ISSN 0006-2979, Biochemistry (Moscow), 2007, Vol. 72, No. 7, pp. 728-734. © Pleiades Publishing, Ltd., 2007.
Published in Russian in Biokhimiya, 2007, Vol. 72, No. 7, pp. 897-904.
Originally published in Biochemistry (Moscow) On-Line Papers in Press, as Manuscript BM07-049, May 27, 2007.

Interaction of ADP and ATP with Noncatalytic Sites of Isolated and Membrane-Bound Chloroplast Coupling Factor CF_1

A. N. Malyan

Institute of Basic Biological Problems, Russian Academy of Sciences, 142290 Pushchino, Moscow Region, Russia; fax: (0967) 330-532; E-mail: Malyan@ibbp.psn.ru

Received March 2, 2007
Revision received March 30, 2007

Abstract—This study of ATP and ADP binding to noncatalytic sites of membrane-bound CF_1 (ATP synthase) revealed two noncatalytic sites with different specificities and affinities for nucleotides. One of these is characterized by a high affinity and specificity to ADP (K_d = 2.6 ± 0.3 μM). However, a certain increase in ADP apparent dissociation constant at high ATP/ADP ratio in the medium allows a possibility that ATP binds to this site as well. The other site displays high specificity to ATP. When the ADP-binding site is vacant, it shows a comparatively low affinity for ATP, which greatly increases with increasing ADP concentration accompanied by filling of the ADP-binding site. The reported specificities of these two sites are independent of thylakoid membrane energization, since both in the dark and in the light the ratios of ATP/ADP tightly bound to the noncatalytic sites were very close. The difference in noncatalytic site affinity for ATP and ADP is shown to depend on the amount of δ-subunit in a particular sample. Thylakoid membrane ATP synthase, with stoichiometric content of δ-subunit (one δ-subunit per CF_1 molecule), showed the maximal difference in ADP and ATP affinities for the noncatalytic sites. For CF_1, with substoichiometric δ-subunit values, this difference was less, and after δ-subunit removal it decreased still more.

DOI: 10.1134/S0006297907070061

Key words: ATP synthase, CF_oCF_1, chloroplast coupling factor CF_1, noncatalytic sites, chloroplasts

In chloroplasts, mitochondria, and bacteria, ATP synthase consists of F_1, its peripheral catalytic part, and F_o responsible for transmembrane transport of protons (sodium ions). F_1 includes subunits α, β, γ, δ, and ε with a stoichiometry of 3 : 3 : 1 : 1 : 1. The minimal composition of F_o implies *a*-, *b*-, and *c*-subunits with a ratio of 1 : 2 : 10 [1]. At the interface between α- and β-subunits, there are six nucleotide binding sites; three of these are "noncatalytic" and located mostly on the α-subunit, while three others are catalytic and hosted by the β-subunit [2]. According to modern knowledge on the mechanism of functioning of ATP-synthases of various origin [3, 4], the transmembrane proton transport is coupled with rotation of protein that contains *c*-subunits associated with γ- and ε-subunits. Rotation of the γ-subunit induces successive structural changes in the three catalytic sites that provide ADP and phosphate binding, their ATP condensation, and ATP dissociation. This process is reversible, since ATP hydrolysis causes rotation of the γ-, ε-, and *c*-subunits and appearance of a transmembrane proton (ion) gradient. Rotation of the α- and β-subunits is prevented by δ- and *b*-subunits, which link the peripheral and membrane parts of the ATP synthase complex. Isolated F_1 catalyzes ATP hydrolysis. In the presence of ADP and Mg^{2+}, it turns inactive [5, 6] with differentiation of properties of its catalytic sites: one of these tightly binds MgADP, another MgATP, and the third one displays much lower affinity for nucleotides [6, 7]. A high ATP concentration or polybasic acid anions added to the reaction mixture can cause dissociation of MgADP and re-activation of F_1-ATPase [8-12].

Evidence is reported [9, 13-20] that the effect of ATP or anions is underlain by their association with F_1 noncatalytic sites. In the absence of transmembrane gradient of electrochemical potentials H^+ ($\Delta\mu_{H^+}$), the effect of ADP and Mg^{2+}, as well as of sulfite (the most efficient anion), on CF_oCF_1 ATPase activity is similar to their effect on CF_1 [21-23], although the extent of inactivation

Abbreviations: CF_oCF_1) chloroplast ATP synthase; DTT) dithiothreitol; PMS) phenazine methosulfate; TCA) trichloroacetic acid.

is notably lower [24]. Generation of $\Delta\mu_{H^+}$ results in dissociation of ADP tightly bound to a catalytic site [25], and switches ATP synthase to active "energized" state [22, 25, 26]. The rate of ADP dissociation is comparable with that of phosphorylation [27]. The ability of F_1 and CF_1 noncatalytic sites to affect ATPase activity suggests a possibility of their participation in regulation of ATP synthase activity, which brings their properties to the focus of our studies.

As we showed previously, energization of the thylakoid membrane causes binding of labeled nucleotides to CF_oCF_1 noncatalytic sites [28]. In the absence of nucleotides, energy-dependent dissociation of endogenous nucleotides was observed in the medium, and its rate was about two orders of magnitude lower than the rate of dissociation from catalytic sites [27, 28]. The literature reports on properties of CF_1 and CF_oCF_1 noncatalytic sites are contradictory. According to [29], one site of CF_1 heat-activated in the presence of ADP predominantly binds ATP, while the other binds ADP. Heat-activated CF_1, as well as thiol-activated CF_1, can bind at its noncatalytic sites from 2.2 to 2.8 molecules of ADP or ATP [18, 29]. This means that there is no marked difference between specificities displayed by CF_1 noncatalytic sites. As reported by Possmayer and colleagues [30], noncatalytic sites of isolated ATP synthase bind only ATP, and each displays a dramatically different affinity. However, kinetic studies of nucleotide binding to thylakoid membrane ATP synthase [31] showed that with medium ADP replaced by ATP, total nucleotide incorporation into noncatalytic sites decreases, which demonstrates a comparatively low affinity of these sites for ATP. The difference in properties of noncatalytic sites may be underlain by asymmetric interactions between α-subunits and γ- or δ-subunits, since for every three α-subunits there is one γ- or δ-subunit. Unlike the γ-subunit that holds the central position relative to α-subunits, the δ-subunit is at the periphery of ATP synthase, and it links CF_1 with the membrane through only one of the three α-subunits [32].

To elucidate noncatalytic site specificity and its probable dependence on membrane components of ATP synthase, the current study considers effect of the medium ATP/ADP ratio on composition of nucleotides incorporated into noncatalytic sites of isolated and membrane-bound CF_1. The effect of membrane energization on composition of bound nucleotides, as well as influence of the δ-subunit on the ratio of ADP to ATP incorporated into noncatalytic sites of isolated CF_1, were also subjects of this study.

MATERIALS AND METHODS

Chloroplast thylakoid membranes were isolated from pea leaves as described by Semenova et al. [33] with a modification described earlier [28]. Chlorophyll concentration was determined according to Arnon [34]. The chloroplast coupling factor CF_1 isolated according to Binder et al. [35] was purified by HPLC with a DEAE 5PW column and stored in 2 M ammonium sulfate in the presence of 1 mM ATP, 1 mM EDTA, and 50 mM Tris-SO_4 (pH 7.8). δ-Deficient CF_1 (CF_1-δ) was obtained by incubation of CF_1 in glycerol–ethanol buffer followed by separation of the δ-subunit using a DEAE 5PW column as described by Richter et al. [36]. Free nucleotides were removed by forced gel filtration using a fine Sephadex G-50 column equilibrated with 50 mM Tris-HCl, pH 7.8, and 50 mM KCl. CF_1 was activated in the presence of 2 μM thioredoxin and 2 mM dithiothreitol (DTT) at room temperature for 30 min. The protein concentration was determined according to Bradford [37]. The molecular weight of CF_1 was assumed to be 400 kD [38]. Nucleotide to CF_1 binding was performed in 50 μl medium containing 50 mM Tris-HCl, pH 7.8, 2 mM $MgCl_2$, 50 mM KCl (TKM-buffer), and [α-^{32}P]ATP or [α-^{32}P]ADP (in some experiments [^{14}C]ADP was used). To maintain [α-^{32}P]ATP concentration, the incubation medium also contained pyruvate kinase and phosphoenolpyruvate. Cessation of binding of labeled nucleotides and their selective dissociation from catalytic sites were performed using the "cold chase" technique of Boyer [39]. Nucleotides were separated by thin layer chromatography [40]. SDS-PAGE of CF_1 preparations was performed according to Laemmli [41]. The gels were stained with 0.05% Coomassie Brilliant Blue R-250 in 14% TCA for 18 h at room temperature and destained with 14% acetic acid.

Thylakoid membranes were illuminated with 560 W/m^2 photosynthetically active radiation for 5 min in a reaction medium containing 20 mM Tricine-KOH (pH 7.8), 0.2 M sucrose, 20 mM KCl, 1 mM $MgCl_2$ (TSKM-buffer), 50 μM PMS (phenazine methosulfate), 10 mM DTT, and 0.2 mg/ml chlorophyll; then they were precipitated by centrifugation and resuspended to give a final chlorophyll concentration of 1-2 mg/ml. To remove endogenous nucleotides, the medium was diluted 1 min before the end of preincubation with 5-10 volumes of TSKM-buffer. Nucleotide binding to CF_0F_1 of thylakoid membranes was performed as follows: the membranes were illuminated with white light (560 W/m^2) at room temperature in 0.7-1.4 ml reaction medium containing [α-^{32}P]ADP or [α-^{32}P]ATP, TSKM buffer, 50 μM PMS, and 100-200 μg/ml chlorophyll. Nucleotides were selectively dissociated from catalytic sites using a modified "cold chase" technique described earlier [28]. The reaction medium was supplemented with solution (0.1 of its volume) containing 22 mM ATP, 0.66 M $KHSO_3$ (pH 7.8), 50 μM diadenosine pentaphosphate, and 50 μM gramicidin D. After 2 min, the mixture was centrifuged for 1 min at 10,000*g*. To remove free nucleotides, the pellet obtained after centrifugation was washed three times and resuspended in 90 μl buffer containing 0.2 M sucrose,

50 mM Tricine-KOH (pH 7.8), 50 mM KCl, and 5 mM $MgCl_2$. For chlorophyll determination, 20-40 μl of the resultant suspension was utilized, and the rest was used for nucleotide extraction by 0.5 M $HClO_4$ followed by neutralization with KOH. Chromatographic separation of nucleotides from $HClO_4$ extracts was carried out by thin layer chromatography. The nucleotide content was counted from radioactivity of nucleotide spots. Data processing was performed using Origin 6.0 (Microcal Software).

RESULTS

Figure 1 shows CF_1 noncatalytic site filling versus ADP and ATP concentrations, and Fig. 2 presents similar curves for CF_oCF_1. As we showed previously, the incubation time required for equilibrium filling of CF_1 noncatalytic sites at nucleotide concentrations above 2 μM is never longer than 30 min [19]. Nucleotide binding to CF_oCF_1 noncatalytic sites takes no more than 5 to 7 min [28]. With varying ATP, the medium ATP/ADP ratio was maintained by an ATP-regenerating system at the level of 12-18 for 30 min. At CF_1 noncatalytic sites, this ratio was 5-7, with a slight increase at higher ATP concentrations (Fig. 1). With varying ADP, the medium ATP/ADP ratio was as low as 0.06-0.08, while for nucleotides bound to noncatalytic sites, it reached a value of 0.08-0.12. The Origin 6.0-computed apparent constant of ATP dissociation is 2.5 ± 0.1 μM, that for ADP is 1.0 ± 0.1 μM. In spite of different affinities for noncatalytic sites, the maximal binding levels of both nucleotides were close to each other. The bound nucleotides appeared to contain up to 5% AMP. However, addition of 4-fold AMP excess to the incubation medium produced no marked effect on ATP or ADP incorporation into the noncatalytic sites. Most probably, AMP forms at the step of tightly bound nucleotide extraction that follows perchloric acid treatment.

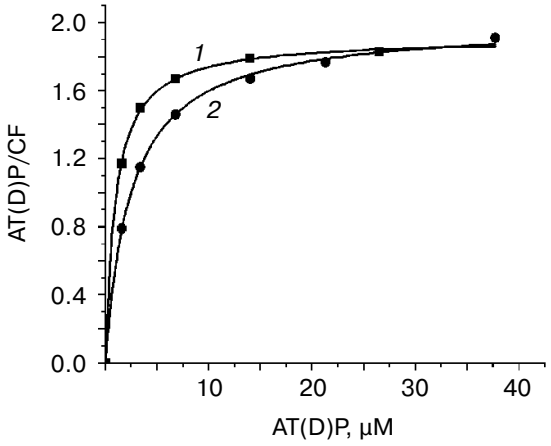

Fig. 1. Concentration dependence of nucleotide binding to CF_1 noncatalytic sites. Thioredoxin-reduced CF_1 (0.08-0.14 mg/ml) was incubated with various concentrations of [α-^{32}P]ADP (*1*) or [α-^{32}P]ATP (*2*) in medium containing 50 mM Tris-HCl, pH 7.8, 2 mM $MgCl_2$, and 50 mM KCl for 30 min. To maintain ATP concentration, the incubation medium contained 1 mM phosphoenol pyruvate and 0.18 mg/ml pyruvate kinase (*2*). To remove labeled nucleotides from the catalytic sites, 1.5 mM unlabeled ATP and 50 mM Na_2SO_3 were added. After 0.5 min, the enzyme was separated from free nucleotides using a Sephadex G-50 column (superfine). The quantity of nucleotides tightly bound to noncatalytic sites was determined as described under "Materials and Methods".

When it comes to ATP synthase of a light-energized thylakoid membrane, the relationship between nucleotide binding and nucleotide composition and concentration is quite different. With varying ATP, the complete saturation was still not achieved even at an ATP concentration of 40.6 μM (Fig. 2, curve *1*). The calculated value of the dissociation constant was 36 ± 9 μM. In spite of 14-fold excess ATP in the medium, ADP binding exceeded that of ATP and occurred within a far lower range of concentrations (Fig. 2, curve *2*). Under these conditions, the apparent dissociation constant of ADP was 5.7 ± 2.1 μM. The calculated maximal binding values for these nucleotides were close and amounted to 0.19 ± 0.04 nmol/mg of chlorophyll. With varying ADP (the medium ATP/ADP ratio ranging from 0.11 to 0.15), the difference in site filling by ATP and ADP, as dependent on their concentrations, was minor (Fig. 2, curves *3* and *4*). The apparent dissociation constant of ADP was 2.6 ± 0.3 μM. The maximal filling value for each nucleotide was 0.22 ± 0.01 nmol/mg of chlorophyll, which, within the limits of experimental error, is the value observed under varying ATP conditions. With varied low concentrations of each nucleotide, the bound ATP/ADP ratio ranged from 0.2 to 0.3, tending to reach 1 at high concentrations. Over the entire range of ATP and ADP concentrations, i.e., for the 100-fold change in the medium ATP/ADP ratio, excess ATP has no effect on the maximal ADP incorporation, and conversely, excess ADP has no effect on the maximal ATP binding by ATP synthase, with both maximal values being virtually equal (about 0.2 nmol/mg of chlorophyll). This suggests that ATP and ADP either do not compete with each other or do that only slightly, each associating with its own specific site.

As known, light energization of the thylakoid membrane produces a considerable effect on nucleotide binding to catalytic and noncatalytic sites of ATP synthase [27, 28]. To learn the extent of membrane energization-determined difference in the described above specificity of noncatalytic sites of CF_1 and CF_oCF_1, we studied nucleotide binding under conditions of a de-energized thylakoid membrane. Thylakoid membranes isolated by a commonly used technique contain about 4 moles of nucleotides per mole of ATP synthase [42]; out of these 2 moles are attributed to noncatalytic sites.

The previously found thylakoid membrane capability of dissociating nucleotides from ATP synthase noncat-

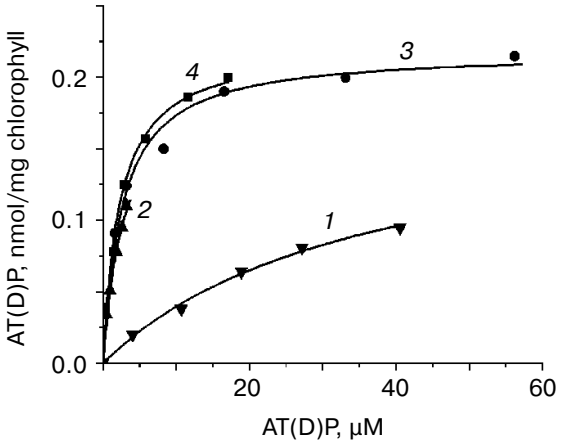

Fig. 2. Concentration dependence of nucleotide binding to noncatalytic sites of ATP synthase. Light- and DTT-activated thylakoid membranes (0.05-0.15 mg chlorophyll/ml) were incubated with various concentrations of [α-^{32}P]ATP or [α-^{32}P]ADP in medium containing TSKM-buffer (pH 7.8) and 50 μM PMS for 7 min. Determination of nucleotide binding to noncatalytic sites was performed as described under "Materials and Methods".

alytic sites [28] underlies selection of conditions providing the enzyme with vacant noncatalytic sites. Since a long light exposure of membranes notably decreases their phosphorylation activity, DTT treatment of ATP synthase was performed concurrently with dissociation of endogenous nucleotides from noncatalytic sites.

As seen from Fig. 3 (curve *1*), at a medium chlorophyll concentration of about 1.5 mg/ml, the subsequent dark incubation yielded only insignificant incorporation of nucleotides into noncatalytic sites. An increase in chlorophyll concentration up to 4.1 mg/ml gave a still lower value of the incorporation (not shown). Optimal conditions were provided by 5 min DTT reduction at 0.2 mg/ml with 5- to 10-fold dilution of the reaction mixture with TKM buffer one minute prior to light cessation (curve *2*). In some samples of thylakoid membranes, such a procedure allowed achieving in the dark the level of light nucleotide incorporation, while mostly, the level of dark incorporation reached 60-80% of the light one (curves *2-4*). This suggests that the increased ability of ATP synthase to incorporate nucleotides into noncatalytic sites after light preincubation at low chlorophyll concentrations may result from a decreased concentration of endogenous nucleotides after dilution of the reaction mixture. To verify this suggestion, the 5-fold mixture dilution was accompanied by addition of 10 μM ADP, which almost completely inhibited the dark incorporation of nucleotides (not shown). The comparison of light incorporation of labeled nucleotides into vacant (curve *4*) and filled (curve *3*) noncatalytic sites shows that previous dissociation of endogenous nucleotides from these sites notably accelerated binding of the radioactive marker. This supports the previous suggestion [28] that the light-dependent incorporation of labeled nucleotides into ATP synthase noncatalytic sites is limited by dissociation of endogenous nucleotides, and in fact, results from their exchange with nucleotides from the reaction mixture. Unlike the light conditions, very slow dark dissociation makes incorporation of labeled nucleotides into ATP synthase noncatalytic sites virtually irreversible [28], which prevents the use of dissociation constants in characterizing the nucleotide affinity for noncatalytic sites.

The analysis of ATP and ADP binding to CF_1 noncatalytic sites revealed that this process comprises a fast and reversible step of loose binding followed by a slow step of tight binding [18]. If this is true also for noncatalytic sites of ATP synthase, then the relative affinity of ATP and ADP may be judged from the tightly bound ATP/ADP ratio, or more specifically, from the difference between of the ratio of ATP and ADP incorporated into noncatalytic sites and their ratio in the incubation mixture. Indeed, in isolated CF_1 the composition of tightly bound nucleotides differed only slightly from that in the mixture (see above), and dissociation constants of ATP and ADP were only 2.5-fold different. In ATP synthase, the composition difference between free and bound nucleotides was many times larger, and their dissociation constants differed greatly. In the experiment shown in Fig. 3, the tightly bound ATP/ADP ratio at noncatalytic sites was almost the same both in the light and in the dark,

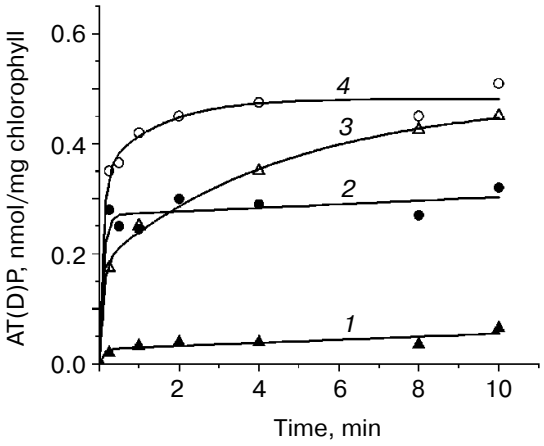

Fig. 3. Effect of chlorophyll concentration at the activation step on labeled nucleotide binding to noncatalytic sites of chloroplast ATP synthase. Thylakoid membranes were incubated in the light with 10 mM DTT for 5 min at chlorophyll concentrations 1.5 mg/ml (curves *1* and *3*) or 0.15 mg/ml (curves *2* and *4*). One minute before light cessation, the mixture was diluted with five volumes of TSKM-buffer. Activated membranes were incubated in the dark (curves *1* and *2*) or in the light (curves *3* and *4*) in the medium containing TSKM-buffer, 9.2 μM [^{14}C]ADP, and 50 μM PMS or 5 μM gramicidin D. Each 1-ml aliquot taken at indicated intervals was mixed with 100 μl of solution containing 22 mM ATP, 0.66 M $KHSO_3$ (pH 7.8), 50 μM diadenosine pentaphosphate, and 50 μM gramicidin D. The quantity of nucleotides tightly bound to noncatalytic sites was determined as described under "Materials and Methods".

Effect of the δ-subunit on ATP/ADP ratio at the CF_1 noncatalytic sites

Enzyme	ATP/ADP in the medium*	AT(D)P/CF_1, mol/mol	ATP/ADP at the noncatalytic sites
CF_1	0.33 ± 0.02 (3)	0.18 ± 0.03	0.15 ± 0.02
CF_1-δ	0.30 ± 0.02 (3)	0.16 ± 0.03	0.32 ± 0.04

Note: CF_1 (0.31 mg/ml) and CF_1-δ (0.16 mg/ml) were incubated for 15 min in the medium containing 50 mM Tris-HCl, pH 7.8, 50 mM KCl, 0.8 μM [^{14}C]ATP, 2.0 μM [^{14}C]ADP, and 2 mM $MgCl_2$. Subunit abundance ratio was determined with the TotalLab v.2.01 program.

* Mean value of ATP/ADP ratio at the beginning and at the end of incubation. Values in parentheses are the numbers of repeats.

and with 1.5-fold excess ATP its value was 0.2-0.3. Such a significant difference between the ATP/ADP ratios in the medium and at noncatalytic sites is typical of a membrane-bound enzyme and indicates that a lower affinity of noncatalytic sites for ATP observed in moving from CF_1 to ATP synthase is caused by binding of isolated CF_1 to membrane components of ATP synthase, but not by light energization of the thylakoid membrane.

Noncatalytic sites are located on α-subunits that via the δ-subunit interact with two *b*-subunits (in terms used for chloroplast ATP synthase, subunits I and II) which pertain to the membrane part of the complex. It is believed that only one of the three α-subunits interacts with the δ-subunit [32]. With high energy of the interaction taken into account [43], it is likely that the structure of this subunit is affected, and hence, the noncatalytic site that it hosts is affected too. Since a number of δ-subunits are known to remain bound to the membrane after isolation of the coupling factor, we used CF_1 samples with various δ-subunit contents to learn the role of this subunit in nucleotide binding properties of the enzyme. The initial CF_1 contained 0.4 mole of δ-subunit per mole of CF_1 (denoted as CF_1). After treatment according to [36], the δ-subunit content decreased 4-fold, with 10% loss of the ε-subunit amount (denoted as CF_1-δ, Fig. 4). As seen from the table, the ratio of ATP to ADP at CF_1-δ noncatalytic sites is very close to that in the medium, while at the sites of initial CF_1 the ATP proportion is almost two times lower. This is indicative of a lower affinity for ATP displayed by the CF_1 noncatalytic site, as compared to that of CF_1-δ.

DISCUSSION

The concentration dependence of ATP and ADP incorporation into noncatalytic sites of ATP synthase with various medium ATP/ADP ratios reveals two sites different in their specificity and affinity for nucleotides. One of these displays high affinity (K_d = 2.6 ± 0.3 μM) and specificity for ADP. However, a certain increase in ADP apparent dissociation constant (K_d = 5.1 ± 2.0 μM)

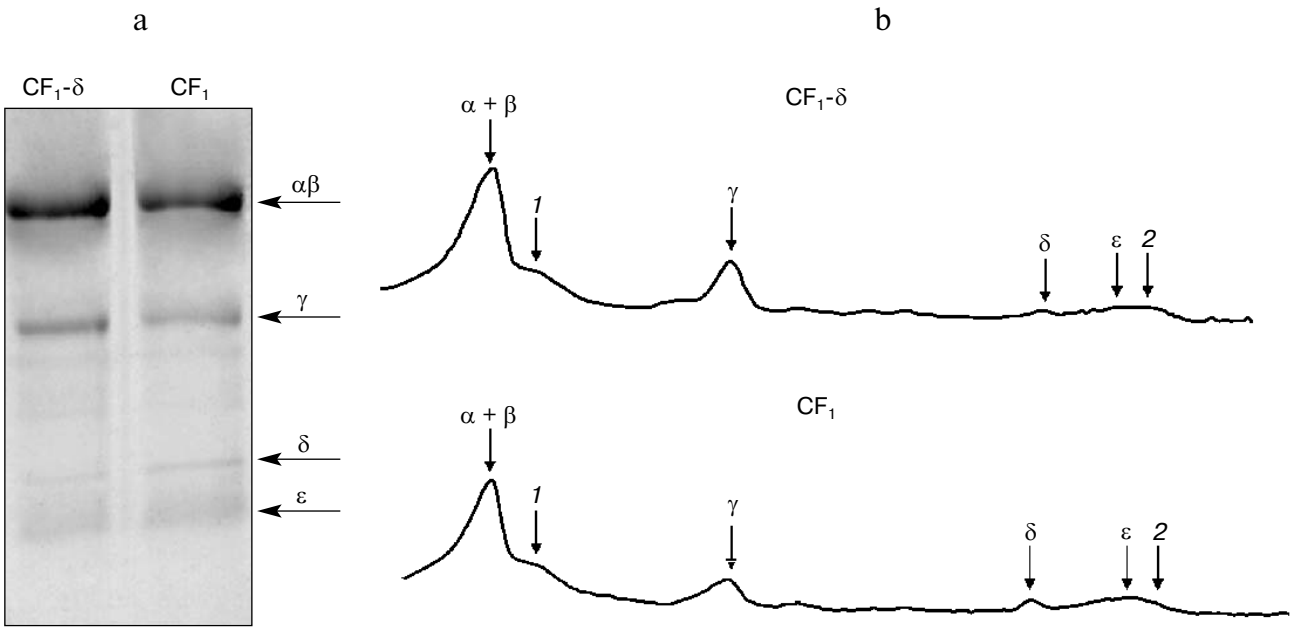

Fig. 4. SDS-PAGE (a) and densitograms (b) of CF_1 preparations. CF_1 or CF_1-δ aliquots equivalent to 12 and 15 μg of protein, respectively, were applied on polyacrylamide gel; electrophoresis was performed as described under "Materials and Methods". Peaks *1* and *2* correspond to large and small subunits of ribuloso-1,5-bisphosphate carboxylase.

at high ATP/ADP ratio in the medium suggests the possibility that ATP binds to this site as well. The other site exhibits a high specificity to ATP. Its affinity for ATP is comparatively low ($K_d = 36 \pm 9$ μM) when the ADP-binding site is vacant, but it rapidly grows with increasing ADP concentration and filling of the ADP-binding site. This distribution of specificities between the two sites seems independent of ATP synthase energization because there is no difference between dark and light bound ATP/bound ADP ratios, and regardless of excess ATP in the medium, it is ADP that predominantly binds to the enzyme.

The values of dissociation constants that we obtained for high medium ATP/ADP ratio are quite close to those (about 50 and 2 μM) reported in [30] for noncatalytic sites of isolated chloroplast ATP synthase (sites 6 and 5, respectively, in terms used by the authors). These were derived from azido-ATP concentrations providing semimaximal site filling after 5 min incubation. Nucleotide incorporation into a third noncatalytic site was neither detected in this study, nor reported in the literature [30]. It is believed [30] that the dissociation constant of this site is equal to a fraction of a nanomole. If this is true, ATP dissociation may require an energy level exceeding thylakoid membrane energization. The latter can be estimated from free energy change of ATP synthesis and in physiological conditions reaches (as well as the corresponding value of $\Delta\mu_{H^+}$ on the membrane) about −12.5 kcal/mol [44]. It is easily calculated that energy of ATP dissociation from the site with a constant below 1 nM amounts to the same or even higher value (above −12.3 kcal/mol), and hence, may be insufficient for light-dependent ATP dissociation from the most tightly binding noncatalytic site.

According to our results, one of the noncatalytic sites of thylakoid membrane ATP synthase predominantly binds ADP even with excess ATP available (Fig. 2, curves *1* and *2*). At its appropriate site of isolated ATP synthase, Tyr385-bound nucleotide was composed of 80% ATP and only 20% ADP [30]. We believe that this difference may be caused by detergents used in ATP synthase isolating procedure that weaken intersubunit interactions. Here it is worth mentioning that the corresponding site of chloroplast coupling factor isolated without detergents displayed a higher affinity for ADP than for ATP (see Fig. 1 and [29]). As it follows from the current study, the δ-subunit plays an important role in differentiation of properties of the noncatalytic sites. Its removal lessens the difference in specificity of CF_1 noncatalytic sites, while the greatest difference was observed for thylakoid membrane samples with a δ-subunit content approaching the stoichiometric value.

The author thanks A. S. Pronin for help in preparation of figures. The study was supported by the Russian Foundation for Basic Research (grant 06-04-49315-a).

REFERENCES

1. Jiang, W., Hermolin, J., and Fillingame, R. H. (2001) *Proc. Natl. Acad. Sci. USA*, **98**, 4966-4971.
2. Abrahams, J. P., Leslie, A. G. W., Lutter, R., and Walker, J. E. (1994) *Nature*, **370**, 621-628.
3. Boyer, P. D. (1993) *Biochim. Biophys. Acta*, **1140**, 215-250.
4. Senior, A. E., Nadanaciva, S., and Weber, J. (2002) *Biochim. Biophys. Acta*, **1553**, 188-211.
5. Minkov, I. B., Fitin, A. F., Vasilyeva, E. A., and Vinogradov, A. D. (1979) *Biochem. Biophys. Res. Commun.*, **89**, 1300-1306.
6. Malyan, A. N., and Vitseva, O. I. (1983) *Biokhimiya*, **48**, 718-724.
7. Malyan, A. N., and Vitseva, O. I. (1990) *Photosynthetica*, **24**, 613-622.
8. Vasilyeva, E. A., Fitin, A. D., Minkov, I. B., and Vinogradov, A. D. (1980) *Biochem. J.*, **188**, 807-815.
9. Jault, J. M., and Allison, W. S. (1993) *J. Biol. Chem.*, **268**, 1558-1566.
10. Lambeth, D. O., and Lardy, H. A. (1971) *Eur. J. Biochem.*, **22**, 355-369.
11. Nelson, N., Nelson, H., and Racker, E. (1972) *J. Biol. Chem.*, **247**, 6506-6510.
12. Malyan, A. N., and Akulova, E. A. (1978) *Biokhimiya*, **43**, 1206-1211.
13. Xue, Z., and Boyer, P. D. (1989) *Eur. J. Biochem.*, **179**, 677-681.
14. Milgrom, Y. M., Ehler, L. L., and Boyer, P. D. (1990) *J. Biol. Chem.*, **265**, 18725-18728.
15. Matsui, T., Muneyuki, E., Honda, M., Allison, W. S., Dou, C., and Yoshida, M. (1997) *J. Biol. Chem.*, **272**, 8215-8221.
16. Murataliev, M. B., and Boyer, P. D. (1992) *Eur. J. Biochem.*, **209**, 681-687.
17. Dou, C., Grodsky, N. B., Matsui, T., Yoshida, M., and Allison, W. S. (1997) *Biochemistry*, **36**, 3719-3727.
18. Malyan, A. N., and Allison, W. S. (2002) *Biochim. Biophys. Acta*, **1554**, 153-158.
19. Malyan, A. N., and Vitseva, O. I. (2001) *Biochemistry (Moscow)*, **66**, 505-510.
20. Malyan, A. N. (2003) *Biochim. Biophys. Acta*, **1607**, 161-166.
21. Carmeli, C., and Lifshitz, Y. (1972) *Biochim. Biophys. Acta*, **267**, 86-95.
22. Bakker-Grunvald, T., and van Dam, K. (1974) *Biochim. Biophys. Acta*, **347**, 290-298.
23. Larson, E. M., and Jagendorf, A. T. (1989) *Biochim. Biophys. Acta*, **973**, 67-77.
24. Malyan, A. N., Vitseva, O. I., and Gubanova, O. N. (1998) *Photosynth. Res.*, **57**, 297-303.
25. Graber, P., Schlodder, E., and Witt, H. T. (1977) *Biochim. Biophys. Acta*, **461**, 426-440.
26. Schafer, G. (1982) *FEBS Lett.*, **139**, 271-275.
27. Lohse, D., and Strotmann, H. (1989) *Biochim. Biophys. Acta*, **976**, 94-101.
28. Malyan, A. N. (2005) *Biochemistry (Moscow)*, **70**, 1245-1250.
29. Milgrom, Y. M., Ehler, L. L., and Boyer, P. D. (1991) *J. Biol. Chem.*, **266**, 11551-11558.
30. Possmayer, F. E., Hartog, A. F., Berden, J. A., and Graeber, P. (2001) *Biochim. Biophys. Acta*, **1510**, 378-400.
31. Malyan, A. N. (2006) *Photosynth. Res.*, **88**, 9-18.

32. Ogilvie, I., Aggeler, R., and Capaldi, R. A. (1997) *J. Biol. Chem.*, **272**, 16652-16656.
33. Semenova, G. A., Agafonov, A. V., and Opanasenko, V. K. (1996) *Biochim. Biophys. Acta*, **1285**, 29-37.
34. Arnon, D. J. (1949) *Plant Physiol.*, **24**, 1-5.
35. Binder, A., Jagendorf, A. T., and Ngo, E. (1978) *J. Biol. Chem.*, **253**, 3094-3100.
36. Richter, M. L., Patrie, W. J., and McCarty, R. E. (1984) *J. Biol. Chem.*, **259**, 7371-7373.
37. Bradford, M. M. (1976) *Analyt. Biochem.*, **72**, 248-254.
38. Moroney, J., Lopresti, L., McEwen, B. F., McCarty, R. E., and Hammes, G. G. (1983) *FEBS Lett.*, **158**, 58-62.
39. Xue, Z., and Boyer, P. D. (1989) *Eur. J. Biochem.*, **179**, 677-681.
40. Shigalova, T., Lehmann, U., Krevet, M., and Strotmann, H. (1985) *Biochim. Biophys. Acta*, **809**, 57-65.
41. Laemmli, U. K. (1970) *Nature*, **227**, 680-685.
42. Harris, D. A., and Slater, E. C. (1975) *Biochim. Biophys. Acta*, **387**, 335-348.
43. Weber, J., Wilke-Mounts, S., and Senior, A. E. (2002) *J. Biol. Chem.*, **277**, 18390-18396.
44. Lehninger, A. L. (1976) *Biochemistry. The Molecular Basis of Cell Structure and Function* (Bayev, A. A., ed.) [Russian translation], Mir, Moscow, pp. 335-360.