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ATPase activity of thylakoid membranes in CTAB-hexanol-octane low water system

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Abstract

Thylakoid membranes transferred into a low water system composed of *n*-octane, the cationic surfactant cetyltrimethylammonium bromide (CTAB), and 1-hexanol as cosurfactant, displayed protein- and substrate-dependent ATPase activities for more than 60 min. This activity was enhanced 7–10-fold and 3–4-fold with 28%-vol. of methanol and 21%-vol of *tert*-butanol present in the polar phase, respectively, in a fashion reminiscent of what occurs in aqueous media. Approximately 25% and 10% of control and methanol-enhanced ATPase activities found in buffer were detected in the low water system, respectively, and both activities showed a pronounced dependency on the amount of water present (between 2.5 and 15% of water (v/v)). ^{1}H-Nuclear Magnetic Resonance (^{1}H-NMR) studies revealed that the bound/free water ratio (a) increased with decreasing concentration of water in the reverse micellar phase and (b) slightly increased in the presence of methanol. The results altogether suggest that the amount and physical state of water significantly contribute to determine the ATPase activity in the low water system. © 1997 Elsevier Science B.V.

Keywords: ATPase; Thylakoid membrane; Low water system

1. Introduction

The central role of water in all non-covalent protein interactions is well recognized and under examination [1,2]. Controlled hydration studies with lysozyme [3,4] and with soluble enzymes suspended in organic solvents [5] have addressed how much water is required for enzyme function. However, a lot needs to be done to better understand the participation of water in protein structure and catalysis. In this regard, reverse micelles can be very useful since their water content can be controlled. They have been employed to study the water dependence of enzyme catalysis, thermostability and protein folding (reviewed in [6]; see also [7–9]).

Few membrane proteins have been studied in reverse micelles (reviewed in [10]). Relevant to the present work are: the mitochondrial ATPase [11–14] and the ATPase from the sarcoplasmic reticulum (SR) [7]. These two ATPases display different properties when transferred to reverse micelles formed with toluene, phospholipids and Triton X-100 (TPT). The mitochondrial ATPase's hydrolytic activity is very

Abbreviations: ATPase, adenosinetriphosphatase; CTAB, cetyltrimethylammoniumbromide; CHO, cetyltrimethylammoniumbromide-1-hexanol-*n*-octane

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low (2–5%), as compared to an all aqueous media. In contrast, the ATPase from SR in this reverse micellar system reaches 50% of its hydrolytic activity in aqueous media. In this regard it seemed interesting to study the catalytic characteristics of another membrane ATPase, in an attempt to explain these differences. In addition, it has been argued that water is important in the catalytic cycle of the chloroplast ATPase [15], thus making this enzyme a good candidate to be studied in reverse micelles.

The chloroplast ATPase is a multimeric enzyme complex with a hydrophilic sector (CF_1), with subunit stechiometry $\alpha_3\beta_3\gamma\delta\epsilon$ [16] containing six nucleotide binding sites [17,18], and a membrane-spanning hydrophobic sector (CF_0) containing 4 different polypeptides known as subunits I–IV [19] acting as a proton channel. The structure and dynamic behavior of both enzyme parts are known in detail [20,21]. The dynamic and complex interactions between the different nucleotide binding sites are now being explored [22,23].

This study presents results on the enzymatic behavior of the multimeric ATPase complex in cetyltrimethylammonium bromide, 1-hexanol and *n*-octane (CHO) reverse micellar system [24]. The TPT low water system successfully used for most ATPase work in reverse micelles was replaced in the present study by the CHO system because it allows a broader range of water solubilization, and has better temperature stability and spectroscopic characteristics [25–28]. Under optimal conditions, the enzyme can hydrolyze ATP in the low water system at a constant rate, close to that found in all water media, for more than 60 min. The latent chloroplast ATPase in reverse micelles is activated by alcohols severalfold, in a similar fashion as it occurs in all water media. The activity depends on protein, substrate and the water content of the system, which was measured using ^{1}H-NMR.

2. Materials and methods

All *chemicals* were obtained from SIGMA or FLUKA and used without any further purification. Pyruvate kinase and lactate dehydrogenase were obtained from BOEHRINGER.

Thylakoid membranes were isolated in the cold as follows: 80 ml of isolation buffer (50 mM tricine, 300 mM NaCl, 3 mM $MgCl_2$, pH 7.5) were added to 18 g of fine cut leaves. After blending the homogenate was filtered through nylon cloth (36 μm) along with muslim cloth, and centrifuged for 3 min at 3900 r.p.m. (HETTICH, Universal/K2S). The pellet was washed once with resuspension buffer (0.2 M sorbitol, 20 mM tricine, 5 mM NaCl, pH 7.5) and finally resuspended in the same buffer at 2.5–3.5 mg chlorophyll/ml (30–40 mg protein/ml). Aliquots were stored in liquid nitrogen without significant loss in ATPase activity.

The *CHO reverse micellar media* was formed with 0.2 M Cetyltrimethylammonium bromide (CTAB) in a mixture of 1 part of 1-Hexanol and 8.7 parts *n*-octane [24]. The aqueous phase for the enzymatic reaction contained 25 mM tricine-KOH pH 8.0, 30 mM KAc, 12 mM $MgCl_2$, 12 mM Na_2-ATP pH 8.0, unless otherwise stated. The reaction mixture was added to CHO and a transparent reverse micellar phase was obtained within seconds of strong vortexing. A desired amount of thylakoids were added to start enzymatic reaction and to achieve the final water content, expressed as *W*o (molar ratio of H_2O to surfactant). A *W*o of 20 corresponds to 7.4% of water (v/v). Experiments in the presence of alcohols (methanol or *tert*-butanol) were designed so that the total volume of the polar phase (reaction mixture, alcohol plus thylakoid membranes) remained constant, e.g. alcohol addition replaced an equal volume of water in the reaction mixture. Reactions were stopped in 0.55 M KOH. The formed ADP was extracted [14] from the low water system with a mixture of water-saturated isobutanol:benzene 1:1 and quantified in a coupled enzymatic assay following NADH-oxidation [29,30] in an assay media containing 25 mM tricine-KOH pH 8.0, 30 mM KAc, 5 mM $MgCl_2$, 5 mM Na_2-ATP pH 8.0, 5 mM phosphoenolpyruvat and 0.22 mM NADH. Coupling was achieved with an excess of pyruvate kinase and lactic dehydrogenase.

Chloroplast ATPase *activity in 100% water* was assayed measuring either formed ADP under the same conditions as in CHO (as indicated above) in the presence of 5 mM $MgCl_2$ and 5 mM ATP, or phosphate production by the Fiske and Subbarow method [31] in a reaction mixture containing 25 mM *N*-tris(hydroxymethyl)methyl-3-aminopropanesulfon-

ic acid (TAPS) pH 8.8, 20 mM NaCl, 50 mM sodium-sulfite, 5 mM $MgCl_2$ and 5 mM Na_2-ATP. Reactions were stopped with 4% TCA.

Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a 60 MHz apparatus (VARIAN EM 360 L) of the University of Geneva (Unité de Chimie Pharmaceutique, Sciences II).

Activity measurements at 25°C in CHO and in 100% water were made in triplicates from at least two different chloroplast preparations. NMR-spectra were taken in duplicate on two separate sample preparations.

3. Results

3.1. Enzyme- and substrate-dependent ATPase activity of thylakoids in the CHO micellar system

ADP formation by the chloroplast ATPase in a reverse micellar system (CHO) with $Wo = 20$ was characterized with respect to enzyme and substrate (ATP) concentration (see Fig. 1A,B). A linear increase in the hydrolytic activity was observed up to 40 μg chlorophyll/ml CHO (corresponding to 370 μg protein/ml CHO). Higher protein concentrations only marginally increased ADP formation despite the much higher (5-fold) protein solubilizing capacity of CHO. The chloroplast ATPase transferred into CHO showed a strong dependence on ATP concentration. An increasing activity was observed up to 12 mM ATP (concentration in the water pool). At [ATP] between 12 and 24 mM, ADP production leveled off. No ADP production was observed without chloroplasts or without ATP in the reverse micellar phase (data not shown). Heat-inactivated chloroplasts (in buffer, 80°C for 4 min) showed no detectable ATPase activity after transfer into CHO.

3.2. Effect of methanol and tert-butanol on chloroplast ATPase activity in reverse micelles

ATP hydrolysis of both, the membrane bound chloroplast ATPase [32] and the isolated coupling factor (CF1) [33,34], is enhanced by methanol and *tert*-butanol. Thus, it seemed worthwhile to study if these alcohols would also activate this enzyme in reverse micelles when present in their aqueous compartment. Fig. 2 shows that the presence of these alcohols in the reaction mixture stimulated several-fold the chloroplast ATPase activity in the low water system. These experiments were carried out in reverse micelles with a $Wo = 20$. Optimal effects were obtained around 25–30% (v/v of aqueous phase)

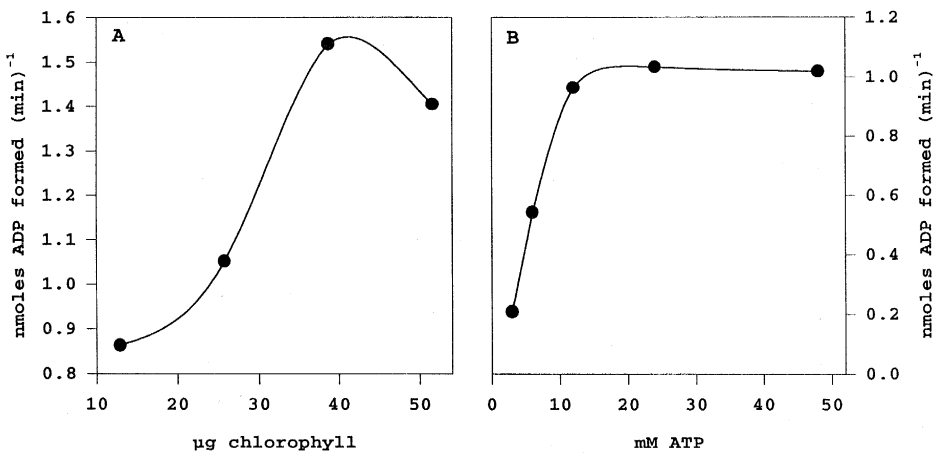

Fig. 1. Enzyme- and substrate-dependent chloroplast ATPase activity in CHO at $Wo = 20$. A: increasing amounts (in μg chlorophyll per 0.8 ml of CHO; 12 mM ATP) of isolated thylakoid membranes in resuspension buffer were added into CHO containing the reaction mixture (final $Wo = 20$), and hydrolytic activity measured (see Section 2). B: variations in ATPase activity with increasing concentrations of ATP present in CHO (14.1 μg Chl/0.8 ml CHO). Enzymatic reactions were started in both cases by adding the chloroplast preparations in resuspension buffer to the reverse micellar phase formed before, and concentrations of ATP expressed in mM were calculated with respect to the total aqueous pool of the reverse micellar phase. Reactions were stopped at the indicated time points and ADP was quantified as described in Section 2.

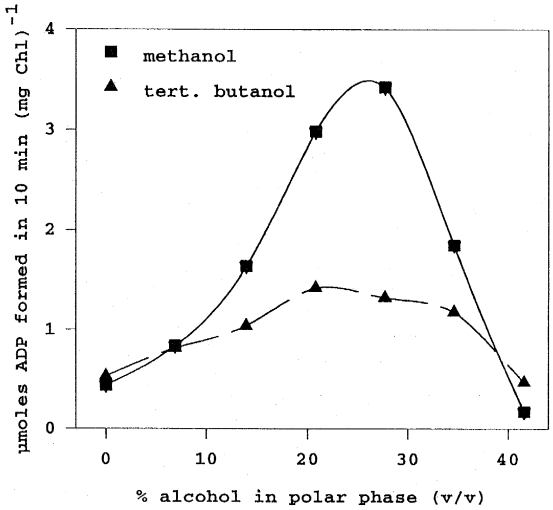

Fig. 2. Stimulation by alcohols of chloroplast ATPase activity in CHO. Varying concentrations of methanol (squares) or tert-butanol (triangles) were present in the reaction mixture. The volume of the polar phase (water, alcohol plus thylakoid membranes) was kept constant and alcohols replaced water in the reaction mixture. Alcohol concentrations were expressed as vol. % of the total polar phase. Reactions were started by the addition of thylakoids (around 25 µg Chl/ml CHO) to the reverse micellar phase formed previously with the reaction mixture. Reactions were stopped after 10 min and ADP was quantified as described in Section 2. The *Wo*-value was 19.5 for experiments without any added alcohol.

with methanol, and around 20% with *tert*-butanol. It is worthwhile mentioning that an increase in the amount of alcohol present in the aqueous phase is paralleled by a decrease in *Wo* (e.g., increasing the alcohol concentration from 0% to 42% decreases *Wo* from 20 to 11). The latent chloroplast ATPase activity in CHO was enhanced maximally 15-fold with 28% methanol at $Wo = 14.1$. In contrast, the chloroplast preparations in buffer showed a 60-fold maximal enhancement at concentrations ~40% (compare in Table 1). The specific activity was enhanced by a factor of 3 with 21% *tert*-butanol. The increase in activity produced by methanol overcomes the activity decrease caused by the water reduction in CHO (compare results on *Wo*-dependent ATPase activities in Fig. 4). Both alcohols inhibited the chloroplast ATPase activity at concentrations above 40% (v/v).

With 28% methanol in the reaction mixture the enzyme activity in CHO was enhanced severalfold (see Fig. 2). Fig. 3 shows that this activity was mostly affected during the first 10 min. Thereafter,

Table 1
Specific activities in µmol·min^{-1}·(mg Chl)$^{-1}$ of chloroplast ATPase in low water system (CHO) and in buffer (100% water) in the absence (latent) and in the presence of methanol (stimulated)

		CHO system	100% water
Latent	(P_i)	–	0.08
	(ADP)	0.04	0.16
Stimulated	(P_i)	–	7.00
	(ADP)	0.62	–

ADP production by the latent an alcohol-stimulated ATPase in CHO, and in buffer for the latent ATPase, was measured under the same conditions (see Section 2). Phosphate (P_i) was determined in a different buffer (for details, see Section 2). ATPase was activated by 28% methanol in CHO (corresponding to $Wo = 14.4$; $Wo = 19.5$ for latent activity) and 40% methanol in buffer. Initial velocities are shown for the activated enzyme. All activities are means of at least three measurements.

the activities in the presence or absence of methanol became similar. The control curve without methanol was measured at $Wo = 19.5$, corresponding to 7.2% of water, whereas the *Wo* decreased to 14.1 (5.2% of water) in the presence of 28% methanol. For comparison, the inset in Fig. 3 shows the effect of methanol on chloroplast ATPase activity in an all water system. The thylakoid preparations used in this work were

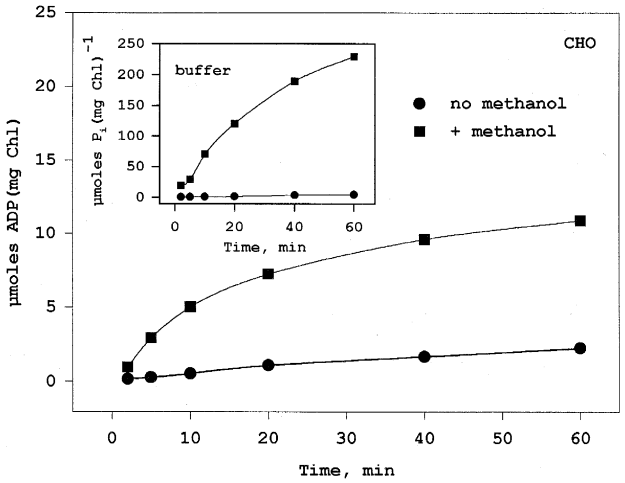

Fig. 3. Kinetics of latent and methanol-enhanced chloroplast ATPase activity. Activities in CHO were measured at $Wo = 19.5$ (circles) and in the presence of 28% methanol (squares, $Wo = 14.1$). The added methanol in CHO replaced water in the reaction mixture. Inset: Control and methanol-stimulated ATPase activity in aqueous buffer. The reaction mixture contained either no methanol (circles) or 40% methanol (squares).

maximally enhanced in the presence of 40% methanol in all water medium and, in contrast to the results from Anthon and Jagendorf [32], remained activated for a longer time. As in the low water system, the kinetics of alcohol activation in buffer were not linear. Table 1 compares results obtained in the low water system with the activities in buffer. Under equivalent conditions, the latent chloroplast ATPase activity found in the low water system at 7.2% of water ($Wo = 19.5$) was half or one fourth of that found in buffer. Methanol enhanced ATPase activity about 15-fold in CHO and 40–85-fold in all water. It must be pointed out that the alcohol-stimulated ATPase activities in aqueous media were determined measuring P_i because of the interference of the alcohol on the ADP enzyme assay. P_i was measured in the presence of sulfite and at a higher pH than ADP (see Section 2), this could contribute to the difference in the alcohol stimulation in the two systems. It was not possible to fully recover P_i from reverse micellar system, precluding the use of this assay in CHO. However, since there is only a factor of two difference between the activity determined measuring ADP and P_i in aqueous media (Table 1), the activation ratios should be somehow comparable.

3.3. Water dependence of the hydrolytic activity of latent and methanol-activated chloroplast ATPase in reverse micelles

As mentioned earlier, one of the advantages of reverse micelles is that it is possible to probe the water dependence of an enzymatic reaction. As Fig. 4 portrays, latent and methanol-activated enzyme activities increased with increasing amounts of water. Below $Wo = 7$ there was essentially no activity detectable. Chloroplast proteins extracted in a low water system composed of phospholipid and isooctane, having a water content of around 1% (10), also did not show any ATPase activity. In the presence of 28% methanol, specific activity increased in CHO at least 7 times for all samples tested. The latent enzyme activity slightly increased over the whole *W*o-range tested. The methanol-activated ATPase displayed optimal behavior at a *W*o between 20 and 30. At $Wo > 30$ (11% H_2O), the methanol-enhanced chloroplast ATPase activity decreased.

3.4. Water behavior in reverse micelles assayed by NMR

Water in reverse micelles does not entirely show the behavior of normal bulk water. This can be seen in Fig. 5 where the resonance shifts (measured from the main $-CH_3$ at 0.87 p.p.m.) of the water protons are plotted against the water content of the reverse micelles. The water resonance, which includes the fast exchanging proton of the –OH group from 1-hexanol, increases from around 3.4–3.75 as the water concentration was raised from 2 to 15% (v/v). Essentially there were no changes detectable in the resonance shifts of all other NMR-active groups in CHO in the indicated water range, with the exception that the $N(CH_3)_3$ of the CTAB slightly moved at $Wo < 10$. The inset in Fig. 5 confirms that all the water added to the low water system is observed in the NMR-measurements as a linear increase in the total area of the water peak. Importantly, the addition of 28% methanol to CHO (methanol replacing water) slightly displaced the resonance shifts to higher values. This effect is more pronounced at higher *W*o-

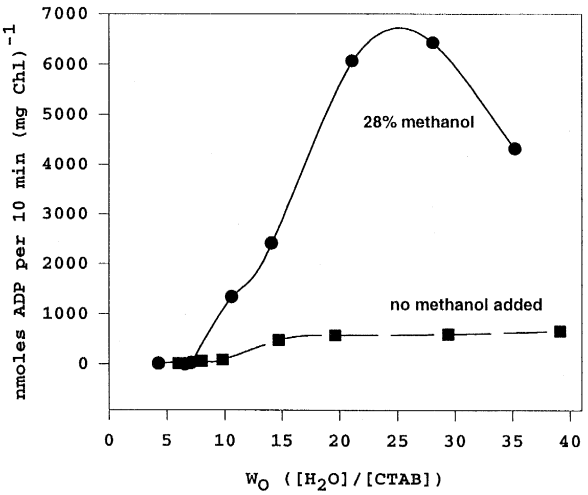

Fig. 4. *W*o-dependent chloroplast ATPase activity in CHO. Enzymatic activity was measured either with 28% or without methanol in the reaction mixture at different water concentrations. Experimental conditions were chosen so that the total volume of water plus methanol remained constant. The experiments were started with 12.5 and 50 μg Chl/ml in the presence or absence of methanol, respectively. Reactions were stopped after 20 min and specific activities were calculated (see Section 2).

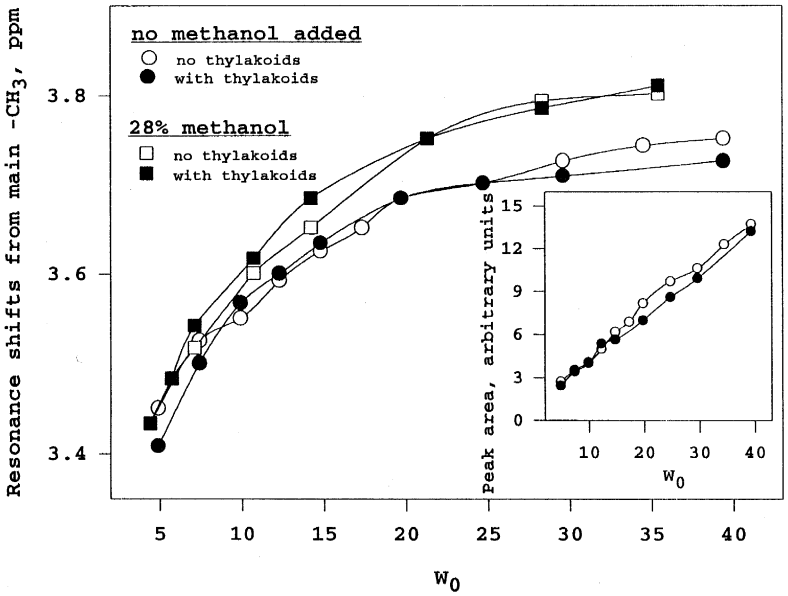

Fig. 5. *Wo*-dependent water resonance shifts in CHO. Proton resonance shifts of H–O–H and –OH (of 1-hexanol), from the main –CH3 resonance band, were determined by ^{1}H-NMR as a function of *Wo* in CHO. Shown are the resonance shifts for CHO with (closed symbols) and without (open symbols) chloroplast protein (230 μg protein/ml CHO), and with 28% methanol (squares) or without (circles). Inset: Peak area in arbitrary units under the water resonance from H–O–H and –OH as a function of *Wo*.

values than at low hydration levels and could indicate that methanol 'transforms' bound water into free water.

4. Discussion

There have been limited functional studies of active membrane-bound enzymes in reverse micelles (reviewed in [10]). This report shows the successful transfer of spinach thylakoid membranes into a low water medium containing the cationic detergent CTAB in a mixture of *n*-octane and 1-hexanol, where they displayed ATPase activity. As has been found for other enzymes in reverse micelles, the chloroplast ATPase activity depended on *Wo*. Barely any ATP-hydrolysis could be detected below $Wo = 7$ (2.6% water, v/v), above this *Wo*, activity increased up to $Wo = 30$ where it reached a plateau. In CHO reverse micelles under optimal conditions, the latent chloroplast ATPase activity was 25–50% of that found in thylakoids suspended in buffer, and remained constant for at least 90 min. Preliminary results with divalent cations indicate that the enzyme in CHO behaves more like the membrane-bound form, highly dependent on Mg^{2+}, in contrast to the Ca^{2+} dependent activity displayed by the detached ATPase.

Methanol and *tert*-butanol, which in aqueous media increase the chloroplast ATPase activity of the membrane bound enzyme [32] as well as the isolated coupling factor [33,34], distinctly enhanced ATP hydrolysis in the low water system (Figs. 2 and 3). Less methanol was required to achieve maximal activation in CHO (28% versus 40% in buffer), particularly considering the possible partition of a small amount of alcohol into the solvent. On the other hand, methanol's effect was 4-fold less potent than in all water media.

How alcohols stimulate the chloroplast ATPase in water media is not fully understood. Anthon and Jagendorf [32] pointed out that methanol enhances the turnover rate of the latent enzyme without acting on the inhibitory protein subunit or on nucleotide binding. It has been suggested that alcohols affect water structure [35], and thereby can induce protein conformational changes. Our results monitoring water behavior by NMR in reverse micelles containing alcohol (Fig. 5), are consistent with this suggestion. Recent reports indicate that organic solvents may alter the hydrophilic/hydrophobic environment at

distinct sites of ATPases in all water media causing activity changes [36–39]. It is likely that the chloroplast ATPase sites affected by alcohols in all water media may be similar and accessible in the low water system.

In agreement with previous findings [40,41], as the water content in reverse micelles was raised, the fraction of bound to free water, monitored by NMR, decreased. These measurements report the overall free water content of the population of empty and protein-filled reverse micelles. In our case more than 90% of the micelles remain empty after entrapment of the thylakoid membranes, therefore, the amount of free water in the protein-filled micelles remains to be determined. However, the latent and alcohol activated chloroplast ATPase activities were enhanced as more free water appeared in CHO, indicating that increases in the overall free-water content of the micelles are reflected in those containing protein. Part of the ATPase activity enhancement induced by methanol could result from the increase in the fraction of free water it causes (compare Fig. 4 and Fig. 5). Polar solvents like ethanol can occupy weaker bound water molecules in the secondary hydration layer of a protein [41,42]. The increase in the resonance shift at $Wo = 20$ between CHO reverse micelles in the absence and presence of methanol is equivalent to an increase in water content of 1.8% or a change in Wo of 5. However, Fig. 4 shows that this apparent increase in bulk water is not enough to explain the large methanol effect on the chloroplast ATPase in CHO. It is not clear at this time why at Wo above 30, where most water appears as free water in the NMR measurements, enzyme activity decreases.

The latent and methanol-enhanced chloroplast ATPase activities recovered in CHO, with respect to all water media, fall within the range of activities found for the mitochondrial (1% [14]) and sarcoplasmic reticulum (50% [7]) ATPases. Why this range is so wide remains unexplained. The mitochondrial and chloroplast ATPases are close relatives [43], yet their activities vary significantly in reverse micelles. Could it be that the water sensitive sites that affect catalysis are different or that they differ in sensitivity to the transfer process into reverse micelles? The catalytic cycle of cytochrome c oxidase [44,45] and of the sarcoplasmic reticulum ATPase [7] involve intermediates with distinct water requirements. Our results with the chloroplast ATPase in reverse micelles, and the reported water movements from the chloroplast membranes to CF1 during the photophosphorylation process [15], indicate that it is worth while to continue exploring the role of water in catalysis of membrane-embedded enzymes.

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References

[1] J.L. Finney, B.J. Gellatly, I.C. Golton, J. Goodfellow, Biophys. J. 32 (1980) 17–33.
[2] P.M. Wiggins, Microbiol. Rev. 54 (1990) 432–449.
[3] J.A. Rupley, Adv. Protein Chem. 41 (1991) 37–172.
[4] G. Careri, E. Gratton, P.-H. Yang, J.A. Rupley, Nature 284 (1980) 572–573.
[5] A. Zaks, A.M. Klibanov, J. Biol. Chem. 263 (1988) 3194–3201.
[6] G. Garza-Ramos, D.A. Fernández-Velasco, M. Tuena de Gómez-Puyou, A. Gómez-Puyou, A., in: A. Gómez-Puyou (Ed.), Biomolecules in Organic Solvents, CRC Press, Orlando, FL, 1992, pp. 113–131.
[7] H. Barrabin, H.M. Scofano, M. Tuena de Gómez-Puyou, A. Gómez-Puyou, Eur. J. Biochem. 213 (1993) 757–763.
[8] A.D. Fernandez-Velasco, M. Sepulveda-Becerra, A. Galina, A. Darszon, M. Tuena de Gomez-Puyou, A. Gomez-Puyou, Biochemistry 34 (1995) 361–369.
[9] M.A. Sepúlveda-Becerra, T.S. Ferreira, R. Strasser, W. Garzón-Rodríguez, C. Beltrán, A. Gómez-Puyou, A. Darszon, Biochemistry 35 (1996) 15915–15922.
[10] A. Darszon, L. Shoshani, in: A. Gómez-Puyou, (Ed.), Biomolecules in Organic Solvents, CRC Press, Orlando, FL, 1992, pp. 35–65.
[11] A. Darszon, A. Gómez-Puyou, Eur. J. Biochem. 121 (1982) 427–433.
[12] V.J. Sánchez-Bustamente, A. Darszon, A. Gómez-Puyou, Eur. J. Biochem. 126 (1982) 611–616.
[13] G. Ayala, M. Tuena de Gómez-Puyou, A. Gómez-Puyou, A. Darszon, FEBS Lett. 203 (1986) 41–43.

[14] G. Garza-Ramos, A. Darszon, M. Tuena de Gómez-Puyou, A. Gómez-Puyou, Biochemistry 29 (1990) 751–757.
[15] E.K. Zolotareva, M.E. Gasparyan, L.S. Yaguzhinsky, FEBS Lett. 272 (1990) 184–186.
[16] R.E. McCarty, G.G. Hammes, Trends Biochem. Sci. 12 (1987) 234–237.
[17] F.A.S. Kironde, R.L. Cross, J. Biol. Chem. 261 (1986) 12544–12549.
[18] A.B. Shapiro, K.D. Gibson, H.A. Scheraga, R.E. McCarty, J. Biol. Chem. 266 (1991) 17276–17285.
[19] I. Grotjohann, P. Gräber, Biochim. Biophys. Acta 1017 (1990) 177–180.
[20] N. Nelson, in: J. Barber (Ed.), Topics in Photosynthesis, vol. 4, Electron Transport and Photophosphorylation, Elsevier Science, Amsterdam/New York/Oxford, 1982, pp. 81–104.
[21] P. Fromme, P. Gräber, Biochem. Biophys. Acta 1020 (1990) 187–194.
[22] P.D. Boyer, Biochim. Biophys. Acta 1140 (1993) 215–250.
[23] D.R. Ort, K. Oxborough, Annu. Rev. Plant. Physiol. Plant Mol. Biol. 43 (1992) 269–291.
[24] B. Tyrakowska, R.M.D. Verhaert, R. Hilhorst, C. Veeger, Eur. J. Biochem. 187 (1990) 81–88.
[25] R. Hilhorst, R. Spruijt, C. Laane, C. Veeger, Eur. J. Biochem. 144 (1984) 459–466.
[26] D.A. Fernández-Velasco, L. Garza-Ramos, L. Ramírez, L. Shoshani, A. Darszon, M. Tuena de Gómez-Puyou, A. Gómez-Puyou, Eur. J. Biochem. 205 (1992) 501–508.
[27] G. Garza-Ramos, D.A. Fernández-Velasco, L. Ramírez, L. Shoshani, A. Darszon, M. Tuena de Gómez-Puyou, A. Gómez-Puyou, Eur. J. Biochem. 205 (1992) 509–517.
[28] L. Ramírez-Silva, M. Tuena de Gómez-Puyou, A. Gómez-Puyou, Biochemistry 32 (1993) 5332–5338.
[29] M.E. Pullman, H.S. Penefsky, A. Datta, E. Racker, J. Biol. Chem. 235 (1960) 3322–3329.
[30] J. Coves, B. Pineau, J. Joyard, R. Douce, Plant. Physiol. Biochem. 26 (1988) 151–163.
[31] C.H. Fiske, Y. Subbarow, Anal. Chem. 34 (1962) 1164–1167.
[32] G.E. Anthon, A.T. Jagendorf, Biochim. Biophys. Acta 723 (1983) 358–365.
[33] G.E. Anthon, A.T. Jagendorf, Biochim. Biophys. Acta 848 (1986) 90–98.
[34] H. Sakurai, K. Shinohara, T. Hisabori, K. Shinohara, J. Biochem. 90 (1981) 95–102.
[35] M. Tuena de Gómez-Puyou, G. Ayala, A. Darszon, A. Gómez-Puyou, J. Biol. Chem. 259 (1984) 9472–9478.
[36] S. Beharry, P.D. Bragg, J. Bioenerg. Biomembranes 24 (1992) 507–514.
[37] M.K. Al-Shawi, A.E. Senior, Biochemistry 31 (1992) 886–891.
[38] S. Edelfors, A. Ravn-Jonsen, Pharmacol. Toxicol. 70 (1992) 181–187.
[39] H. Wolosker, J.H. Petretski, L. de Meis, Eur. J. Biochem. 193 (1990) 873–877.
[40] A. Maitra, J. Phys. Chem. 88 (1984) 5122–5125.
[41] J.A. Rupley, E. Gratton, G. Careri, Trends Biochem. Sci. 8 (1983) 18–22.
[42] P.J. Halling, Biochim. Biophys. Acta 1040 (1990) 225–228.
[43] N. Nelson, C. Beltrán, F. Supek, H. Nelson, Cell Physiol. Biochem 2 (1992) 150–152.
[44] E. Escamilla, G. Ayala, M. Tuena de Gómez-Puyou, A. Gómez-Puyou, L. Millán, A. Darszon, Arch. Biochem. Biophys. 272 (1989) 332–343.
[45] J.A. Kornblatt, M.J. Kornblatt, Biochim. Biophys. Acta 1099 (1992) 182–184.