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ACTIVATION OF THE CF_0-CF_1, ATP SYNTHASE FROM SPINACH CHLOROPLASTS BY CHLOROPLAST LIPIDS

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The interactions of CF_0-CF_1 with different lipids were studied by following the stimulation of Mg-ATPase and of P_i-ATP exchange activities of reconstituted CF_0-CF_1 proteoliposomes. The following results were obtained: (1) Both P_i-ATP exchange and Mg-ATPase activities are stimulated by lipids. Furthermore, the inhibition of Mg-ATPase by *N,N'*-dicyclohexylcarbodiimide is dependent on the interactions of CF_0-CF_1 with lipids. (2) A polar lipid extract of thylakoid membranes stimulates Mg-ATPase activity of CF_0-CF_1 more efficiently than phospholipids. The relative effectiveness of Mg-ATPase stimulation is: chloroplast lipids > soybean phospholipids > phosphatidylcholine/phosphatidylserine (4:1) > phosphatidylcholine. The rate of P_i-ATP exchange in chloroplast lipids CF_0-CF_1 proteoliposomes is, however, lower than in soybean lipids CF_0-CF_1 proteoliposomes, due to their higher permeability to protons. Addition of 10% phosphatidylserine to chloroplast lipids reduces their permeability to protons and stimulates P_i-ATP exchange. (3) The kinetic mechanism of ATPase stimulation by chloroplast lipids is by decreasing the K_m (ATP) and by increasing V_{max} in comparison to soybean lipid proteoliposomes. This may explain the low affinity for ATP and the slow turnover rate of the purified enzyme in artificial lipids in comparison to the native enzyme in chloroplast thylakoids. (4) Chloroplast lipids lacking monogalactosyldiacylglycerols only poorly activate CF_0-CF_1. A large stimulation of P_i-ATP exchange is obtained by a mixture of 60% monogalactosyldiacylglycerol and 40% of the rest of the chloroplast lipids, but not by mixtures of monogalactosyldiacylglycerol with phospholipids. Hydrogenation of the unsaturated fatty acids of monogalactosyldiacylglycerol inhibits the activation of CF_0-CF_1. (5) The results suggest that: (a) interactions of specific chloroplast lipids with CF_0-CF_1 activates the enzyme by increasing its turnover and its affinity for ATP; (b) specific requirements for CF_0-CF_1 activation are the presence of monogalactosyldiacylglycerols together with another chloroplast lipid component and of highly unsaturated fatty acids.

Introduction

The lipid composition of thylakoid membranes of higher plant chloroplasts is unique in having a high content of monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) (which make up approx. 70% of the polar lipid

Abbreviations: PC, phosphatidylcholine; PS, phosphatidylserine; PG, phosphatidylglycerol; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; CF_0-CF_1, chloroplast ATP synthase complex; DCCD, *N,N'*-dicyclohexylcarbodiimide; S-13, 5,chloro-3-*tert*-butyl-2'-chloro-4'-nitrosalicylanilide; SF-6847, 3,5-di-*tert*-butyl-4-hydroxybenzylidenemalonitrile; TLC, thin-layer chromatography; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]-glycine.

content) and also in the high proportion of unsaturated fatty acids, mainly linolenic acid [1]. Also the physical properties of chloroplast lipids are unusual. Freeze-fracture studies performed on aqueous dispersions of chloroplast lipid extracts [2–4] revealed a large range of non-bilayer structures such as spherical and cylindrical inverted micelles which probably result from a phase separation of the non-bilayer forming lipids.

Since the lipid composition of thylakoids is so unusual, it could be expected that interactions of specific lipids with proteins and enzymes in this membrane should play an important role in photosynthesis. Indeed, several recent reports suggest specific interactions between certain chloroplast proteins such as the chlorophyll *a/b* complexes of Photosystem II [5], oxygen evolving thylakoid fragments [6] and cytochrome *b*-559 [7] with specific lipids in thylakoid membranes.

Interactions of lipids with membrane proteins have been studied also in many other systems mainly by following the immobilization of spin-labeled lipids by ESR spectra [8,9]. One example is the Ca-ATPase from sarcoplasmic reticulum in which 30 lipids interact with each protein [9,10]. Preferential interactions of membrane proteins with specific lipid components have also been demonstrated for several proteins such as between cardiolipin and cytochrome oxidase [11].

This report demonstrates that specific chloroplast lipids modify the catalytic properties of the CF_0-CF_1 ATP synthase purified from spinach thylakoids. It is shown that the activating effect of chloroplast lipids on the enzyme depends both on the properties of the polar-head group and of the unsaturated fatty acids of the lipid.

Methods and Materials

Isolation of chloroplast lipids. Spinach thylakoids were isolated as described in Ref. 12 and lipids were extracted using the method of Bligh and Dyer [13]. Pigments and neutral lipids were removed from the extract on silicic acid columns [14]. The total polar lipid thus obtained was separated into lipid classes by chromatography on acid-washed Florisil columns, and were further purified by preparative thin-layer chromatography (TLC). The purity of the lipid classes was verified by TLC on ammonium-impregnated plates as described in Ref. 15. Saturation of lipids was achieved by catalytic hydrogenation in the presence of Adams' catalyst as described in Ref. 16. Quantification of lipids was carried out by gas-liquid chromatography of their fatty-acid methyl-esters as previously described [17]. The major polar lipid constituents as described in Ref. 4 are: MGDG (38%), DGDG (29%), sulphoquinovosyl diacylglycerol (18%), PG (11%) and PC (3%).

Isolation of the CF_0-CF_1 ATP synthase. CF_0-CF_1 was isolated from spinach leaves (*Spinaca olerace*) as previously described [18,19]. Most studies were carried out with the enzyme fraction precipitated between 35 and 45% saturation ammonium sulphate. Identical results were obtained with the sucrose-gradient-purified enzyme.

Reconstitutions and assays. Reconstitution of CF_0-CF_1 ATP synthase with lipids was performed by the cholate-dilution procedure [20] as follows: lipid mixtures dissolved in chloroform were dried under a stream of nitrogen, redissolved in peroxide-free diethylether, dried again under nitrogen and mixed with a vortex mixer in a buffer containing 0.2 M sucrose/20 mM Na-Tricine (pH 8)/3 mM $MgCl_2$ (20 mg lipid/ml). Sodium cholate (7 mg cholate/ml) was added to the turbid liposome suspension and the mixture was sonicated to clarity (1–2 min) under a nitrogen stream in an ultrasonic cylindrical tank [19]. CF_0-CF_1 ATP synthase (1.5 mg/ml) was added to the lipid-cholate mixture and the suspension was incubated 20–30 min on ice. 20-μl Samples of the protein/lipid/cholate mixture were added to glass tubes containing in 1 ml 80 mM Na-Tricine (pH 8), 3 mM $MgCl_2$, 3 mM ATP, 2 mM sodium phosphate containing $^{32}P_i$ (0.2 μCi/μmol P_i; P_i-ATP exchange assay) or 80 mM Na-Tricine (pH 8), 1 mM $MgCl_2$, 1 mM ATP containing [γ-^{32}P]ATP (0.5 μCi/μmol ATP, ATPase assay). The tubes were incubated for 30 min at 37°C and the amount of $^{32}P_i$ incorporated into ATP (P_i-ATP exchange) or of $^{32}P_i$ released (ATPase) was determined by the isobutanol/benzene extraction procedure [21] as previously described [19,22].

ΔpH measurements. CF_0-CF_1 proteoliposomes used for ΔpH measurements were transferred through Sephadex G-50 columns preequilibrated with valinomycin in the following way: 30–50-μl

samples of CF_0-CF_1 protein/lipid/cholate (1.5 : 20 : 7) mixtures were applied to a Sephadex G-50 column (25 × 1 cm) preequilibrated at 25°C with 30 mM KCl, 10^{-8} M valinomycin, 1 mM P_i, 3 mM $MgCl_2$ and either 20 mM Na Tricine (pH 8; ATP-induced ΔpH) or 10 mM sodium succinate (pH 5.2; artificially induced Δ pH). The flow rate was adjusted to 2.0 ml/min. The proteoliposomes were collected into a measuring cuvette containing in a total volume of 2.0 ml the column equilibration buffer and 1 μM 9-aminoacridine hydrochloride. Fluorescence was followed at 25°C in an Perkin-Elmer MPF-44 spectrofluorimeter. The excitation and emission wavelengths were 420 and 465 nm, respectively, and the slits were adjusted to 10 nm each. Fluorescence changes in response to ATP (0.15 mM) or to NaOH (final pH 8.1–8.3) were followed. A more complete description of this procedure is described by Admon et al. [23].

Measurement-trapping capacity of proteoliposomes. The trapping capacity of CF_0-CF_1 proteoliposomes was measured by entrapment of ^{32}Pi followed by separation on Sephadex G-50 columns by the centrifugation procedure [24] as previously described [25].

Materials. Egg phosphatidylcholine was purchased from Makor Chemical, Jerusalem. Soybean phospholipids (soybean phosphatidylcholine, fraction V), brain phosphatidylserine and other chemicals and detergents were obtained from Sigma Chemicals. Sodium cholate was purified and recrystallized according to Kagawa and Racker [26]. [γ-^{32}P]ATP was synthetized by photophosphorylation with spinach thylakoids in the presence of ^{32}P and ADP followed by separation on poly(ethylene)imino-cellulose column according to Magnusson et al. [27].

Results

Lipid requirement for the reconstitution of CF_0-CF_1 ATP synthase

It has been demonstrated that the capacity of purified CF_0-CF_1 ATP synthase to catalyse P_i-ATP exchange necessitates reconstitution with phospholipids [28]. However, since the P_i-ATP exchange reaction requires the formation of tight vesicles, which are impermeable to protons, it is not possible to conclude from these results whether or not phospholipids are needed also in order to activate the enzyme.

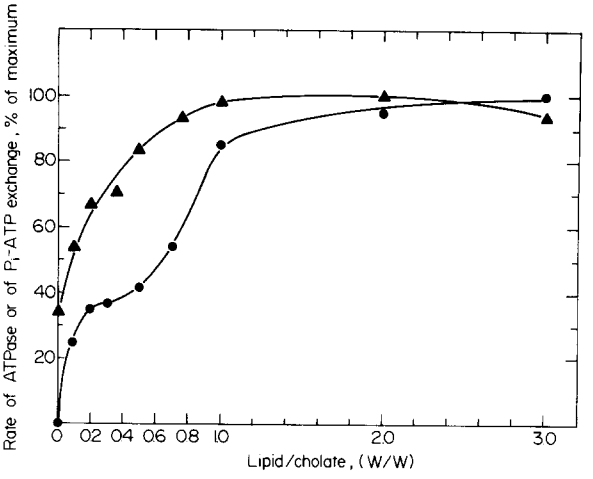

Fig. 1. Lipid-requirement for P_i-ATP exchange (●———●) and ATPase activities (▲———▲) of CF_0-CF_1. Reconstitution of CF_0-CF_1 with soybean lipids was performed by incubation of CF_0-CF_1 (1.5 mg/ml) for 25 min at 4°C with mixtures of sodium cholate (7 mg/ml) and different concentrations of soybean lipids (0–20 mg/ml), followed by a 50-fold dilution into the reaction mixture as described under Materials and Methods. The control rates of P_i-ATP exchange and Mg-ATPase were 110 and 90 nmol/mg protein per min, respectively.

Fig. 1 demonstrates that the presence of soybean phospholipids during reconstitution is indeed essential for the stimulation of P_i-ATP exchange and also to enhance the Mg-ATPase activity of CF_0-CF_1. These results suggest that phospholipids may be required also for the activation of the enzyme.

The sensitivity of Mg-ATPase activity of CF_0-CF_1 to the inhibitor DCCD also depends on the

TABLE I

LIPID-REQUIREMENT FOR DCCD INHIBITION OF CF_0-CF_1 Mg-ATPase

CF_0-CF_1 was reconstituted with different mixtures of cholate with soybean phospholipids as in Fig. 1 and was incubated for 30 min at 4°C with or without 50 μM DCCD, prior to the assay of Mg-ATPase activity.

Lipid/cholate (w/w)	Rate of ATP hydrolysis		Percentage inhibition
	control (nmol/mg)	+ DCCD (protein/min)	
0	110	122	0
0.2	195	110	44
3.0	310	103	67

presence of phospholipids during the reconstitution (Table I). Without the addition of phospholipids the rate of ATP hydrolysis is low and is insensitive to DCCD, while a partial sensitivity to DCCD is observed at a limiting phospholipid concentration which induces a suboptimal ATPase activation.

The effect of chloroplast lipids on P_i-ATP exchange and on Mg-ATPase activity of CF_0-CF_1 ATP synthase

In order to find out whether chloroplast lipids have a specific effect on CF_0-CF_1, the effects of chloroplast lipid mixtures were compared with that of different phospholipids on the activation of Mg-ATPase and of P_i-ATP exchange. For this purpose, CF_0-CF_1 was reconstituted by the cholate-dilution procedure with saturating concentrations of different lipid mixtures. Table II (upper part) demonstrates that CF_0-CF_1 proteoliposomes made from a polar chloroplast lipid extract have a lower P_i-ATP exchange rate but a much higher Mg-ATPase activity than the soybean phospholipid proteoliposomes. Since the decreased P_i-ATP exchange and increased Mg-ATPase in chloroplast lipids proteoliposomes may be due to a partial uncoupling, because of a higher permeability of chloroplast lipids to protons, the proton permeabilities and the extent of the ATP-induced ΔpH formation in chloroplast lipids and in soybean lipids proteoliposomes were compared by the 9-aminoacridine fluorescence quenching technique [27].

For this purpose, CF_0-CF_1 proteoliposomes, in which the major component of ATP-induced $\Delta\tilde{u}_{H^+}$ is ΔpH, were compared. Such proteoliposomes can be formed by transferring the protein/lipid/cholate mixture through a Sephadex G-50 column preequilibrated with valinomycin. CF_0-CF_1 proteoliposomes prepared by this procedure have similar activities (P_i-ATP exchange, Mg-ATPase) to proteoliposomes prepared by the cholate-dilution reconstitution technique. However, in contrast to cholate-dilution proteoliposomes, in which the ATP induces the formation of a large membrane potential ($\Delta\psi$) and of a small ΔpH, in the Sephadex-proteoliposomes ATP induces the formation of a large ΔpH and of a small $\Delta\psi$ [23].

Addition of ATP to soybean proteoliposomes induces a large fluorescence quenching (40%) indicating the formation of a large pH gradient across the liposome membrane (Fig. 2A). A much smaller fluorescence quenching is induced by ATP in chloroplast lipid proteoliposomes, in spite of their

TABLE II

THE EFFECT OF CHLOROPLAST LIPIDS ON P_i-ATP EXCHANGE AND ON ATPase ACTIVITIES OF CF_0-CF_1 ATP SYNTHASE

Lipid mixture	Addition to assay	P_i-ATP exchange (nmol/mg protein per min)	DCCD-sensitive Mg-ATPase (nmol/mg protein per min)
Soybean lipids		125	120
Chloroplast lipids		71	460
MGDG-deficient chloroplast lipids		20	68
MGDG-deficient chloroplast lipids/MGDG (1 : 1)		49	250
MGDG-deficient chloroplast lipids/ hydrogenated MGDG (1 : 1)		19	–
PC		3	15
PC/MGDG (1 : 1)		7	17
PC/PS (8 : 2)		12	35
PC/PS/MGDG (4 : 1 : 5)		14	36
Soybean lipids/MGDG (1 : 1)		66	140
Soybean lipids	–	132	100
Soybean lipids	0.2 μM SF-6847	16	160
Chloroplast lipids	–	82	380
Chloroplast lipids	0.2 μM SF-6847	13	450

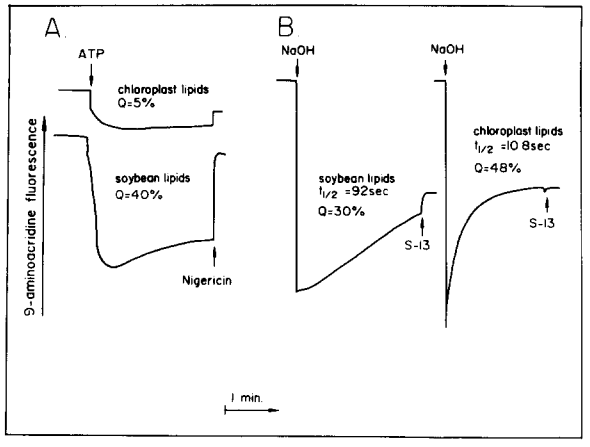

Fig. 2. Comparison of ATP-induced and of artificially induced pH gradients in CF_0-CF_1 proteoliposomes of soybean and of chloroplast thylakoid lipids. CF_0-CF_1 proteoliposomes were transferred through a Sephadex G-50 column preequilibrated with 10^{-8} M valinomycine as described under Materials and Methods. The pH of the incubation medium was (A) 8 or (B) 5.2 and the temperature was (A) 23°C or (B) 10°C. 9-Aminoacridine fluorescence quenching was induced by injection of (A) 0.15 mM ATP or (B) 40 μl 1 M NaOH. The final pH in (B) was 8.3. The final concentrations of Nigericine and of S-13 were 1 μM. $t_{1/2}$ represents the half-time of decay of the 9-aminoacridine fluorescence quenching signal (B) and Q represents the extent of fluorescence quenching as a percentage of the total fluorescence.

higher rate of ATP hydrolysis (Table II, upper part), indicating the formation of a smaller ΔpH. These results suggest that the permeability of chloroplast lipids to protons is indeed higher than that of soybean. A more direct comparison of the proton permeability of the two preparations is shown in fig. 2B. An artificial pH gradient was created by preequilibration of the proteoliposomes at 10°C in an acidic pH (5.3) followed by injection of NaOH (final pH, 8.2). The decay of the signal in chloroplast lipids proteoliposomes ($t_{1/2} = 11$ s) is much faster than the decay in soybean lipids proteoliposomes ($t_{1/2} = 92$ s) indicating that chloroplast lipids are indeed considerably more permeable to protons. However, the high permeability of chloroplast lipids to protons provides only a partial explanation for their much higher rate of Mg-ATPase in comparison to soybean lipids, since even fully uncoupled lipids proteoliposomes hydrolyze ATP at a much slower rate than fully uncoupled chloroplast lipids proteoliposomes (Table II, bottom part). These results suggest, therefore, that the high rate of Mg-ATPase in chloroplast lipid proteoliposomes is mainly due to activation of the enzyme by chloroplast lipids. A similar conclusion may be reached from the poor correlation between proton permeability and Mg-ATPase activity bteween phospholipids and chloroplast lipid proteoliposomes which can be seen from comparison of Tables II (upper part) and III. The permeability of chloroplast lipids to protons is comparable to that of PC or PC/PS (4 : 1) proteoliposomes (Table III), while their Mg-ATPase activities are vastly different (Table II, upper part).

In the absence of monogalactosyldiacylglycerol (MGDG), which constitutes about 50% of the total chloroplast lipids [1,5], there is a sharp decrease in both P_i-ATP exchange and Mg-ATPase activities (Table II, upper part). The poor activity of the MGDG-deficient chloroplast lipids cannot be due to inability of these lipids to form liposomes, since these lipids form with CF_0-CF_1 proteoliposomes which have an even larger trapping capacity (3.2 μl/mg) than comparable proteoliposomes made from soybean phospholipids (1.1 μl/mg) or from total chloroplast lipids (1.9 μl/mg) by the same procedure. Readdition of MGDG to the MGDG-deficient chloroplast lipids largely restores both P_i-ATP exchange and Mg-ATPase activity. Optimal activation of ATP hydrolysis is obtained by a mixture at a ratio of 6 : 4 of MGDG/MGDG-deficient chloroplast lipids, while a larger excess of MGDG decreases the effectiveness of reconstitu-

TABLE III

DIFFERENTIAL PERMEABILITY TO PROTONS OF DIFFERENT LIPID PROTEOLIPOSOMES

Induction of pH gradients in different proteoliposome preparations were performed by injection of NaOH (final pH, 8.1–8.3) to acidified proteoliposomes (pH 5.2) as in Fig. 2B. Q and $t_{1/2}$ are the extents and the half times of decay of the 9 aminoacridine fluorescence quenching signal, respectively.

Lipid mixture	$t_{1/2}$ (s)	Q (%)
Soybean lipids	92	30
Chloroplast lipids	11	48
Chloroplast lipids/PS (9 : 1)	27	42
PC	5	14
PC/PS (4 : 1)	24	28

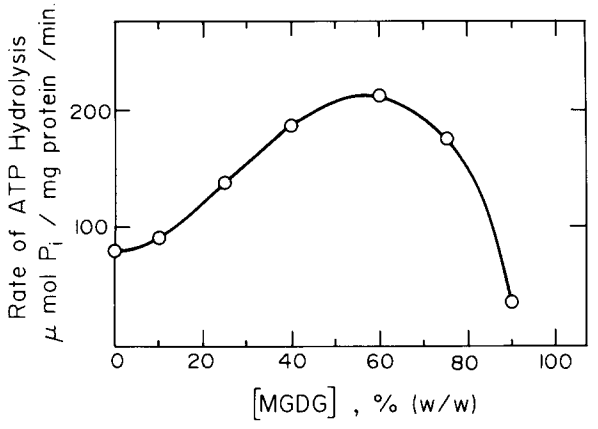

Fig. 3. Activation of CF_0-CF_1 ATPase by a mixture of MGDG and of MGDG-deficient chloroplast lipids. Reconstitution of CF_0-CF_1 with different mixtures of MGDG and MGDG-deficient chloroplast lipids fraction and ATPase assay were performed as described under Materials and Methods.

tion (Fig. 3). In contrast, hydrogenated MGDG in which the unsaturated fatty acids have been fully reduced, is ineffective in the reactivation of P_i-ATP exchange of MGDG-deficient chloroplast lipids (see Table II, upper part). This result indicates that the unsaturated fatty acids in MGDG may have an important role in the activation of the enzyme.

Egg phosphatidylcholine (PC) is ineffective in the reconstitution of catalytically active CF_0-CF_1 proteoliposomes but a supplement of 20% phosphatidylserine (PS) significantly stimulates both P_i-ATP exchange and Mg-ATPase activity of the proteoliposomes (Table II, upper part). The enhancement of P_i-ATP exchange seems to be largely due to the fact that PC/PS proteoliposomes are less permeable to protons in comparison to pure PC proteoliposomes (Table III). 1 : 1 mixtures of MGDG with either PC or PC/PS only slightly stimulate P_i-ATP exchange or Mg-ATPase of CF_0-CF_1 proteoliposomes in contrast to the large stimulation by MGDG and by MGDG-deficient chloroplast lipids (Table II, upper part; Fig. 3). These results indicate that the combination of MGDG with another specific component (or components) of the chloroplast lipid extract at an optimal ratio of close to 1 : 1 is essential for the activation of CF_0-CF_1.

Since P_i-ATP exchange activity of chloroplast lipids proteoliposomes seems to be limited by their high permeability to protons, and since small amounts of PS seem to decrease the permeability of PC vesicles to protons (Table III), an attempt to increase P_i-ATP exchange of chloroplast lipids proteoliposomes was made by the addition of PS. Fig. 4 demonstrates that low PS concentrations indeed enhance P_i-ATP exchange of chloroplast lipids proteoliposomes. The optimal stimulation of P_i-ATP exchange is obtained by addition of 10% PS (which also significantly decreases the permeability of chloroplast lipids to protons, Table III). A partial stimulation is obtained by higher PS concentrations up to a 75% mole ratio with a sharp drop in pure PS proteoliposomes. The observation that the amount of chloroplast lipids can be reduced 4-fold, with only slightly reducing the activity, may suggest that the actual amount of chloroplast lipids needed for activation of CF_0-CF_1 is significantly lower than the amount of lipids needed to form tight-proteoliposomes with the enzyme.

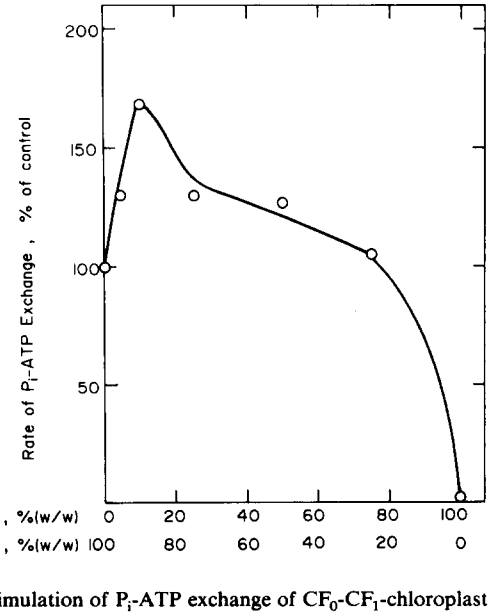

Fig. 4. Stimulation of P_i-ATP exchange of CF_0-CF_1-chloroplast lipid proteoliposomes by PS. CF_0-CF_1 proteoliposomes were reconstituted with different mixtures of chloroplast lipids and phosphatidylserine (PS). P_i-ATP exchange was measured as described under Materials and Methods. The control rate (100%) was 70 nmol/mg protein per min.

In order to check whether the large differences in the activities of the CF_0-CF_1 proteoliposomes between the different types of lipid mixtures are not a result of ineffective reconstitution of the protein, two different reconstitution procedures were compared – the cholate dilution and the freeze-thaw sonication [30] in which a higher lipid/protein ratio and no cholate is sued for reconstitution. Comparisons of soybean lipids, chloroplast lipids, MGDG-deficient chloroplast lipids, PC and PC/PS in the reconstitution of CF_0-CF_1 proteoliposomes by the two techniques gave very similar results indicating that the differences in activities between proteoliposomes made from the different lipid mixtures are mainly due to the interactions of the lipids with the enzyme.

In previous experiments we have analyzed by an electron microscope CF_0-CF_1 proteoliposome preparations reconstituted with pure egg-PC phosphatidylcholine and with soybean lipids by the freeze-thaw reconstitution procedure. Both preparations appear very similar with respect to the orientation of CF_0-CF_1 in liposome membrane (about 60% CF_1 facing out and 40% facing in) and in that essentially all the enzyme was incorporated in the liposome membrane (Pick, U., unpublished data). These results also support the conclusion that the differential effects of lipids on the activity of CF_0-CF_1 are not due to ineffective reconstitution or to a preferentially asymmetric orientation in the lipid bilayer but to direct interactions of the lipid with the enzyme.

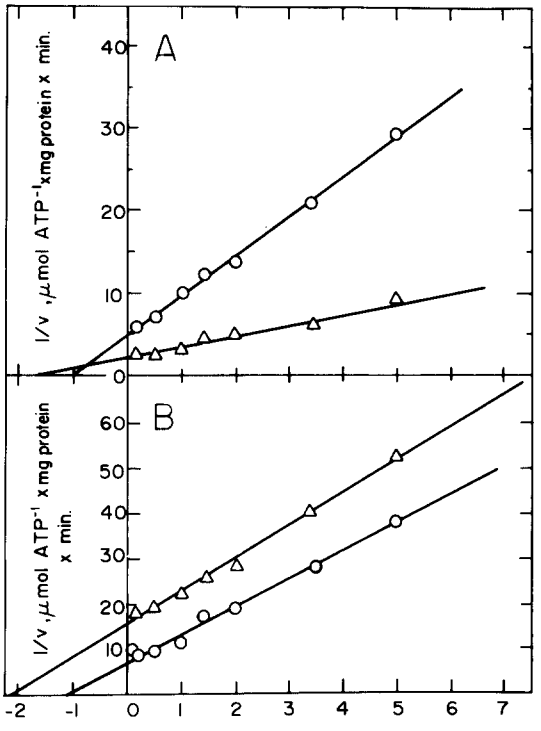

Fig. 5. Differential effects of soybean (○———○) and of chloroplast (△———△) lipids on the K_m and V of CF_0-CF_1 for ATP. The initial rates of (A) ATP hydrolysis (soybean: $K_m = 1$ mM, $V_{max} = 212$ mM/min; chloroplast: $K_m = 0.6$ mM, $V_{max} = 490$ mM/min) and of (B) P_i-ATP exchange (soybean: $K_m = 0.46$ mM, $V_{max} = 65$ mM/min; chloroplast: $K_m = 0.9$ mM, $V_{max} = 138$ mM/min) were measured at different ATP concentrations (0.2–10 mM) and the reciprocal rates were plotted as a function of the reciprocal ATP concentrations.

Comparison of the kinetic parameters of ATP hydrolysis and of P_i-ATP exchange in soybean lipids and in chloroplast lipid proteoliposomes

The results described in the previous sections indicate that chloroplast lipids have a specific effect on the catalytic capacity of CF_0-CF_1. In order to find out how this effect is expressed in the kinetics of ATP hydrolysis and of P_i-ATP exchange, the ATP concentration dependence of both reactions in soybean lipids and in chloroplast lipid proteoliposomes was compared. The analysis in Fig. 5 demonstrates that the K_m for ATP in chloroplast lipids is about 2-fold lower than in soybean lipid proteoliposomes for both reactions indicating a higher affinity for ATP in chloroplast lipids. The analysis also demonstrates a higher V_{max} for Mg-ATPase and a lower V_{max} in P_i-ATP exchange in chloroplast lipids, the latter probably resulting from the high permeability of chloroplast lipids proteoliposomes to protons. In order to find out to what extent does the degree of coupling, namely, the $\Delta\tilde{u}_{H^+}$ influence the K_m for ATP the effect of uncoupler on the K_m and V_{max} for P_i-ATP exchange in soybean lipid proteoliposomes was tested. 1.5 μM SF-6847 reduces the V_{max} by 55% but affects very little the K_m for ATP (1.1 mM, data not shown). The differences in K_m (ATP) between chloroplast lipids and soybean lipid proteoliposomes cannot be due, therefore, to the higher permeability of the former to protons.

Discussion

The interactions of chloroplast lipids with the purified CF_0-CF_1 ATP synthase was studied by taking advantage of the fact that specific lipids affect the catalytic properties of the enzyme. It was demonstrated that chloroplast lipids in comparison to phospholipids activate the enzyme as expressed by acceleration of the turnover rate and by a decrease in the K_m for ATP. The observation that chloroplast lipids, which are supposedly in contact with CF_0, influence the catalytic properties of CF_1 demonstrates that the lipids in the thylakoid membrane have specific functions in addition to acting as a proton-impermeable barrier.

The effect of chloroplast lipids on the kinetics of ATP hydrolysis by CF_0-CF_1 may provide a partial explanation for the low ATPase activity and for the low affinity for ATP of purified CF_0-CF_1 – phospholipid proteoliposomes in comparison to the kinetic parameters of ATP hydrolysis by light-activated thylakoids. Thus the K_m (ATP) for Mg-ATPase in light-activated thylakoids is 100–200 μM (Ref. 31 and Pick, U., unpublished data) in comparison to about 1 mM in CF_0-CF_1 soybean lipids proteoliposomes (Fig. 5). Similarly, the rate of Mg-ATPase activity in light-activated thylakoids is approx. 3000 μmol/mg CF_0-CF_1 protein per min (by assuming 0.5 mg CF_0-CF_1 protein/mg chlorophyll, Pick, U., unpublished results) in comparison to 100–400 μmol/mg protein per min in CF_0-CF_1 soybean lipids proteoliposomes.

Two unusual properties of chloroplast lipids are the high content of MGDG and DGDG (70% of the total lipid composition, Refs. 1 and 4) and the high content of unsaturated fatty acids (mainly linolenic acid, Ref. 1). Both of these properties seem to be essential for the activation of CF_0-CF_1. This was demonstrated by the MGDG requirement for effective activation of CF_0-CF_1 and by the observation that hydrogenation of the unsaturated fatty acids in MGDG reduces its effectiveness in activating the enzyme (Table II, upper part). The requirment for unsaturated MGDG may indicate that the activation of CF_0-CF_1 depends on: (a) specific structural properties of this lipid (packing) which are modified by hydrogenation or (b) that MGDG should be in a liquid crystalline phase (high fluidity), since saturated MGDG exists in a gel-phase at room temperature. In addition to MGDG, another chloroplast lipid component (possibly DGDG), seems to be essential for CF_0-CF_1 activation since combinations of MGDG with phospholipids were ineffective (Table II, upper part).

One of the puzzling observations in this work is the relatively high permeability of chloroplast lipids to protons. Intact thylakoids have a much lower permeability to protons under comparable conditions. The half-time for the decay of light-induced pH gradients in thylakoids at 10°C is approx. 0.5–1 min [32]. The increased permeability of chloroplast lipids may be due to an alteration of their structure and properties because of the absence of specific membranal proteins. Recent studies have demonstrated clear differences in the physical properties between chloroplast lipid extracts and the lipids in the intact thylakoid membrane [2]. Mixtures of MGDG/DGDG [3] as well as total chloroplast lipid extracts in the presence of physiological concentrations of Mg^{2+} [2] tend to form non-bilayer structures as a result of phase separations of certain lipid components. Such structures have not been observed in thylakoids under normal conditions [33]. It was suggested that proteins in the thylakoid membrane may have a restraining effect on the lipid and prevent the formation of non-bilayer structures [34]. It is possible, therefore, that the high permeability of chloroplast lipid proteoliposomes results from the formation of inverted-micellar and other non-bilayer structures which are not formed in the thylakoid membrane. It has to be remembered that polar lipid/protein weight ratio is about 1 : 2 in thylakoids [4] and at least 10 : 1 in the reconstituted system. It is not too surprising, therefore, that the absence of many structural proteins of thylakoid membranes and the relatively low protein content in the reconstituted system should affect the organization of the lipids. In this respect the effect of PS on the permeability (Table III) and on the activity (Fig. 4) of chloroplast lipid proteoliposomes may suggest that one of the missing factors conferred by membranal proteins is negative charge.

Finally, the specific effects of chloroplast lipids on CF_0-CF_1 activity may be related to the activa-

tion of a Mg-specific ATPase in soluble CF_1 by octylglucoside micelles [22,35]. We have previously demonstrated that octylglucoside micelles activate CF_1 in a manner similar to the light activation of CF_0-CF_1 in the thylakoid membrane [22,35–37] and speculated that the detergent micelles may bind to a region in CF_1 which in illuminated chloroplasts comes in contact with the thylakoid membrane interface. At present we have no evidence to indicate whether the activating effect of chloroplast lipids on CF_0-CF_1 is via CF_0 or by a more direct interaction with CF_1. However, it may be noted that Nelson et al. [38] and Sigrist-Nelson and Azzi [39] have previously reported that chloroplast galactolipids are required in order to induce a DCCD-sensitive proton channel by the purified subunit III of CF_0 indicating a specific interaction between CF_0 and galactolipids in the thylakoid membrane.

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