Purification and Characterization of Chloroplast F_1-ATPase in *Acetabularia acetabulum*

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Abbreviations: FITC, fluorescein isothiocyanate; DCCD, *N,N'*-dicyclohexylcarbodiimide; Pipes, 1,4-piperazinediethanesulfonic acid; Tris, tris (hydroxymethyl) aminomethane; FPLC, fast protein liquid chromatography; PMSF, phenylmethanesulfonyl fluoride; DTT, dithiothreitol; NEM, *N*-ethylmaleimide; DES, diethylstilbestrol; NBD-Cl, 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole; PCR, polymerase chain reaction; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase

SUMMARY

ATPases were isolated from the chloroplast of the unicellular marine alga *A. acetabulum*. Three preparations of ATPase, chloroplast-enriched fraction, $\alpha\beta$-subcomplex, and $\alpha\beta\gamma\delta$-complex were compared. The $\alpha\beta\gamma\delta$-complex was released into an EDTA solution and purified by ion-exchange chromatography. The subunit composition of this enzyme appeared to be 52-53 (α), 51 (β), 40 (γ), and 28kDa (δ) both from SDS-PAGE and immunoblot.

The catalytic properties of the $\alpha\beta\gamma\delta$-complex were as follows: pH optimum at 7.5; substrate specificity, ATP>ITP, GTP>UTP=CTP (Km for ATP 0.2mM); divalent cation requirement, $Mg^{2+}=Mn^{2+}=Co^{2+}>Zn^{2+}>Ni^{2+}>Ca^{2+}$; ATPase activity was inhibited by monovalent anions (NO_3^-, SCN^-), while monovalent cations had neither inhibitory nor stimulatory effects. Orthovanadate had no inhibitory effect on the both activities of $\alpha\beta$- and $\alpha\beta\gamma\delta$-complex. Azide was the most effective inhibitor of the $\alpha\beta\gamma\delta$-complex, but did not inhibit the activity in the $\alpha\beta$-subcomplex. N-Terminal amino acid sequences of the α and β subunits were not obtained and appeared to be blocked. The γ subunit gave sequence of AGLKE, which showed 60% similarity to the γ subunit of spinach chloroplast ATPase, 80% to the subunit of *Chlamydomonas reinhardtii* CF_1-ATPase and EF_1-ATPase.

INTRODUCTION

ATPases play an important role in energy conversion both in prokaryotes and eukaryotes. They have been well studied biochemically and molecular biologically in

connection with their cation-translocating activities. They are classified into three categories: P,V, and F type.[1] Archaebacterial ATPase has been reported to be closely related to the V type and not related to the F type.[2-4] Mukohata *et al.* proposed that ATPases in archaebacteria be given an independent classification, A type in the ATPase family. Evolutionary aspects of the ATPase family have been one of the most current topics in the field. One hypothesis is that common progenotes may have been three different progenitors of ATPase (V,A, and F types).[5-7]

Acetabularia acetabulum, a unicellular marine alga, belongs to *Dasycladaceae,* and is one of the most ancient eukaryotes. ATPases present in this organism are, therefore, of great interest. We have already isolated and/or characterized a Cl^--translocating ATPase[8,9] and a vacuolar ATPase from this organism,[10] both of which showed unique properties [confer the refs.] .

Chloroplast-bound ATPase has been also isolated from *A. acetabulum* in an inactive state,[8] but the preparations (peaks II and III in ref. 8) were mostly contaminated by Rubisco through protein chemical experiments [unpublished data] .

Isolation of chloroplast-enriched fraction, partial purification of the chloroplast ATPase subcomplex ($\alpha\beta$) and F_1-complex ($\alpha\beta\gamma\delta$) in an active state are described in this paper. Several catalytic properties of the respective preparation are also presented.

EXPERIMENTAL PROCEDURES

Materials—Most of chemicals were the same as described in our previous reports.[8-10]

The FPLC-system and a Mono Q HR5/5 column from Pharmacia (Uppsala, Sweden) were used for isolation of chloroplast F_1-complex.

The N-terminal sequencing of the isolated F_1-complex was performed on a Model 477A protein/peptide sequencer and an online PTH analyzer, Model 120A-01 (Applied Bio-Systems Co., Foster City, U.S.A.) after electroblotting onto siliconized glass-fiber sheets.[11] Antibodies against the $\alpha,\beta,\gamma,\delta$, and ε subunits of spinach chloroplast F_1-ATPase were prepared as described in our previous paper.[12]

Preparation of chloroplast-enriched fractions — Isolation of chloroplast-enriched fractions was carried out according to the method described by Bidwell *et al.,*[13] which was established for *A. acetabulum.* Class I chloroplast fraction was stored at −20℃ and used for experiments.

Partial purification of the chloroplast ATPase $\alpha\beta$-subcomplex — Axenic cells (3 to 5 cm in length, 20 g wet weight) were pulverized in liquid nitrogen using a Waring blender. The green powder was extracted in a mortar with a homogenization buffer until greenish color in cell debris almost disappeared (*ca.* 200 ml, three to four times extraction). The homogenization buffer consisted of 50 mM Pipes-Tris (pH 7.6), 0.55 M sorbitol, 0.5 mM $MgSO_4$, 1 mM EGTA and 2 mM DTT. The extract was filtered through four layers of cheesecloth and the filtrate was centrifuged at $9,000\times g$ for 10 min. The supernatant was brought to 80% (w/v) saturation with ammonium sulfate.

After centrifugation at 9,000×*g* for 30 min, the precipitate was dissolved in a minimal volume of the homogenization buffer and was subsequently desalted by ultrafiltration. The desalted solution was layered onto three 30 ml sucrose gradient tubes (5 to 25%, w/v) and centrifuged at 50,000×*g* for 12 h at 15 ℃. After centrifugation, the bottom of the centrifuge tube was punctured, 1.2 ml aliquots were collected and assayed for ATPase activity.

Purification of the chloroplast ATPase F_1-complex ($\alpha\beta\gamma\delta$-complex) — Axenic cells (3 to 5 cm in length, 50 g wet weight) were cut into small pieces and extracted with the homogenization buffer (pH 7.0) without EGTA (100 ml×three times) under vigorous stirring at room temperature for 5 min. The extract was filtered through four layers of cheesecloth and the filtrate was centrifuged at 5,000×*g* for 2 min. Further three steps were in principle carried out according to the method of Binder *et al.*[14] The precipitate was then suspended in 50 ml of 10 mM NaCl and centrifuged at 12,000×*g* for 10 min. The washing procedure with 10 mM NaCl was repeated twice. The washed precipitates were suspended in 140 ml of 0.75 mM EDTA (1 ml for 0.1 mg chlorophyl), the pH was adjusted to 7.8 with 0.1 N NaOH and stirred at room temperature for 10 min. After centrifugation at 12,000×*g* for 10 min, the precipitate was reextracted with 0.75 mM EDTA in the same manner. The supernatants were combined, added to 10 ml of a DEAE-cellulose previously equilibrated in 50 mM Tris-HCl (pH 7.8), 2 mM EDTA and 1 mM ATP and stirred at room temperature for 20 min. The suspension was filtered through a Miracloth (60 μm) and the cellulose was washed three times with 50 ml of the above equilibration buffer. The washed cellulose was packed into a column(2.4 cmϕ×5 cm). Elution of proteins was conducted with a buffer containing 50 mM Tri-HCl (pH 7.8), 0.4 M NaCl, 2 mM EDTA and 1 mM ATP. Fractions with ATPase activity were pooled (*ca.* 50 ml) and concentrated by ultrafiltration. The concentrate (*ca.* 6 ml) was brought to 50% saturation with ammonium sulfate and kept at 4 ℃ overnight. The precipitates were collected by centrifugation at 10,000×*g* for 20 min, dissolved in an equilibration buffer for Mono Q (see below) and desalted by ultrafiltration with Centricon YM-30. The desalted concentrate (*ca.* 600 μl) was applied to a Mono Q column (1ml bed volume) previously equilibrated with a buffer consisting of 25 mM Pipes-Tris (pH 7.0), 0.25 M sorbitol, 6 mM $MgSO_4$, 1 mM EGTA, 2 mM DTT, 12.5 μM PMSF and 1 mM ATP. Proteins were eluted by a linear increase of Na_2SO_4 in the buffer from 0 to 0.2 M as described previously.[8] The active fractions were pooled, concentrated and desalted by ultrafilttration. The final preparation was kept at −70 ℃ and used for further experiments.

Assay and analytical procedures — ATPase activity was measured as described previously,[8] except that the pH of the Pipes-Tris buffer was 8.0, methanol was added with a final concentration of 20% (v/v) and incubation was performed at 37 ℃. Protein determination, analytical SDS-PAGE, and western blotting and reactions with antibodies were all carried out as described previously.[8,10]

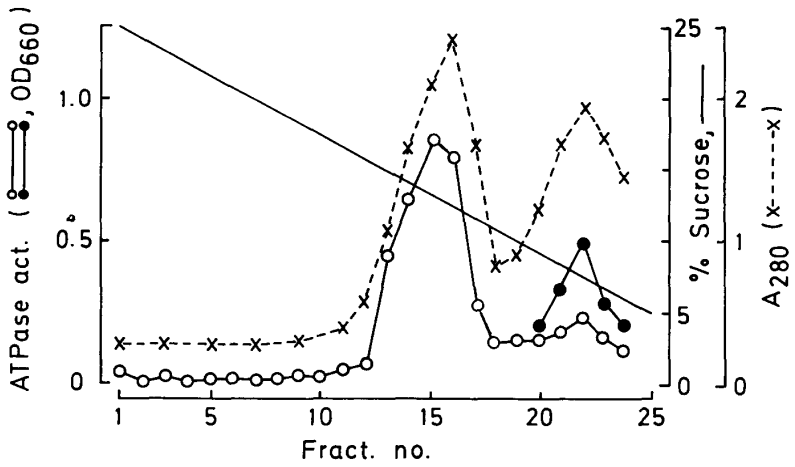

Fig. 1. Separation of ATPase activity by sucrose density gradient centrifugation. An aliquot (20 μl) of each fraction was assayed for ATPase activity at pH 8.9 (●—●) and at pH 7.0 (○——○). A broken line represents absorbance at 280 nm for monitoring protein concentrations. ATPase activity was presented as $OD_{660}/20$ μl enzme sources.

RESULTS

Isolation of the chloroplast ATPase αβ-subcomplex — A typical elution profile of the ATPase activity after the sucrose density gradient centrifugation is shown in Fig. 1. Two ATP-hydrolyzing activities were separated, one between 5 and 9% sucrose in the buffer(Fr. B) and the other between 11 and 15% (Fr. A). The activity in the Fr. A was attributable to apyrase in the cytosolic fraction, since ADP and AMP were also good substrates for the enzyme. Total enzyme activity in the Fr. A and B was recovered with a yield of 89% of the activity in the ammonium sulfate fraction. The specific activity of the Fr. B ranged from 10 to 30 mU/mg protein.

SDS-PAGE showed essentially the same electrophoretograms as observed for the peaks II and III in ref. 8. No appreciable band around 40 kDa (γ subunit) was observed, even when excess amount of protein in the Fr. B was analyzed by SDS-PAGE. These two polypeptides (*ca.* 52 and 51 kDa) were also subjected to immunoblot and the reactions with the anti-α and -β sera of EF_1-ATPase were detected (data not shown). The Fr. B is thus believed to contain an $\alpha\beta$-subcomplex of the chloroplast ATPase. Further protein chemical characterization, however, revealed that the preparation was mostly contaminated with a Rubisco large subunit (confer the ref. 12).

Purification of chloroplast ATPase αβγδ-complex — One ATPase-containing fraction was obtained through DEAE-cellulose and Mono Q chromatography. The elution profile of the ATPase activity on Mono Q chromatography is shown in Fig. 2. The activity was eluted from the Mono Q column between 160 and 180 mM Na_2SO_4. As summarized in Table I, the ATPase was about 130-fold enriched in 61% yield, and had a specific activityof 10.6 units/mg protein at 37 ℃.

Figure 3a shows SDS-polyacrylamide gel electrophoretograms of the crude chloroplast fraction, the supernatant after EDTA extraction, concentrates after DEAE-

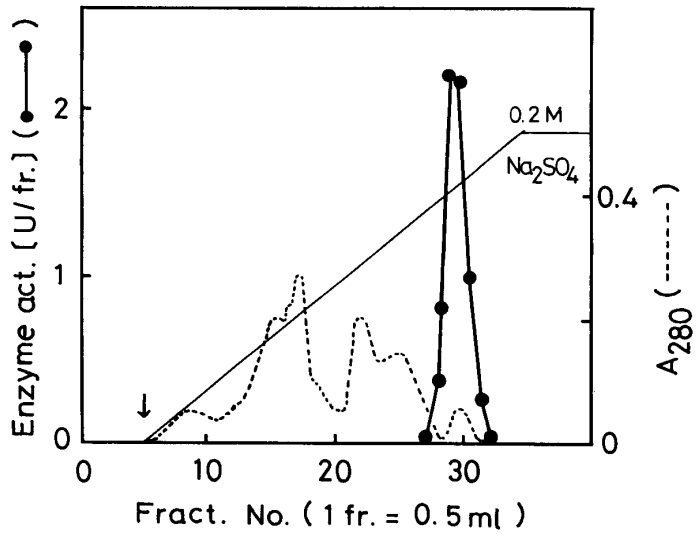

Fig. 2. Elution profile on Mono Q chromatography. The desalted concentrate after a DEAE-cellulose column was applied to a Mono Q column. Each fraction was assayed for ATPase activity (●——●), and protein was monitored by its absorption at 280 nm [A_{280} (------)] . The solid line indicates the linear salt gradient of Na_2SO_4 used for chromatography and the arrow shows the start of the linear gradient from 0 to 0.2 M Na_2SO_4.

Table I. Purification of the chloroplast ATPase F_1-complex ($\alpha\beta\gamma\delta$-complex) Axenic cells (50 g of wet weight) were subjected to the purification. Enzyme activity at each step was assayed at pH8.0 using ATP-Na (3 mM) and $MgSO_4$ (3 mM) as substrates and in the presence of 0.25 M sorbitol, 20% (v/v) methanol at 37 ℃.

step	total volume (ml)	total protein (mg)	total activity (units)	specific activity (units/mg of protein)	yield (%)	purification (x-fold)
(1) crude chloroplast fraction	50	74.5	5.7	0.08	100	1
(2) 10 mM NaCl washed thylakoid membrane	290	42.1	3.8	0.09	67	1.1
(3) DEAE column (concentrate)	6	2.8	7.2	2.5	126	31.3
(4) Ammonium sulfate precipitation	0.58	2.3	6.0	2.6	106	32.5
(5) Mono Q column (concentrate)	0.24	0.33	3.5	10.6	61	130

cellulose column and after a Mono Q column. The final preparation mainly shows 4 polypeptides with molecular masses of 52-53, 51, 41, and 28kDa. Cross-reactivities of the Mono Q fraction were also tested using the antiserum against the respective α,β, γ, and δ subunits of spinach chloroplast F_1-ATPase, and the results are shown in Fig. 3b. Clear cross-reactions with the anti-α, -β, and -γ sera were observed, but the reaction with the anti-δ serum was weak. Immunoblot also showed that the decomposition of the α and β subunits occurred during purification procedures. Both data

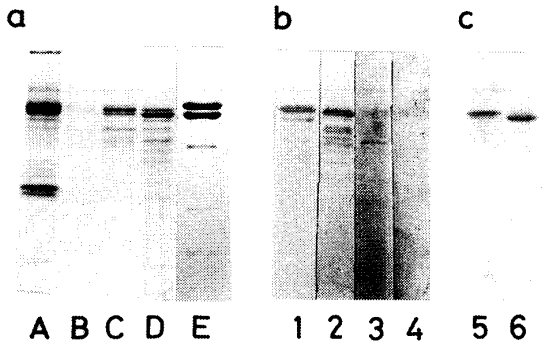

Fig. 3. SDS-Polyacrylamide gel electrophoretograms (Coomassie stain) and immunoblot of the Mono Q fraction and thylakoid membrane. a. Coomassie stain: Lane A, crude chloroplast fraction (15 μg protein); lane B, extract with 0.75 mM EDTA (1 μg protein); lane C, concentrate after a DEAE-cellulose column (4 μg protein); lane D, concentrate after a Mono Q column (3 μg protein); lane E, spinach CF_1-complex (10 μg protein). b. Reactions of the Mono Q fraction with antibodies against spinach CF_1-complex: Lanes 1 to 4, reaction of the lane D with the anti-α, -β, -γ, and δ subunit, respectively. c. Reactions of the chloroplast-enriched fraction with antibodies against spinach CF_1-complex: Lanes 5 and 6, reaction of the lane A with the anti-α, -β subunit, respectively.

supported that the Mono Q fraction mainly consisted of the α,β,γ, and δ subunits of the chloroplast F_1-ATPase.

Catalytic properties of the chloroplast-enriched fraction, the isolated $\alpha\beta$-subcomplex, and $\alpha\beta\gamma\delta$-complex as ATPase — The chloroplast-enriched fraction, the isolated $\alpha\beta$-subcomplex and $\alpha\beta\gamma\delta$-complex were characterized as ATPase, and the results are summarized in Table II. All the data are presented as relative values from duplicate tests.

(A) pH optimum. The pH profiles of enzyme activity in the three preparations are shown in Fig. 4. The pH optima of the ATPase activities in the chloroplast-enriched fraction and in the isolated $\alpha\beta$-subcomplex were around 9.0, while the pH optimum of the isolated $\alpha\beta\gamma\delta$-complex around 7.5.

(B) Substrate specificity and inhibitors. As summarized in Table II, ATP was the best substrate both for the isolated $\alpha\beta$-subcomplex and $\alpha\beta\gamma\delta$-complex. The Km value of the $\alpha\beta\gamma\delta$-complex was 0.2 mM.

Table II also lists number of compounds tested as inhibitors for the ATPase activity in the three preparations. They showed different susceptibilities to the inhibitors, except for orthovanadate. Azide, a typical inhibitor of F type ATPase, inhibited the activity in the isolated $\alpha\beta\gamma\delta$-complex and in the chloroplast-enriched fraction, but did not inhibit the activity in the isolated $\alpha\beta$-subcomplex (see Fig. 5).

(C) Cation and anion requirement. Several divalent cations were tested for the isolated complexes as replacement of Mg^{2+}, and the results are also listed in Table II. In the

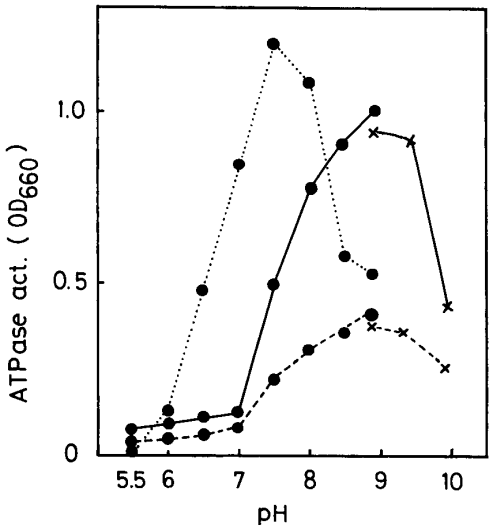

Fig. 4. pH Profiles of ATPase activity in the chloroplast-enriched fraction, the isolate $\alpha\beta$-subcomplex, and $\alpha\beta\gamma\delta$-complex. An aliquot (2.8 μg protein for the chloroplast-enriched fraction, 87 μg for the $\alpha\beta$-subcomplex and 0.14 μg for the $\alpha\beta\gamma\delta$-complex) was assayed for the ATPase activity at various pH values. Each value was mean of duplicate determinations. ●, Pipes-Tris buffer; ×, CHES-Tris buffer. ——, ATPase activity in the chloroplast-enriched fraction; ·····, ATPase activity in the isolated $\alpha\beta$-subcomplex; ·····, ATPase activity in the isolated $\alpha\beta\gamma\delta$-complex.

absence of Mg^{2+}, the ATPase had almost no activity in the both preparations. Mn^{2+} and Ca^{2+} could substitute for Mg^{2+} in the isolated $\alpha\beta$-subcomplex, while Mn^{2+}, Co^{2+}, Zn^{2+}, and Ni^{2+} could subsitute for Mg^{2+} in the isolated $\alpha\beta\gamma\delta$-complex. A number of monovalent cations were tested and shown to have no significant stimulatory effects on the ATPase activity in the both preparations. The effects of monovalent anions tested are also summarized in the table. NO_3^- and SCN^- showed inhibitory effects on the activity in the three preparations. ID_{50} concentrations of NO_3^- and SCN^- were 15 mM and 0.2 mM for the isolated $\alpha\beta\gamma\delta$-complex, respectively.

(D) Effects of methanol and ethanol on the ATPase activities in the three preparations. The enzyme activity in the isolated $\alpha\beta$-subcomplex and $\alpha\beta\gamma\delta$-complex was stimulated by addition of methanol as shown in Fig. 6, while the activity in the chloroplast-enriched fraction was not affected by methanol (data not shown). Ethanol also stimulated the activity in the isolated $\alpha\beta\gamma\delta$-complex (see also Fig. 6). Without addition of methanol or ethanol the isolated preparations had almost no ATPase activity.

N-Terminal amino acid sequences of the α, β, and γ subunits of the Mono Q fraction. — Amino acid sequences of the α and β subunits were not obtained and appeared to be blocked. The γ subunit gave sequence of AGLKE, which showed 60% similarity to the γ subunit of spinach chloroplast ATPase, 80% to the subunit of *Chlamydomonas reinhardtii* CF_1- and EF_1-ATPase.

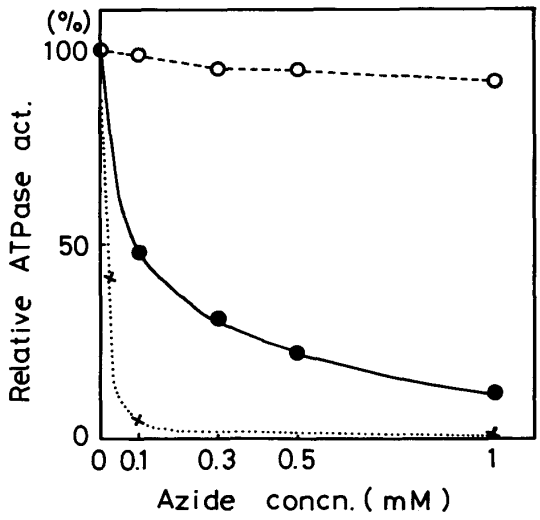

Fig. 5. Effects of azide on ATPase activity in the chloroplast-enriched fraction, the isolated $\alpha\beta$-subcomplex, and $\alpha\beta\gamma\delta$-complex. ATPase activity was assayed for the three preparations in the presence of various amounts of azide. Ten percent methanol was added to the reaction mixture of the isolated $\alpha\beta$-subcomplex and 20% to the mixture of the isolated $\alpha\beta\gamma\delta$-complex. The values were from duplicate determinations. ●——●, ATPase activity in the chloroplast-enriched fraction; O·····O, ATPase activity in the isolated $\alpha\beta$-subcomplex; ×·····×, ATPase activity in the isolated $\alpha\beta\gamma\delta$-complex.

DISCUSSION

Chloroplast ATPase in an active state was isolated and characterized in this paper. The subunit composition of the F_1-portion appeared to be 52-53 (α), 51 (β), 40 (γ), and 28kDa (δ) both from SDS-PAGE and immunoblot. The ε subunit was not confirmed in the isolated preparation. The N-terminal amino acid sequence of the putative γ subunit also supported that the ATPase complex was originated from chloroplasts. Decompositions of especially the α and β subunits were drastic during purification, and serine-protease inhibitor, PMSF, could not prevent decompositions.

Catalytic properties of the three preparations presented here were quite different especially for the pH optimum and for sensitivity to inhibitors. An acidic shift of the pH optimum occurred during purification. This phenomenon has been reported for plasmalemma ATPase in *Dunaliella* by Smahel *et al.*[15] Differences in susceptibilities to the inhibitors might be attributable to the pH shift. Azide had no appreciable inhibitory effect on the activity in the $\alpha\beta$-subcomplex, while concentration-dependent inhibition was observed for the activities in the chloroplast-enriched fraction and for the isolated $\alpha\beta\gamma\delta$-complex. Kagawa *et al.*,[16] and Miwa and Yoshida[17] have recently reported that the $\alpha_3\beta_3$ complex of thermophilic ATP synthase is the catalytic core of TF_1-ATPase and that the ATPase activity in the $\alpha_3\beta_3$ complex was not inhibited by azide, whereas the activity in the $\alpha_3\beta_3\gamma$ complex inhibited by azide. The inhibitory

Table II. Catalytic properties of the chloroplast ATPase $\alpha\beta$-subcomplex and $\alpha\beta\gamma\delta$-complex (% Activity) Substrate specificity, inhibitor effects, divalent cation requirements, and monovalent cation and anion effects were tested for the chloroplast ATPase in an assay mixture containing 25 mM Pipes-Tris buffer, 0.25 M sorbitol, 3 mM each of $MgSO_4$ and ATP-Na, and 10% methanol for $\alpha\beta$-subcmplex, 20% ethanol for $\alpha\beta\gamma\delta$-complex. Except for the pH dependence, the buffer was pH8.9 for $\alpha\beta$-subcomplex, pH7.5 for $\alpha\beta\gamma\delta$-complex. For the inhibitor studies, the preincubation of each inhibitor with the enzyme was carried out at 37℃ for 5min, and the reaction was started by addition of ATP. Results are the mean of duplicate measurements. *not detectable **ATPase activity in thylakoid membranes.

	Chloroplast ATPase			Chloroplast ATPase	
	$\alpha\beta$-subcomplex	$\alpha\beta\gamma\delta$-complex		$\alpha\beta$-subcomplex	$\alpha\beta\gamma\delta$-complex
Substrate specificity(%)			Divalent cation requirement		
ATP (3 mM)	100	100	None	n.d.*	n.d.*
GTP (3 mM)	33	67	Mg^{2+} (3 mM)	100	100
ITP (3 mM)	49	75	Mn^{2+} (3 mM)	106	103
UTP (3 mM)	21	17	Ca^{2+} (3 mM)	62	10
CTP (3 mM)	18	2	Zn^{2+} (3 mM)	13	53
ADP (3 mM)	8	1	Ni^{2+} (3 mM)	16	31
AMP (3 mM)	n.d.*	1	Co^{2+} (3 mM)	23	115
p-Nitrophenyl phosphate (3 mM)	10	1	Effect of monovalent ions		
			Li^+ (10 mM)	87	111
Inhibitors			K^+ (10 mM)	83	93
None	100	100	Na^+ (10 mM)	100	101
Orthovanadate (100 μM)	92(114)**	102	NH_4^+ (10 mM)	90	83
Azide (100 μM)	104(59)**	8	Choline (10 mM)	100	100
DCCD (100 μM)	45(76)**	110			
DES (100 μM)	41(75)**	85	Cl^- (10 mM)	100	70
FITC (100 μM)	48(79)**	81	Br^- (10 mM)	72	87
NBD-CI (100 μM)	25(36)**	89	I^- (10 mM)	60	93
NEM (100 μM)	49(80)**	117	HCO_3^- (10 mM)	84	101
			F^- (10 mM)	76	52
			NO_3^- (10 mM)	68(57)**	42
			SCN^- (10 mM)	52(54)**	5

effects of monovalent anions such as NO_3^- and SCN^- on the activities in the three preparations have not been observed for the F type of ATPase, but typical for the V type of ATPase.

As mentioned in Introduction, evolutionary aspects of the ATPase family are the most current topics. Cloning of the genes encoding the α and β subunits of the chloroplast ATPase is now in progress. cDNA library from *A. acetabulum* was directly amplified by PCR using primers for the conserved regions of the α and β subunits of the F type ATPase in eukaryotes, the fragments were subcloned into a plasmid and sequenced. The partial amino acid sequences obtained for the α and β subunits (about 90 amino acids) showed higher similarities to those of higher plants than those of *Chlamydomonas* and liverwort. Cloning of the whole genes encoding the both subunits will give more informations on the evolution of the ATPase family.

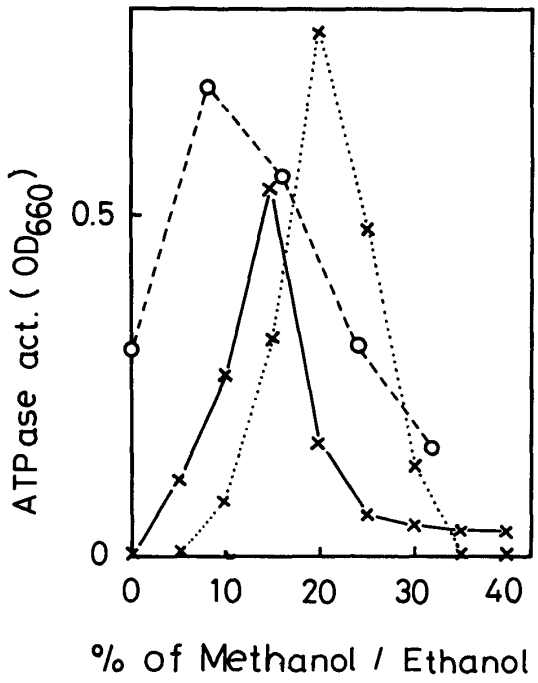

Fig. 6. Effects of methanol and ethanol on ATPase activity in the isolated $\alpha\beta$-subcomplex and in the $\alpha\beta\gamma\delta$-complex. An aliquot (87 μg protein for the $\alpha\beta$-subcomplex and 0.14 μg for the $\alpha\beta\gamma\delta$-complex) was assayed for ATPase activity in the presence of various amounts of methanol and ethanol. Each value was mean of duplicate determinations. ○·····○, ATPase activity in the isolated $\alpha\beta$-subcomplex in the presence of methanol; ×·····×, ATPase activity in the isolated $\alpha\beta\gamma\delta$-complex in the presence of methanol; ×——×, ATPase activity in the isolated $\alpha\beta\gamma\delta$-complex in the presence of ethanol.

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REFERENCES

1) Pedersen, P.L. & Carafoli, E. (1987) *Trends Biochem. Sci.*, **12**, 146-150.
2) Mukohata, Y., Ihara, K., Yoshida, M., Konishi, J. Sugiyama, Y., & Yoshida, M. (1987) *Arch. Biochem. Biophys.*, **259**, 650-653.
3) Denda, K., Konishi, J., Oshima, T., Date, T., & Yoshida, M. (1988) *J. Biol. Chem.*, **263**, 6012-6015.
4) Inatomi, K., Eya, S., Maeda, M., & Futai, M. (1989) *J. Biol. Chem.*, **264**, 10954-10959.
5) Nelson, N. & Taiz, L. (1989) *Trends Biochem. Sci.*, **12**, 186-189.
6) Gogarten, J.P., Kibak, H., Dittrich, P., Taiz, L., Bowman, E.J., Bowman, B.J., Manolson, M.F., Poole, R.J., Date, T., Oshima, T., Konishi, J., Denda, K., & Yoshida, M. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 6661-6665.

7) Mukohata, Y. & Ihara, K. (1990) *in Bioenergetics;* molecular biology, biochemistry and pathology (Kim, C.H. & Ozawa, T., eds.), pp. 205-216, Plenum Press, NY.
8) Ikeda, M., Schmid, R., & Oesterhelt, D. (1990) *Biochemistry,* **29**, 2057-2065.
9) Ikeda, M. & Oesterhelt, D. (1990) Biochemistry, **29**, 2065-2070.
10) Ikeda, M., Satoh, S., Kimura, M., Maeshima, M., Mukohata, Y., & Moritani, C. (1991) *Biochim. Biophys. Acta,* **1070**, 77-82.
11) Eckerskorn, C., Mewes, W., Goretzki, H., & Lottspeich, F. (1988) *Eur. J. Biochem.,* **176**, 509-519.
12) Ohhashi, T., Moritani, C., Andoh, H., Satoh, S., Ohmori, S., Lottspeich, F., & Ikeda, M. (1991) *J. Chromatogr.,* **585**, 153-159.
13) Bidwell, R.G.S., Levin, W.B., & Sephard, D.C. (1969) *Plant Physiol.,* **44**, 946-954.
14) Binder, A., Jagendorf, A., & Ngo, E. (1978) *J. Biol. Chem.,* **253**, 3094-3100.
15) Smahel, M., Hamann, A., & Gradmann, D. (1990) *Planta,* **181**, 496-504 .
16) Kagawa, Y., Ohta, S., & Otawara-Hamamoto, Y. (1989) *FEBS Lett.,* **249**, 67-69.
17) Miwa, K. & Yoshida, M. (1989) *Proc. Natl. Acad. Sci. USA,* **86**, 6484-6487.