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Structure of the active metal-adenosine 5′-triphosphate chelate complex used by light-triggered chloroplast ATPase

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By employing phosphorothioate analogs of ATP in the presence of Mg^{2+} and Mn^{2+} as substrates in ATP hydrolysis, catalyzed by light and dithiothreitol-activated chloroplast ATPase, the structure of the reactive metal-nucleotide complex has been determined. Mg(S_P)-ATPαS and Mn(S_P)-ATPαS, in contrast to the corresponding R_P-isomers, are substrates in ATP hydrolysis. No metal-dependent change of specificity was observed. Mg(S_P)-ATPβS, having the Δ configuration, and Mn(S_P)-ATPβS and Mn(R_P)-ATPβS, consisting of a mixture of Δ and Λ configurations, were better substrates than Mg(R_P)-ATPβS, the isomer with almost exclusive Λ chelate structure. The same results were obtained when the competitive effect of the analogs on hydrolysis of ATP was studied. The competitive effect of the diastereomers on tight binding of ATP by membrane-associated CF_1 was investigated in the presence of Mg^{2+} and Cd^{2+}. Mg(S_P)-ATPβS and Cd(R_P)-ATPβS, which both exhibit Δ structure, were more effective than Mg(R_P)-ATPβS and Cd(S_P)-ATPβS, showing the Λ configuration. No metal-dependent change of the preferred S_P-ATPαS specificity was detected. These results permit the conclusion that the actual substrate used by chloroplast ATPase is the β,γ-Δ-bidentate nucleotide chelate. Moreover, a stereospecific direct ionic interaction between the protein and α-phosphate is likely.

Introduction

ATP hydrolysis catalyzed by the 'light-triggered' ATPase of thylakoid membranes, like photophosphorylation, requires Mg^{2+} ions [1,2], suggesting that the Mg-ATP chelate complex may be the actual substrate in this enzyme reaction.

Under physiological conditions divalent cations are chelated to the phosphate chain of ATP. Different stereoisomers of these chelate complexes should exist because the α- and β-phosphorus become chiral upon coordination. It is not possible to tell how many isomers exist in a solution or to discriminate them because the various coordination isomers of Mg-ATP equilibrate more rapidly than the NMR time-scale [3]. It has been shown, however, by several examples, that ATP-dependent enzymes usually accept a specific configuration of the nucleotide-metal ion complex [4]. This problem can be experimentally approached with metal-nucleotide complexes of ATP or ATP analogs in which the ligand exchange is lowered (Co^{3+} and Cr^{3+} complexes of ATP) [3,5,6] or with nucleotide analogs containing a fixed chirality at the α- or β-phosphorus by chemical modification. In the present paper, phosphorothioate analogs of ATP were employed which contain one sulfur atom instead of one of the non-bridging oxygen

Abbreviations: ATPαS, adenosine 5′-*O*-(1-thiotriphosphate); ATPβS, adenosine 5′-*O*-(2-thiotriphosphate); FCCP, carboxyl cyanide *p*-trifluoromethoxyphenylhydrazone; NTP, nucleotide triphosphate; Tricine, *N*-tris(hydroxymethyl)methylglycine; CF_1, coupling factor 1.

atoms either in the α- or β-phosphate group [7]. ATPαS and ATPβS each exist in the form of two diastereomers (A and B) which can be separated [8]. Their absolute configurations have been determined [9]: the A forms of ATPαS and ATPβS have the S_P-configuration, while the B forms have the R_P-configuration. The existence of different screw sense Me^{2+} complex structures is based on the fact that various metal ions show different tendencies to coordinate either to oxygen or to sulfur.

Jaffe and Cohn [10] found by ^{32}P-NMR studies, that in phosphorothioates Mg^{2+} preferentially coordinates to oxygen and Cd^{2+} to sulfur while Mn^{2+}, Co^{2+} and Zn^{2+} coordinate to both. Therefore, the β,γ-Mg complex of S_P-ATPβS (A-form) is equivalent to the Cd-complex of R_P-ATPβS (B-form) with respect to the geometry of the phosphate ligands to the metal ion or screw sense of the chelate. According to Cornelius and Cleland [6,11] Mg(S_P)-ATPβS and Cd(R_P)-ATPβS each have Δ screw sense, while Mg(R_P)-ATPβS and Cd(S_P)-ATPβS both show Λ screw sense if the metal remains coordinated to the phosphorothioate during the enzymatic reaction. The stereospecificity of the enzyme for one of the diastereomers in the presence of Mg^{2+} would be inverted when Mg^{2+} was replaced by Cd^{2+}. This has been demonstrated for a series of ATP-utilizing enzymes [4,12]. If the isomer selectivity, however, is independent of the metal ion employed, this may be taken as an indication that the nucleotide is bound to the catalytic site without coordinated metal ion. As Mn^{2+} can coordinate to both sulfur and oxygen, Mn(S_P)-ATPβS and Mn(R_P)-ATPβS exist as mixtures of compounds with both Λ and Δ screw sense. Therefore Mn^{2+} should abolish a stereospecificity of an enzyme reaction exhibited in the presence of Mg^{2+}.

The diastereomers of ATPαS and ATPβS have been employed as substrates for chloroplast ATPase together with Mg^{2+}, and a preference of the enzyme for the two S_P-isomers has been reported [13]. In the light-triggered ATPase reaction, only Mn^{2+} is able to replace Mg^{2+}, while in the presence of either Cd^{2+} or Co^{2+} no activity could be measured. The divalent metal specificity of tight nucleotide binding compared to ATPase activity is low, and Mg^{2+} can be replaced by many other cations, also by Cd^{2+}, which is employed in the present study. Recently, it was independently demonstrated by two groups [14,15,16] that the three nucleotide-binding sites present on CF_1 are identical with regard to their protein structure, suggesting that all of them can assume the properties of a catalytically active site as well as the conformation of a binding site containing a tightly bound nucleotide [17]. This may justify the present study in order to learn more about the properties of the catalytic site by investigating the stereospecificity of the binding of substrate.

The results presented here allow conclusions to be drawn about the actual structure of the substrate in ATP hydrolysis catalyzed by CF_1 and in the process of nucletide binding.

Experimental

Chloroplasts were isolated from spinach leaves as described earlier, but without adding Mg^{2+} [18].

The chloroplasts were washed once with isolation medium and a further three times with a medium (A) which comprised 50 mM NaCl/2 mM Tricine buffer (pH 7.8). For experiments with cations other than Mg^{2+}, the chloroplasts were washed once with 50 mM NaCl/2 mM Tricine (pH 7.8)/5 mM EDTA (pH 8.0) and a further two times with medium A.

The phosphorothioate analogs of ATP employed here (ATPαS, A- and B-form, ATPβS A- and B-form) were gifts from Prof. Dr. F. Eckstein. The NH_4^+ salts of the analogs were changed into the Na^+ salts by column chromatography on Dowex 100–200 mesh (Na^+ form) and then lyophilized.

Hydrolysis of ATP and ATP analogs by light-triggered chloroplasts was followed in a medium containing 25 mM Tricine (pH 8)/50 mM NaCl/ 50 μM *N*-methylphenazonium methosulfate, 5 mM dithiothreitol and the indicated concentrations of $MgCl_2$ or $MnCl_2$. After addition of chloroplasts (50 μg/ml) the light was switched on for 2 min. The nucleoside triphosphates were added immediately after illumination. The final volume was 0.2 ml and the temperature 25°C. After the indicated time in the dark, the samples were deproteinized by 1 M perchlorid acid. P_i was determined colorimetrically in aliquots of the supernatants [19].

Inhibition of ATP-hydrolysis by phosphorothioate analogues was carried out under identical conditions, except that γ-^{32}P-labeled ATP was used and added together with the phosphorothioates immediately after switching off the light. In this case the $^{32}P_i$-contents were assayed in the isobutanol/toluol extracts of the phosphomolybdate complex [20]. γ-^{32}P-labeled ATP was synthesized by photophosphorylation of ADP as described in [21]. $^{32}P_i$ was purchased from Amersham Buchler.

Binding of [^{14}C]ATP by light-triggered chloroplasts was carried out in the dark after 1 min illumination followed by the addition of 5 μM (8-^{14}C ATP) (Amersham Buchler, spec. act. 50 μCi/μmol) together with the indicated analog and metal ions after 15 s in the dark. The medium was identical to that described above except that no dithiothreitol was added, the final volume was 0.25 ml and the temperature 21°C. After the indicated time in the dark, ATPase activity was interrupted by adding a quenching solution which comprised 10 mM ADP/50 μM FCCP/5 mM EDTA (pH 8.0) [22]. The final chlorophyll concentration was about 0.2 mg/ml. In order to remove the free label, the pellets were washed three times with washing solution [18]. The radioactivity of the washed resuspended pellets was measured by liquid scintillation counting [18].

Results

ATPαS and ATPβS as substrates of light-triggered ATPase

In this section, the kinetic constants in light-triggered hydrolysis by chloroplasts of the four diastereomers of ATPαS and ATPβS in the presence of Mg^{2+} and Mn^{2+} are compared.

In Table I K_m and V_{max} values for ATP and the phosphorothioate analogs are presented. In case of the S_P- and R_P-ATPαS, no change in specificity could be detected by replacing Mg^{2+} by Mn^{2+} as cation, as shown by the ratios $V_{max}(S_P)/V_{max}(R_P)$, which are comparable for both metal ions. While S_P-ATPαS exhibits about one-third of the maximal velocity obtained with ATP, R_P-ATPαS is an extremely poor substrate for the chloroplast ATPase with Mg^{2+} as well as with Mn^{2+} as the chelating ion. The rates of hydrolysis of ATPβS isomers with either metal ion are very low compared to those of ATP and S_P-ATPαS. For this reason the corresponding K_m values can not be determined with satisfying confidence. The $V_{max}(S_P)/V_{max}(R_P)$ ratios of ATPβS are 12 in the case of the Mg^{2+}-chelated isomer and 1.4 in the case of the Mn^{2+} chelates, i.e. there is a significant change in the reactivity of these thiophosphate analogs when employing Mn^{2+} instead of Mg^{2+}.

TABLE I

KINETIC CONSTANTS FOR ATP AND THE PHOSPHOROTHIOATE ANALOGS IN THE PRESENCE OF Mg^{2+} AND Mn^{2+}

The concentrations of $MgCl_2$ and $MnCl_2$ were 1 mM, the concentrations of the indicated nucleotides were varied between 0.02 and 5 mM.

Nucleotide	K_m (μM)	V_{max} (μmol P_i/ mg Chl per h)	$\frac{V_{max}(S_P)}{V_{max}(R_P)}$
Mg-ATP	97	107	
Mn-ATP	84	92	
Mg-(S_P)-ATPαS	185	39	≥ 19.5
Mg-(R_P)-ATPαS	-	≤ 2	
Mn-(S_P)-ATPαS	160	26	≥ 17.3
Mn-(R_P)-ATPαS	-	≤ 1.5	
Mg-(S_P)-ATPβS	-	12	≥ 12
Mg-(R_P)-ATPβS	-	≤ 1	
Mn-(S_P)-ATPβS	-	7	1.4
Mn-(R_P)-ATPβS	-	5	

ATPαS and ATPβS as competitor of ATP hydrolysis

In Table II the effects of each of the two isomers of ATPαS and ATPβS on light-induced dark hydrolysis of [γ-^{32}P]ATP are shown. These experiments demonstrate that all phosphorothioate analogs compete with ATP on the catalytic site of ATPase, with S_P-ATPαS (A) as the strongest (apparent K_i 20 μM) and R_P-ATPαS (B) as the weakest inhibitor (K_i 250 μM). Again, no significant change in the effectiveness of the α-thiophosphate analogs could be observed when Mn^{2+} was employed instead of Mg^{2+} as the divalent metal ion. Surprisingly the apparent K_i values of the β-isomers are comparatively low, indicating relatively high affinities in spite of the low

activities as substrates (Table I). The largely inactive β-isomers, obviously, are able to bind nonproductively to the enzyme. The apparent K_i for R_P-ATPβS is, by a factor of about 2, larger than the K_i for S_P-ATPβS. In the case of the β-isomers, Mn^{2+} induces a small change of the $K_i(R_P)/K_i(S_P)$ ratio towards 1.

Competitive inhibition of [^{14}C]ATP binding by ATPαS and ATPβS

The kinetics of tight binding of ATP or ADP by CF_1 can be followed by incorporation of the radioactively labeled nucleotide in the dark after preillumination of thylakoids [23]. Under such conditions, ADP binding is a time- and concentration-dependent irreversible reaction which leads to deactivation of the ATPase [24]. ATP binding which does not cause inactivation [22] under the experimental conditions employed here (preillumination in the absence of dithiothreitol) likewise is an essentially irreversible reaction. ADP as well as ATP binding are, to some extent, independent of added divalent metal ions. However, the initial rates of nucleotide incorporation are enhanced by Mg^{2+}. The divalent cation specificity is low, Mg^{2+} can be replaced by other ions like Mn^{2+}, Co^{2+}, Ca^{2+} (not shown) without a loss of binding activity. A slightly lower but significant stimulation of ATP binding is observed in the presence of Cd^{2+}.

TABLE II

EFFECTS OF PHOSPHOROTHIOATE ANALOGS OF ATP ON LIGHT-INDUCED DARK HYDROLYSIS OF [^{32}P]ATP

The concentration of [^{32}P]ATP was varied between 25 and 500 μM; concentrations of $MgCl_2$ and $MnCl_2$ were 1 mM; for other details see Experimental.

ATP analog	Apparent K_i (μM)	$\frac{K_i(R_P)}{K_i(S_P)}$
Mg-(S_P)-ATPαS	19	
Mg-(R_P)-ATPαS	240	12.6
Mn-(S_P)-ATPαS	20	
Mn-(R_P)-ATPαS	256	12.8
Mg-(S_P)-ATPβS	45	
Mg-(R_P)-ATPβS	82	1.8
Mn-(S_P)-ATPβS	101	
Mn-(R_P)-ATPβS	122	1.2

In Table III initial rates of binding of [^{14}C]ATP with Mg^{2+}, Cd^{2+} and without divalent cations (EDTA-washed chloroplasts) are shown. Since Cd^{2+} might reverse a possible stereoselectivity of the enzyme for ATPαS and ATPβS, this reaction may be of particular interest for the determination of the relevant complex structure.

In a previous paper [13], the competitive type of inhibition of [^{14}C]ATP binding by the thiophosphate analogs has been demonstrated in the presence of Mg^{2+}. These experiments are extended here by including Cd^{2+} as well as the controls obtained in the complete absence of divalent cations. Table IV shows C_{150} values of the two pairs of diastereomers for the inhibition of the initial rate (measured after 20 s) of binding of [^{14}C]ATP.

The initial velocities of the two competing reactions

$$E + ATP \xrightarrow{k} E\text{-}ATP \quad (1)$$

$$E + NTP \xrightarrow{k'} E\text{-}NTP \quad (2)$$

are given by

$$v_0 = k \cdot [E_0] \cdot [ATP] \quad (3)$$

and

$$v_0' = k' \cdot [E_0] \cdot [NTP] \quad (4)$$

where k and k' mean apparent rate constants and $[E_0]$ the initial concentration of the nucleotide-de-

TABLE III

INITIAL RATES OF [^{14}C]ATP BINDING IN THE DARK AFTER PRE-ILLUMINATION OF CHLOROPLASTS WITH Mg^{2+}, Cd^{2+} AND WITHOUT DIVALENT CATIONS

The concentration of [^{14}C]ATP was 5 μM and the concentration of $MgCl_2$ and $Cd(NO_3)_2$ were 1 mM.

	Initial rate (nmol [^{14}C]ATP bound/mg Chl·h)	$t_{1/2}$ (s)	k (1/mmol·s)
Without cations	20.7	120	1.2
+ Cd^{2+}	31.9	94	1.5
+ Mg^{2+}	39.2	65	2.1

TABLE IV

EFFECTS OF PHOSPHOROTHIOATE ANALOGS OF ATP ON DARK RE-BINDING OF [^{14}C]ATP AFTER PRE-ILLUMINATION OF CHLOROPLASTS

The concentration of [^{14}C]ATP was 5 μM; divalent cation concentration 1 mM; and nucleotide analogs were varied between 0 and 100 μM.

ATP analog	C_{150} (μM)	k' (1/mmol·s)	$\frac{k'(S_P)}{k'(R_P)}$	$\frac{k'(Me^{2+})}{k'(-)}$
–-(S_P)-ATPαS	24	0.250	2.25	
–-(R_P)-ATPαS	54	0.111		
Mg-(S_P)-ATPαS	16	0.656	2.50	2.6
Mg-(R_P)-ATPαS	40	0.263		2.4
Cd-(S_P)-ATPαS	22	0.341	2.27	1.4
Cd-(R_P)-ATPαS	50	0.150		1.4
–-(S_P)-ATPβS	24	0.250	3.75	
–-(R_P)-ATPβS	90	0.067		
Mg-(S_P)-ATPβS	10	1.050	6.0	4.2
Mg-(R_P)-ATPβS	60	0.175		2.6
Cd-(S_P)-ATPβS	54	0.139	1.22	0.6
Cd-(R_P)-ATPβS	66	0.114		1.7

pleted enzyme. If binding of ATP is inhibited by 50%, $v_0 = v_0'$ and

$$k' = k \cdot \frac{[ATP]}{[NTP]}$$

From the kinetics of [^{14}C]ATP binding [24], k has been determined according to

$$k = \frac{\ln 2}{[ATP] \cdot t_{1/2}}$$

The calculated velocity constants (k) under the three different experimental conditions are summarized in Table III. The calculated rate constants (k') for binding of the phosphorothioate analogs are shown in Table IV. The results demonstrate that all of the analogs tested interact with the ATP binding site, in spite of their different screw senses, but with rather different apparent rate constants. The ratios of the rate constants, $k'(S_P)/k'(R_P)$, for ATPαS are essentially the same for the Mg^{2+}, Cd^{2+} and unchelated compounds (2.3–2.5). In contrast, large differences for the ATPβS analogs are observed: $k'(S_P)/k'(R_P)$ is 3.8 in the absence of added metal ions, it increases to 6.0 in the presence of Mg^{2+}, but decreases to 1.2 in the presence of Cd^{2+}.

In the last column of Table IV, the ratios of the rate constants for the nucleotide-metal complexes divided by the corresponding rate constants in the absence of a metal ion are shown. This ratio indicates the factor by which binding of a given ATP analog is accelerated by complexation with the metal ions. This factor is the same for S_P- and R_P-ATPαS both in the presence of Mg^{2+} and Cd^{2+}. In case of ATPβS on the other hand, acceleration is larger with the S_P than with the R_P form in the presence of Mg^{2+}. However, if these analogs are complexed with Cd^{2+}, the R_P form is preferred over the S_P form.

Discussion

Neither photophosphorylation nor light-triggered ATPase activity is observed in the presence of Cd^{2+}. Rather, Cd^{2+} acts as an energy transfer inhibitor in photophosphorylation, diminishing coupled electron transport to the basal rate [26]. For this reason, ATPase experiments described here were carried out with Mn^{2+} which has been reported to coordinate to oxygen as well as sulfur in the thiophosphate analogs of ATP [10,11]. Based on this idea, the previously determined stereo-

specificity for Mg(S_P)-ATPαS and Mg(S_P)-ATPβS [13] could be expected to be abolished if Mn^{2+} was employed instead of Mg^{2+}. The S_P-diastereomer of ATPαS is a substrate for the ATPase with either Mg^{2+} or Mn^{2+} while the R_P isomer with either cation is only a very poor substitute for ATP. No metal-induced change of enzyme specificity could be detected, as is demonstrated by the ratio $V_{max}(S_P)/V_{max}(R_P)$, which is the same in the presence of Mg^{2+} and Mn^{2+}. Corresponding ratios were also obtained when apparent K_i values for the competition of the two diastereomers with ATP in ATP hydrolysis were measured in the presence of these two metal ions. The stability constant of Mg-ATP (log $K_a = 4.7$) is much closer to that of Mg-ATPαS (log $K_a = 4.47$) than that of Mg-ATPβS (log $K_a = 4.04$) [27]. This suggests that even in aqueous solution the preferred Mg^{2+} coordination may be through the β,γ-phosphates. A significant α-phosphate coordination occurs with Cd-ATPαS because of the strong Cd-sulfur interaction [27]. For the same reason, a slightly increased proportion of α-coordinated metal might be found in Mn-ATPαS. However, no data on respective stability constants are available. The results reported here permit the conclusion that neither Mg^{2+} nor Mn^{2+} is coordinated to α-phosphate when ATPαS is bound in the active site of the enzyme. On the other hand, the high selectivity for the S_P isomer of ATPαS suggests that a negative charge in the α-position interacts stereospecifically with a positively charged group of the protein. Of interest in this context are the positively charged basic amino acids, lysine and arginine, which have been shown to play a role in substrate interaction in the chloroplast ATPase [28,29,30]. The negative charge is probably on the non-bridging oxygen, since in a non-complexed thiophosphate group the double-bond is largely fixed between phosphorus and sulfur [27].

The stability constants of Mg-ATP and Mg-ATPαS differ by a factor of 1.7 [27], which is comparable to the kinetic constants observed in the enzymatic hydrolysis reaction: V_{max} of Mg(S_P)-ATPαS is diminished in comparison to Mg-ATP by a factor of 2.7 and K_m raised by 1.9. In a former paper, photophosphorylation experiments with ADPαS, A and B in the presence of Mg^{2+} have been published [13]. Similar experiments in the presence of either Mn^{2+} or Co^{2+} (not published) showed that neither Mn^{2+} nor Co^{2+} is able to change the selectivity of the enzyme for the A isomer of ADPαS. This leads to the conclusion that the substrate of photophosphorylation is not an α,β bidentate complex, but a free negatively charged oxygen has to be at the α-position in the *S*-configuration.

In contrast to the diastereomers of the α-substituted phosphorothioate analogs of ATP, the β-substituted ones show a metal-dependent change of specificity in the ATPase reaction. Mg(S_P)-ATPβS, Mn(S_P)-ATPβS and Mn(R_P)-ATPβS, all having Δ-configurations to different extents, are substrates for chloroplast ATPase, with V_{max} values in the order of 5–10% of the parental nucleotide, while Mg(R_P)-ATPβS, the only one with exclusive Λ-structure, is not significantly hydrolyzed by the enzyme. These results indicate metal coordination to the β-phosphate during the reaction and suggest a β,γ-bidentate structure for the substrate. Together with the above conclusions, these results suggest, furthermore, that the active transition state includes an ionic protein-substrate interaction through oxygen, in the *S*-configuration of α-phosphate as well as β,γ-Δ-bidentate metal complexation. The relatively low activity of ATPβS isomers, in comparison to ATP and S_P-ATPαS, could be explained by the more bulky sulfur in the β-position. The low efficiency of β-S-substituted ATP as substrate of ATPase is in variance with their relatively high effectiveness as competitive inhibitors in ATP hydrolysis. If the obtained K_i values mainly reflect the dissociation constants of the enzyme-analog complexes, we have to conclude that it is the catalytic rate constant, rather than the binding constant, which is affected by S-substitution in β-phosphate. Nevertheless, acceptance of the catalytically active β,γ-Δ-structure is preferred over Λ-structure as is obvious from the $K_i(R_P)/K_i(S_P)$ ratio and the decrease of this ratio when Mn^{2+} is employed instead of Mg^{2+}.

Essentially, the same conclusion may be drawn from experiments of tight ATP binding. In this reaction Mg^{2+} can be replaced by a variety of other divalent cations, including Cd^{2+}. However, the results are complicated by the fact that ATP binding, in part, is independent of an added cation. Girault et al. [31] found about 0.6 Mg^{2+}/CF_1

associated with isolated CF_1 even after extensive dialysis, which is probably bound in the vicinity of a nucleotide binding site. Thus, one may speculate that CF_1 molecules which contain a bound Mg^{2+} are able to bind free ATP, forming a mixed protein-Mg-substrate complex, whereas Mg^{2+}-free enzyme molecules need a metal-chelated ATP. This may be supported by the finding that not only the rate but also the final amount of bound ATP is larger in the presence than in the absence of added divalent cations. The I_{50} values calculated from competition experiments indicate that Cd^{2+} may invert, to some extent, the specificity for $Mg(S_P)$ATPβS. However, an inhibitory effect of Cd^{2+} is obviously superimposed, as can be concluded from the reduction of the rate constant for $Cd(S_P)$-ATPβS binding in comparison to the unchelated (S_P)-ATPβS. Nevertheless, a clear difference between the isomers of ATPβS, in contrast to the isomers of ATPαS, in the presence of Mg^{2+} and Cd^{2+}, respectively, is evident if the ratios of the velocity constants fo the chelated to the unchelated analogs are considered.

The significance of tight nucleotide binding for the catalytic process is a matter of controversy [32]. Recent papers [14,15,16] have shown that the three nucleotide-binding sites present on CF_1 exhibit the same peptide structure, although they may be discriminated by nucleotide-binding kinetics [17]. This is supported from the substrate side by the results reported here. Frasch and Selman [33] recently proposed, from investigations with exchange-inert chromium(II)-ADP complexes and isolated CF_1, that the Λ bidentate metal ADP complex is the substrate for phosphorylation to form a tridentate metal-ATP as the product. A similar study with phosphorothioate analogs of ATP has been undertaken by Senter et al. [34] with the isolated ATPase TF_1 of the thermophilic bacterium PS3. In accordance with the results shown here for chloroplast ATPase, the Δ-chelate structure of ATPβS was a better substrate than the Λ structure. Moreover, the S_P-isomer of ATPαS as in CF_1 was preferred over the R_P-isomer in the presence of both Mg^{2+} and Cd^{2+}. Thus the active sites of proton-translocating ATPases seem to be largely identical in these completely different organisms.

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