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THE PHOSPHATE TRANSLOCATOR OF THE CHLOROPLAST ENVELOPE

ISOLATION OF THE CARRIER PROTEIN AND RECONSTITUTION OF TRANSPORT

U.I. FLÜGGE and H.W. HELDT

Lehrstuhl für Biochemie der Pflanze, Universität Göttingen, Untere Karspüle 2, 3400 Göttingen (F.R.G.)

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This report describes the solubilization and purification of the phosphate translocator of spinach chloroplasts and the reconstitution of its activity by incorporation into liposomes. (1) Prior to the isolation, the carrier is specifically labelled by treatment with 2,4,6-trinitrobenzenesulfonic acid and NaB[^{3}H]H$_4$. (2) After preextraction of purified envelope membranes with Brij 58 for removing other loosely bound membrane proteins, the phosphate translocator is extracted with Triton X-100. After passing the resulting extract over a DEAE-Sepharose column followed by sucrose density gradient ultracentrifugation, the translocator protein is purified to apparent homogeneity. The 5–6-fold purification thus obtained concurs with earlier findings that the phosphate translocator protein represents 15–20% of the envelope membrane protein. This highly purified protein is suitable for studies of the hydrodynamic parameters of the translocator. (3) Since the exposure to detergents affects the activity of the translocator protein, alternatively, a rapid batch procedure for the purification of the translocator protein employing hydroxyapatite is used, yielding within 15 min the phosphate translocator protein of about 70% purity. (4) After incorporation of this protein fraction into liposomes, a specific transport of phosphate into these liposomes is observed, which van be terminated by inhibitor stop with pyridoxal 5′-phosphate. This uptake is only observed when the liposomes have been preloaded with phosphate or 3-phosphoglycerate, but not with 2-phosphoglycerate. Thus, like in intact chloroplasts, also the reconstituted transport facilitates an obligatory and specific counter exchange of anions. The apparent K_m for the transport of phosphate by this reconstituted system is about 0.8 mM, which is comparable to the corresponding value in intact chloroplasts. The calculated turnover of 150–300 min^{-1} (20°C) accounts for 3–6% of the original activity.

Introduction

The chloroplasts envelope separating the chloroplast stroma from the cytosol contains a specific translocator for the transport of phosphate, 3-phosphoglycerate and triose phosphate [1]. This phosphate translocator enables the export of the fixed carbon from the stroma to the cytosol in the form of triose phosphate in exchange for phosphate and is therefore an important step in the overall reaction of CO_2 fixation. The kinetic properties of this transport have been studied in detail [2]. In preparations of envelope membranes, the corresponding membrane protein has been identified as a polypeptide with an apparent molecular weight, obtained by SDS-polyacrylamide gel electrophoresis, of 29 000 [3–5]. This polypeptide was shown to contain a lysine residue and probably also an arginine residue at its phosphate-binding site [6]. In order to label the carrier protein, 2,4,6-trinitrobenzenesulfonate was added to the intact chloroplasts. It binds to the lysine residue at the substrate-binding site and the subsequent reduction of this adduct by NaB[^{3}H]H$_4$ leads to a

Abbreviations: SDS, sodium dodecyl sulfate; Mops, 4-morpholinepropanesulfonic acid.

specific incorporation of a radioactive label into the carrier protein [5].

The aim of the present work is two-fold: firstly, to describe a method for isolating the phosphate translocator to apparent homogenity. This is a prerequisite for chemical studies as well as for studies of the hydrodynamic parameters of this transport protein. Secondly, to reconstitute the phosphate translocator into liposomes in order to allow studies of its function in a simplified model system. The experiments provide evidence for the functional integrity of the protein previously identified as the phosphate translocator.

Materials and Methods

Triton X-100 was obtained from the Sigma Chemical Co., Brij 58 from Serva (Heidelberg, F.R.G.), egg yolk phosphatidylcholine from E. Merck (Darmstadt, F.R.G.) and the radiochemicals from Amersham-Buchler (Braunschweig, F.R.G.). Sephadex G-75 and DEAE-Sepharose CL-6B were purchased from Pharmacia (Uppsala, Sweden) and NCS from Amersham Searle. Hydroxyapatite was prepared as described by Bernardi [7]. All other reagents were of the highest purity available.

Isolation of the phosphate translocator

By treatment of intact chloroplasts with 2,4,6-trinitrobenzenesulfonate and $NaB[^3H]H_4$, the phosphate translocator was specifically labelled as previously described [5]. Purified envelope membranes were prepared according to the method of Douce et al. [8] from labelled and unlabelled spinach chloroplasts. Unlabelled envelope membranes (4.8 mg/ml) were mixed with a small amount of radioactively labelled membranes in 5.6 ml of a medium containing 10 mM Mops adjusted with KOH to pH 7.4 and 0.02% NaN_3 (buffer A). The ^3H activity was $118 \cdot 10^3$ dpm/mg envelope protein. All the following procedures were carried out at 4°C in the dark. The mixed purified envelope membranes were treated for 30 min with 0.4 ml of a 20% Brij 58 solution. After centrifugation at 120000 $\times g$ for 1 h the supernatant was discarded and the pellet resuspended and centrifuged again. The resulting sediment was extracted for 1 h with 3.8 ml of buffer A containing 6% Triton X-100. Undissolved material was removed by centrifugation at 120000 $\times g$ for 1 h. The resulting extract was applied to a DEAE-Sepharose CL-6B column (1 × 30 cm) equilibrated with a medium containing buffer A, 0.5% Triton X-100 and 0.2 M NaCl, and the column was washed with the same medium afterwards. The resulting eluant was brought to 5 mM dithiothreitol and 0.5 mM EDTA, and concentrated by ultrafiltration to about 1.5 ml. For further purification, this fraction was layered on top of a continuous sucrose gradient (10–20%, w/w; 30 ml) in a medium containing 250 mM NaCl, 0.02% NaN_3, 0.1mM EDTA, 0.1% Triton X-100, 5 mM dithiothreitol and 10 mM Mops, pH 7.4, and was centrifuged for 14 h at 420000 $\times g$ and 5°C (rotor 65.38, Kontron). The gradient was collected in 0.9 ml fractions and the phosphate translocator was recovered halfway down the gradient in four to six of these fractions.

The method for the rapid purification of the phosphate translocator is essentially similar to the procedure described by Krämer et al. [9] for the one-step purification of the mitochondrial ATP/ADP translocator. About 0.2 ml of envelope membranes (4 mg/ml) was treated for 2–10 min with 1.5–4% Triton X-100 in medium A. The mixture was then added to a suspension of hydroxyapatite in the above-mentioned solubilization medium. After 5 min the sample was centrifuged for 1 min at 20000 $\times g$ and the clear supernatant was immediately used for reconstitution experiments.

Reconstitution of phosphate transport

For the preparation of liposomes, acetone-washed phosphatidylcholine from egg yolk (50 mg/ml) was sonicated (Branson sonifier with microtip) for 20 min in 0.5 s intervals in a solution containing 25 mM Mops buffer, pH 7.4, and 90 mM KH_2PO_4 or 3-phosphoglycerate. Incorporation of the phosphate translocator was achieved by the freeze-thaw technique of Kasahara and Hinkle [10]. 0.3 ml of the supernatant from the hydroxyapatite batch procedure containing about 0.4 mg protein/ml was combined with the sonicated phospholipids, mixed and frozen in liquid nitrogen. After being thawed at 0°C, the liposomes were sonicated in a 1.4 ml plastic test-tube for 18 s while being kept in an ice bath. This was then passed over a Sephadex G-75 column (1 × 30 cm) which had been equilibrated with a medium containing 150 mM KCl and 2 mM Mops, pH 7.4, and which was washed with the same medium. The liposomes in the eluant were ready for phosphate-uptake experiments.

Measurement of phosphate uptake

Uptake was measured by adding radioactively labelled phosphate (0.1–1 mM, spec. act. 5 Ci/mol) to the liposomes (20°C). The uptake was terminated by the addition of pyridoxal 5′-phosphate (final concentration 6.5 mM). Incubation with pyridoxal 5′-phosphate before the addition of labelled phosphate yielded the corresponding blank. Immediately afterwards, the mixture (0.4 ml) was placed on a Dowex AG 1 X 8 (acetate form, 200–400 mesh) anion-exchange column (0.5 X 5 cm) and eluted with 0.9 ml H_2O. Aliquots of the eluted liposomes were counted for ^{32}P radioactivity.

Analytical methods

Protein was measured by the method of Neuhoff et al. [11] using bovine serum albumin as standard. SDS-polyacrylamide gel electrophoresis was performed as described by Neville [12]. Gels with 12% acrylamide and 0.1% *N,N′*-methylenebisacrylamide were used. Radioactivity was analyzed by slicing and digestion of the gels with NCS [13] followed by liquid scintillation counting.

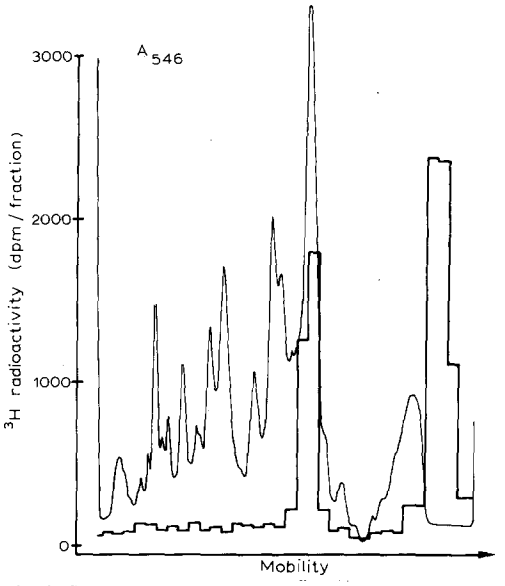

Fig. 1. SDS-polyacrylamide gel electrophoresis of envelope membrane proteins. The labelling of intact chloroplasts with 2,4,6-trinitrobenzenesulfonic acid/NaB[^{3}H]H_4 and the isolation of envelope membranes were carried out as described in Materials and Methods. Membranes equivalent to 100 μg protein were subjected to SDS-polyacrylamide gel electrophoresis. Separate gels were used for the absorbance scan (continuous curve) and for the radioactivity scan (discrete lines).

Results and Discussion

Solubilization and purification

Envelope membranes from spinach chloroplasts were used as the starting material for solubilization and purification of the phosphate translocator membrane protein. In order to identify the carrier protein, these envelope membranes were mixed with a small portion of envelope membranes, which had been treated earlier with 2,4,6-trinitrobenzenesulfonate and NaB[^{3}H]H_4. SDS-polyacrylamide gel electrophoresis (Fig. 1) showed that about one-third of this radioactive label was incorporated specifically into the phosphate translocator protein. The remaining two-thirds of the radioactivity were found in the lipid fraction at the front of the gel. Aliquots were therefore analyzed by SDS-polyacrylamide gel electrophoresis at each stage of the purification procedure in order to measure the radioactivity in the 29 000 dalton band as a marker for the purification of the phosphate translocator. It was checked that the purification techniques did not separate the labelled and unlabelled phosphate translocator protein.

In experiments not shown here, it was found that a number of ionic and nonionic detergents, such as cholate, deoxycholate, Triton X-100, Aminoxide WS-35 and Emulphogen, could solubilize the carrier protein. Fig. 2 shows the effect of Brij 58 and Triton X-100. These two polyglycol detergents are known to have only a relatively small effect on the conformational state of native membrane polypeptides. Brij 58, a relatively mild detergent, solubilized about 30–40% of the total membrane proteins without releasing the phosphate translocator protein from the membrane to any large extent. Triton X-100, a much stronger detergent, achieved a very effective solubilization of the phosphate translocator protein as well as of the total membrane protein. These different solubilizing effects of the two detergents were utilized for a first purification step. The envelope membranes were first preextracted with Brij 58 in order to remove the loosely bound proteins, and from the remaining membranes the translocator protein was extracted afterwards by Triton X-100. Using 6% Triton X-100, 90–95% of the total membrane protein and about 80% of the phosphate translocator were extracted. This ex-

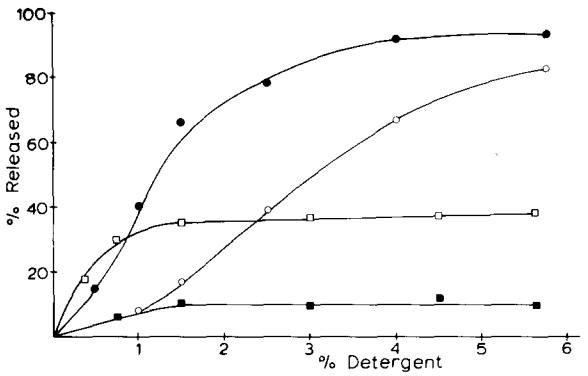

Fig. 2. Effect of Triton X-100 (●, ○) and Brij 58 (■, □) on the solubilization of the total membrane protein (●, □) and the phosphate translocator (○, ■). The samples (4.5 mg protein/ml) were incubated with increasing concentrations of detergent for 1 h (0°C) and centrifuged at 120 000 × *g* for 1 h. The supernatant was assayed for protein and the phosphate translocator as described in Materials and Methods.

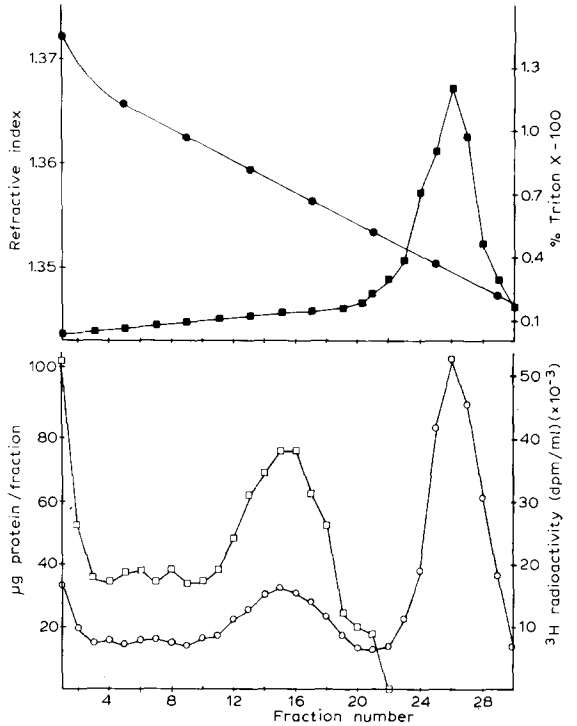

Fig. 3. Sucrose density gradient centrigugation of the phosphate translocator. Fot details see text. (□———□) µg protein/fraction, (○———○) ^{3}H radioactivity, (●———●) refractive index, (■———■) % Triton X-100.

traction can be carried out with a medium of low ionic strength, since the extent of solubilization does not change by varying the NaCl concentration in the extraction medium from 0 to 0.8 M (data not shown).

After centrifugation to remove undissolved material, the clear yellow supernatant was applied to a DEAE-Sepharose CL-6B column. In contrast to most other membrane proteins, the phosphate translocator protein was not bound to the column and appeared in the column eluant. The elution volume was then concentrated by ultrafiltration in the presence of dithiothreitol to prevent aggregation, and the phosphate translocator was then further purified by sucrose density gradient centrifugation. With the chosen centrifugation time, the phosphate translocator protein sedimented into the middle of the gradient, whereas the bulk of the lipids and carotenoids remained at the top as did that portion of the Triton X-100 which was not bound to proteins (Fig. 3). The material found at the bottom of the gradient represented mainly aggregated membrane proteins. When the fraction from the sucrose gradient, which contained the phosphate translocator, was analyzed by SDS-polyacrylamide gel electrophoresis, only one protein band was found, and all the radioactivity was recovered within this band (Fig. 4). The specific content of the preparation could not be increased by a second density gradient centrifugation or gel chromatography. It therefore appears that the phosphate translocator protein was purified to apparent homogeneity. Table I summarizes the results of the various purification steps. The phosphate translocator protein was enriched 5.2-fold, with an overall yield of about 8%. In other purification experiments the increase in specific content was also 5–6-fold, indicating that the translocator represented 15–20% of the total protein, which is in agreement with earlier results [6]. This highly purified protein is suitable for studies of the hydrodynamic parameters, i.e., the estimation of the Stokes' radius, sedimentation coefficient, partial specific volume of the Triton X-100/protein micelle and Triton X-100 binding. Results will be shown in a later publication.

Since the functional integrity of the phosphate translocator protein was gradually decreased during exposure to detergents (see later), a batch procedure was applied, in order to obtain a functionally active

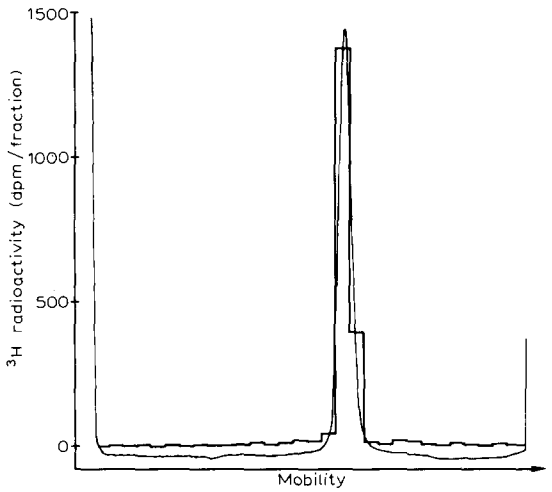

Fig. 4. SDS-polyacrylamide gel electrophoresis of the purified phosphate translocator. Separate gels were used for the absorbance scan (continuous curve) and the radioactivity scan (discrete lines).

transport protein. This procedure had originally been developed for the rapid isolation of the ATP/ADP translocator in mitochondria [9]. For this purpose, envelope membranes were treated with 1.5–4% Triton X-100. Shortly afterwards hydroxyapatite was added to the mixture in order to bind most of the solubilized membrane proteins. The phosphate translocator protein remained dissolved in the Triton X-100 phase and could be separated by centrifugation. This extract from envelope membranes could be obtained within 15 min and was utilized for the reconstitution experiments. It contained besides lipids and carotenoids the phosphate translocator of 70% purity (Fig. 5) with a protein yield of about 7%.

Reconstitution experiments

To measure the activity of isolated translocator proteins, it is necessary to reinsert them into the membranes. Liposomes have been successfully utilized for the reconstitution of several metabolite-transport systems [10,14–21]. Our method for reconstitution of the chloroplast phosphate translocator is very similar to that described earlier for the reconstitution of the mitochondrial ATP/ADP translocator [14,15]. To liposomes which had been formed by sonicating phosphatidylcholine, the Triton X-100 extract containing the phosphate translocator was added. Freezing the mixture, followed by thawing and short sonication caused the translocator protein to be incorporated into the liposomal membranes. As the chloroplast phosphate translocator catalyzed a counterexchange of anions, these liposomes had to be prepared to contain exchangable anions, such as P_i or 3-phosphoglycerate.

The transport measurements were carried out by an inhibitor stop method. Transport was initiated by adding ^{32}P-labelled phosphate and was terminated by adding an inhibitor. The remaining unexchanged [^{32}P]phosphate in the external medium was then removed from the liposomes by passing them through an anion-exchange column. The radioactively labelled

TABLE I

ISOLATION OF PHOSPHATE TRANSLOCATOR

Isolation of the phosphate translocator from 2,4,6-trinitrobenzenesulfonic acid/NaB[^{3}H]H_4-labelled envelope membranes. The purification steps are detailed in Materials and Methods. PEG, poly(ethylene glycol).

Preparation	Volume (ml)	Protein (mg)	^{3}H radioactivity (dpm) ($\times 10^{-2}$)	Specific content (dpm/mg protein) ($\times 10^{-2}$)	Yield (%)
Starting material	6	27	8 530	410	100
Sediment after Brij 58 extraction	3.8	16.4	6 670	545	78
Supernatant of the Triton X-100 extraction	3.6	9.2	5 380	665	63
DEAE Sepharose CL-6B pass-through (after ultrafiltration)	1.8	1.0	1 370	1 550	16
Gradient centrifugation (after PEG dialysis)	0.6	0.4	680	2 140	8

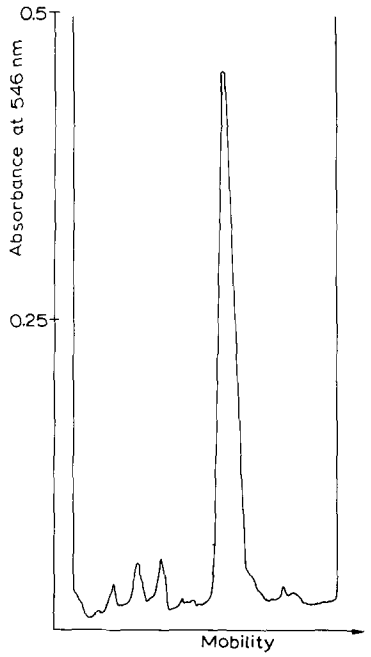

Fig. 5. Densitometric trace of SDS-polyacrylamide gel electrophoresis of the extract obtained after the hydroxyapatite batch procedure. For details see text.

phosphate inside the liposomes did not have access to the ion exchanger and appeared therefore in the column eluant. The inhibitor stop was carried out by adding pyridoxal 5′-phosphate, a substance shown earlier to be a potent inhibitor of the phosphate translocator in chloroplasts [5]. Table II shows the result of an experiment measuring the uptake of [^{32}P]phosphate into liposomes. A certain amount of ^{32}P was taken up in all cases, which did not require the incorporation of the translocator protein and seemed to be due to unspecific [^{32}P]phosphate binding to the liposomes. The incorporation of the phosphate translocator into liposomes had a stimulatory effect on [^{32}P]phosphate uptake, but only when the liposomes were loaded with phosphate, and this stimulation was prevented if pyridoxal 5′-phosphate was added prior to the start of the incubation. In intact chloroplasts the phosphate translocator is known to catalyze an obligatory counterexchange of anions [2]. This demonstration, that the membrane protein mediating [^{32}P]phosphate uptake into liposomes is dependent on internal phosphate, and is inhibited by pyridoxal 5′-phosphate, clearly shows that a reconstitution of phosphate translocation has been achieved.

Accurate measurements of phosphate uptake by the reconstituted system require an effective inhibitor stop. The experiment of Fig. 6 investigated whether pyridoxal 5′-phosphate was suitable for performing such an inhibitor stop. In the experiment of Fig. 6A [^{32}P]phosphate was added at zero time to the liposomes, and the uptake was terminated at various times by adding pyridoxal 5′-phosphate. To obtain a

TABLE II

DEPENDENCE OF THE RECONSTITUTED TRANSPORT ACTIVITY ON THE INCORPORATION OF THE PHOSPHATE TRANSLOCATOR INTO LIPOSOMES

The liposomes had been preloaded with either KCl or 3-phosphoglycerate. PLP, pyridoxal 5′-phosphate. (For details see text).

	^{32}P radioactivity taken up (cpm/sample)		
	PLP added before [^{32}P]phosphate	PLP added 5 min after addition or [^{32}P]phosphate	Specific uptake
(1) Liposomes loaded with 150 mM KCl			
(a) without protein	50	50	0
(b) phosphate translocator incorporated	60	80	20
(2) Liposomes loaded with 90 mM 3-phosphoglycerate			
(a) without protein	50	50	0
(b) phosphate translocator incorporated	160	7 330	7 170

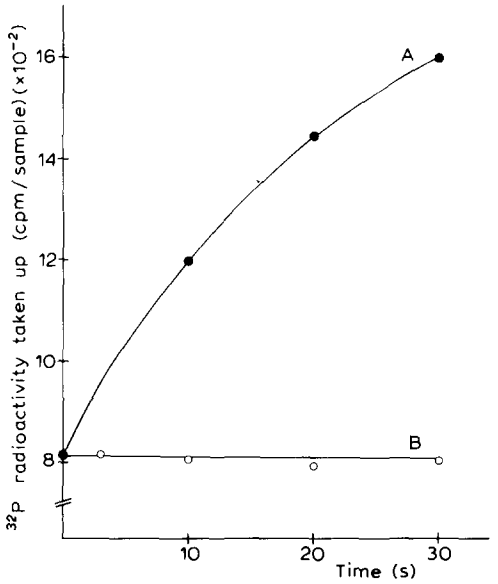

Fig. 6. Time course of the uptake of [^{32}P]phosphate into liposomes. The reaction was stopped by adding pyridoxal 5′-phosphate at the indicated times. (A, •———•). Alternatively, first pyridoxal 5′-phosphate was added at zero time, and [^{32}P]phosphate afterwards at the times indicated (B, o———o). The samples were assayed as described in Materials and Methods.

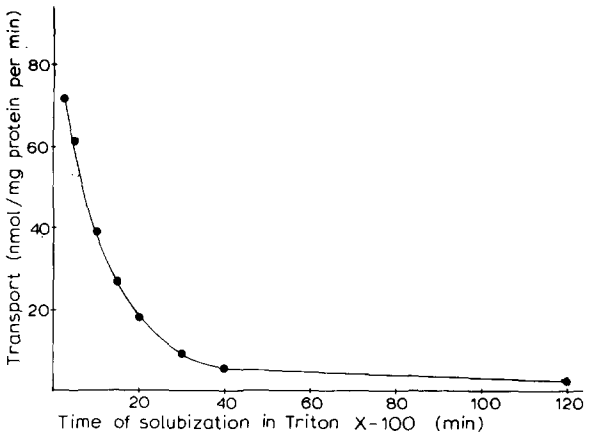

Fig. 7. Influence of the solubilization time in Triton X-100 on the reconstituted phosphate transport activity. Envelope membranes (5 mg/ml) were incubated with 1.5% Triton X-100. At the times indicated, aliquots were reconstituted into liposomes and their rates of [^{32}P]phosphate uptake measured as described in Materials and Methods.

blank value representing unspecific binding of phosphate, pyridoxal 5′-phosphate was added 30 s before zero time. Afterwards, the samples were applied to ion-exchange columns for elimination of external phosphate. The resultant time course for the uptake shows a time-dependent rise in exchanged [^{32}P]phosphate. In the experiment of Fig. 6B, pyridoxal 5′-phosphate was added at zero time and [^{32}P]phosphate 2–30 s afterwards. In this case, none of the measured values for [^{32}P]phosphate radioactivity uptake differed from the blank value obtained in the experiment of Fig. 6A, indicaing that specific [^{32}P]phosphate uptake had been blocked completely by pyridoxal 5′-phosphate in less than 2 s. This result clearly demonstrates that pyridoxal 5′-phosphate is a suitable terminating agent for measuring phosphate uptake in the reconstituted phosphate translocator system.

Like the mitochondrial ATP/ADP translocator protein [9], the chloroplasts phosphate translocator protein gradually loses its structural integrity during prolonged exposure to detergents. In the experiment shown in Fig. 7 purified envelope membranes were treated with 1.5% Triton X-100 and then at the times indicated aliquots of the extract were reconstituted into liposomes, and their rates of [^{32}P]phosphate uptake measured. The carrier protein was inactivated in Triton X-100 with a half-time of only a few minutes. Similar inactivation was found with other detergents, such as aminoxide WS-35, deoxycholate or Zwittergent 3–14 (Calbiochem) (not shown). These results stress the importance of a short solubilization time for obtaining a functional carrier protein. Once the carrier protein has been incorporated into liposomes, however, the transport activity is considerably more stable.

Detergents also affect the stability of liposomes. Therefore, it had to be checked that the detergent contained in the membrane protein extract, used for the reconstitution, did not influence the intactness and transport activity of the liposomes. In the experiments of Fig. 8, the effect of decreasing the phospholipid/Triton X-100 ratio on the intactness of liposomes and the reconstituted transport activity was studied. During the reconstitution procedure, various amounts of Triton X-100 were added together with the membrane protein extract to the liposomes. The liposomes had been loaded with [^{32}P]phosphate and the ^{32}P radioactivity contained in the liposomes after separation from the external medium was taken as

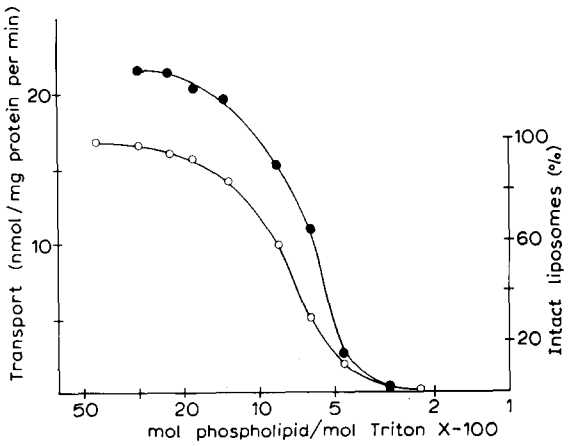

Fig. 8. Influence of Triton X-100 on the intactness of liposomes (○———○) and the reconstituted activity (●———●). Increasing amounts of Triton X-100 were added to liposomes with incorporated protein and containing either 90 mM KH_2PO_4 or 90 mM $KH_2^{32}PO_4$. The reconstituted activity was measured as described in Materials and Methods. For measurement of the intactness of the liposomes, aliquots of the [^{32}P]phosphate-labelled liposomes were passed over a Dowex AG 1 X 8 column (chloride form) and the eluted radioactivity was used as a measure of the amount of intact liposomes.

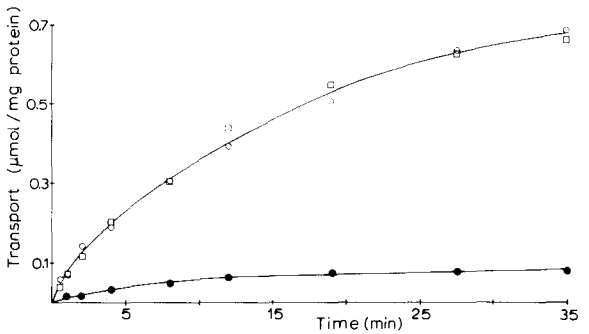

Fig. 9. Time course of [^{32}P]phosphate uptake in reconstituted liposomes. The liposomes had been preloaded with 90 mM KH_2PO_4 (□), 90 mM 3-phosphoglycerate (○) or 90 mM 2-phosphoglycerate (●).

a relative measure of intactness. It appears from these data that about 50% of the vesicles collapse at a phospholipid/Triton X-100 molar ratio of 7; in other experiments these ratios were 3–6. In parallel experiments with liposomes which had been preloaded with unlabelled 3-phosphoglycerate to allow counterexchange a similar effect on the transport activity was found. In order to avoid this damaging effect of Triton X-100, the phospholipid/detergent ratio in the reconstitution experiments was always kept above 15. It may also be noted that the transport activity was sensitive to the duration of the sonication after the freeze-thaw step. The optimal length of time was found to be 15–20 s. A longer sonication time lowered the transport activity. This is probably due to the decrease in vesicle size and reduced internal volume [10]. Fig. 9 shows the time course for the specific uptake of [^{32}P]phosphate. Those liposomes which had been preloaded with P_i or 3-phosphoglycerate showed a marked [^{32}P]phosphate uptake occurring with exponential kinetics. From work with isolated chloroplasts the phosphate translocator is known to transport 3-phosphoglycerate as well as P_i, whereas 2-phosphoglycerate requires a 10–20-fold higher concentration for half-saturation of the carrier and is therefore transported much more slowly [2]. In agreement with these earlier findings, the uptake of the labelled phosphate by the liposomes was largely restricted when these had been preloaded with 2-phosphoglycerate instead of 3-phosphoglycerate or phosphate. Obviously, the reconstituted translocator has the same substrate specificity as the native chloroplasts transport system. In the experiment of Fig. 10 the concentration dependence of the transport was investigated by varying the external phosphate concentration. The double-reciprocal plot yields a linear function, indicating substrate saturation of the transport. The data allow the evaluation of K_m (substrate concentration causing half-maximal rate of transport) and of V (maximal velocity of transport). In different experiments for the transport of phosphate, K_m values between 0.6 and 1.0 mM (pH 7.4) were obtained. These values are remarkably similar to the K_m value for transport of phosphate found in intact chloroplasts (0.7 mM, pH 7.4 [2]).

The efficiency of the reconstitution appears rather low. From the maximal velocity of phosphate uptake measured in Fig. 10, the purity of the carrier protein used (Fig. 5) and the apparent molecular weight of the phosphate translocator protein of 29 000, a turnover number of about 170 min^{-1} (20°C) was obtained. This is 3.5% of the turnover number extrapolated for the phosphate translocator protein in vivo [6]. In other experiments the efficiency was up to 6%. The low turnover may be in part due to the high

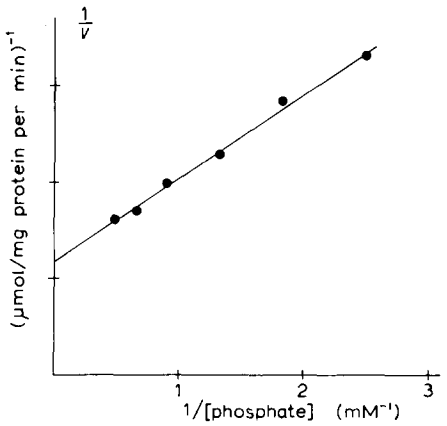

Fig. 10. Concentration dependence of the uptake of P_i into the reconstituted liposomes which had been preloaded with 3-phosphoglycerate. Temperature, 20°C. After freeze-thawing the liposomes were passed over a Dowex 1 X 8 column (acetate form) equilibrated with 5 mM Mops buffer, pH 7.4, containing 330 mM sucrose. In a preceding uptake experiment, the linearity with time of the reaction was checked with the phosphate concentrations used in order to confirm that initial rates were measured. The uptake reactions were stopped after 7 s.

sensitivity of the carrier protein to inactivation by detergents (see Fig. 8) and also the unknown fraction of the carrier protein which is incorporated into liposomes with the right polarity. Other successful reconstitution experiments with purified membrane proteins yielded results for the mitochondrial ATP/ADP translocator [15], mitochondrial phosphate translocator [18] and erythrocyte glucose carrier [10,16, 17] of 5, 3 and 4–16%, respectively, of the estimated turnover numbers in vivo. It may be noted that the reconstitution of the chloroplasts phosphate translocator has not yet been optimized with respect to the lipid composition of the liposomes employed. In chloroplast envelope membranes galactolipids are a major constituent. It seems likely that the phosphatidylcholine liposomes, used in our reconstitution studies are not optimal for this purpose. A possible improvement of the system requires systematic studies on the dependence of the transport on the lipid composition of the liposomes employed.

The reconstituted system seems to be well suited to elucidate how ion gradients may influence the preferential direction of phosphate, triose phosphate and 3-phosphoglycerate transport across the chloroplast envelope.

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