J. Biochem. 126*, 61–68.

Anthon, G. E., & Jagendorf, A. T. (1984) *Biochim. Biophys. Acta 766*, 354–362.

Bruist, M. F., & Hammes, G. G. (1981) *Biochemistry 20*, 6298–6305.

Cross, R. L., & Nalin, C. M. (1982) *J. Biol. Chem. 257*, 2874–2881.

Cross, R. L., Cunningham, D., Miller, C. G., Xue, Z., Zhou, J.-M., & Boyer, P. D. (1987) *Proc. Natl. Acad. Sci. U.S.A.* (in press).

Czarnecki, J. J. (1984) *Biochim. Biophys. Acta 800*, 41–51.

Dunham, K. R., & Selman, B. R. (1981) *J. Biol. Chem. 256*, 212–218.

Feldman, R. I., & Boyer, P. D. (1985) *J. Biol. Chem. 260*, 13088–13094.

Galmiche, J. M., Girault, G., & Lemaire, C. (1985) *Photochem. Photobiol. 41*, 707–713.

Garin, J., Boulay, F., Issartel, J.-P., Lunardi, J., & Vignais, P. V. (1986) *Biochemistry 25*, 4431–4437.

Gromet-Elhanan, Z., & Khananshvili, D. (1984) *Biochemistry 23*, 1022–1028.

Hisabori, T., & Sakurai, H. (1985) *Plant Cell Physiol. 26*, 505–514.

Kironde, F. A. S., & Cross, R. L. (1986) *J. Biol. Chem. 261*, 12544–12549.

Kironde, F. A. S., & Cross, R. L. (1987) *J. Biol. Chem. 262*, 3488–3495.

Leckband, B., & Hammes, G. G. (1987) *Biochemistry 26*, 2306–2312.

Lein, S., & Racker, E. (1971) *Methods Enzymol. 23*, 547–555.

Melese, T., & Boyer, P. D. (1985) *J. Biol. Chem. 260*, 15398–15401.

Merchant, S., & Selman, B. (1985) *Photosynth. Res. 6*, 3–31.

Moroney, J. V., Lopreseti, L., McEwan, B. F., McCarty, R. E., & Hammes, G. B. (1983) *FEBS Lett. 158*, 58–62.

Moudrianakis, E. N., & Tiefert, M. A. (1976) *J. Biol. Chem. 251*, 7796–7801.

Penefsky, H. S. (1977) *J. Biol. Chem. 252*, 2891–2899.

Rosen, G., Gresser, M., Vinkler, C., & Boyer, P. D. (1979) *J. Biol. Chem. 254*, 10654–10661.

Shoshan, V., Shavit, N., & Chipman, D. M. (1978) *Biochim. Biophys. Acta 504*, 108–122.

Strotmann, H., & Bickel-Sandkotter, S. (1984) *Annu. Rev. Plant Physiol. 35*, 97–120.

Strotmann, H., Bickel-Sandkotter, S., & Shoshan, V. (1979) *FEBS Lett. 101*, 316–326.

Tal, M., Silberstein, A., & Nusser, E. (1985) *J. Biol. Chem. 260*, 9976–9980.

Wagenvoord, R. J., Verschoor, G. J., & Kemp, A. (1981) *Biochim. Biophys. Acta 634*, 229–236.

Walker, J. E., Carne, A. F., Runswick, M. J., Bridgen, J., & Harris, J. I. (1980) *Eur. J. Biochem. 108*, 549–565.

Wise, J. G., Duncan, T. M., Latchney, L. R., Cox, D. N., & Senior, A. E. (1983) *Biochem. J. 215*, 343–350.