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Amino Acid Permeability of the Chloroplast Envelope as Measured by Light Scattering, Volumetry and Amino Acid Uptake*

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Summary. The amino acid permeability of the envelope of intact, functional spinach (*Spinacia oleracea* L.) chloroplasts was investigated by light scattering, volumetry and uptake of ^{14}C-labelled amino acids. The criterion for the functionally of the chloroplasts was their ability to reduce CO_2, PGA and oxaloacetate in the light at high rates.

Net uptake into the chloroplast interior of neutral amino acids such as alanine, glycine, serine, proline, threonine or valine occurred only at very low rates. The uptake was concentration dependent, indicating unspecific diffusion rather than carrier-mediated transport. The slowness of uptake is emphasized by the capability of neutral amino acids to provide osmotic support for intact chloroplasts during a considerable length of time. Back-exchange experiments also failed to indicate the existence of specific exchange carriers for the transport of neutral amino acids such as alanine or glycine through the envelope of intact chloroplasts. Dicarboxylic amino-acids are known to be taken up by the so-called dicarboxylate translocator. The same carrier was found to catalyze also the transfer of asparagine and glutamine.

The data do not support current assumptions concerning fast carrier-mediated transport of neutral amino acids and their role in the transfer of carbon during photosynthesis.

Introduction

Chloroplasts of leaf cells possess the complete machinery for synthesizing proteins from amino acids (Parthier, 1964; Heber, 1962). In contrast, their capability to synthesize amino acids appears to be severely limited. Isolated intact chloroplasts from spinach leaves which reduce added $^{14}CO_2$ at rates comparable to maximal rates of *in vivo* photosynthesis, incorporate little or no radioactivity into amino acids in the light (M. Kirk, private communication and own observations). Kirk and Leech (1972) concluded that, while transamination reactions can take place inside the chloroplasts, the carbon skeletons of a number of amino acids found inside the chloroplasts have to be provided by extrachloroplast

* Dedicated to Professor W. Simonis on the occasion of his 65th birthday.

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metabolism. This implies transport of the precursors into the chloroplasts. Alternatively, amino acids themselves may be imported. Indeed, Nobel and Wang (1970) reported rapid transport of amino acids across the envelope of intact chloroplasts: Up to high concentrations of amino acids (50 mM) almost no osmotic response was seen, indicating fast net uptake of amino acids. Osmotic shrinkage at higher concentrations suggested saturation of uptake (Nobel and Cheung, 1972). From this and from competition experiments the existence of two specific carriers mediating fast net uptake of amino acids was postulated. Other types of chloroplast carrier are the *adenylate translocator* (Heldt, 1969), the *dicarboxylate translocator* and the *phosphate translocator* (Heldt and Rapley, 1970). However, the results of Nobel and his group need to be reconciled with the observation that free amino acids are not lost from intact chloroplasts during isolation (Aach and Heber, 1967; Kirk and Leech, 1972): Their concentration in isolated chloroplasts were found to be not much lower than in chloroplasts *in situ*. Direct measurements of glycine and alanine penetration through the chloroplast envelope indicated only slow uptake (Heldt, personal communication). Observed rates were in the order of 1 μmol mg^{-1} chlorophyll h^{-1}, corresponding to about 10^{-3} μmol cm^{-2} chloroplast envelope area h^{-1}. In contrast, the reflection coefficients measured in the experiments of Nobel and coworkers, suggest a mass flow of amino acids across the chloroplast envelope almost comparable to that of water (Nobel and Wang, 1970; Nobel and Cheung, 1972). In view of the postulate that amino acids play an important role in carbon transfer across the chloroplast envelope during photosynthesis (Roberts *et al.*, 1970, compare also Ongun and Stocking, 1965) these controversial findings prompted us to reinvestigate the permeation of amino acids into intact isolated chloroplasts of spinach using both osmotic and direct methods to measure uptake.

Methods

Preparation and Properties of Chloroplasts. Chloroplasts with intact envelopes ("Type A" in the nomenclature of Hall, 1972) were isolated from young leaves of spinach (*Spinacia oleracea* L.) according to the method of Jensen and Bassham (1966), as modified slightly by Heber (1973). The integrity of the chloroplasts was routinely tested by measuring the reduction of ferricyanide (Heber and Santarius, 1970). Between 70 and 90% of the chloroplasts were intact in different preparations. Photosynthetic CO_2 reduction, and reduction of phosphoglycerate and of oxaloacetate was measured polarographically (Heber, 1973).

Determination of Chloroplast Osmotic Volumes. Osmotic volumes of the chloroplasts were determined by a micro-volumetric method. Chloroplasts corresponding to about 60 μm of chlorophyll were suspended in media of different osmolarity and were then filled into calibrated, disposable micropipettes (Brand, Wertheim Germany). These pipettes were closed on one side by fusion and centrifuged at $2000 \times g$ for 10 min. The height of sediments and supernatants was measured with

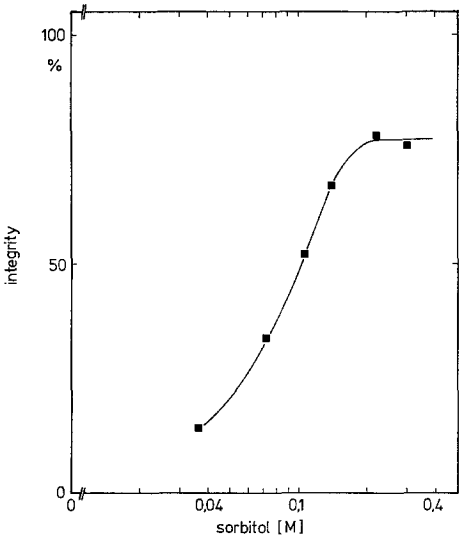

Fig. 1. Integrity of chloroplasts as a function of the sorbitol concentration in the medium. Indicator of integrity was non-reactivity of added ferricyanide in the light (Heber and Santarius, 1970). The integrity of the chloroplasts was 75% at the beginning of this experiment. Osmolarity of the medium with 0.3 M sorbitol was 0.38

the aid of an enlarger. The basic medium used in these experiments was half-strength solution "C" ("C" to H_2O 1:1) (Jensen and Bassham, 1966). Its osmolarity was 0.18, its main component 0.15 M sorbitol. The integrity of the chloroplasts remained largely unchanged in this medium (see Fig. 1). Different amounts of solutes were added to the chloroplast suspension. After sedimentation of the chloroplasts the volumes of the sediments V were plotted against the reciprocal of the osmolarity of the medium, $1/C$. The resulting straigt line was extrapolated to zero and the intercept of the ordinate was taken as the non-osmotic volume b of the sediment V; the osmotic volume is then $V-b$. The reciprocal osmotic volumes $\frac{1}{V-b}$ were plotted as a function of the osmolarity of the reaction medium.

Measurements of Chloroplast "Transmission" Changes. Changes in "transmission" at 535 nm of the chloroplast suspensions were measured with a Zeiss spectrophotometer (PMQ II). The distance between the sample and the photomultiplier was 9 cm. The "transmission" of a sample (2 ml of a medium with the osmolarity of 0.18 containing about 100 µg of chlorophyll) was recorded in a 1 cm cuvette under continuous stirring for a few minutes. Small amounts of 2.0 M solutions of amino acids, sorbitol, ribose etc., containing the same concentration of chloroplasts as the measuring sample (in order no to change the chlorophyll concentrations) were added and the change in "transmission" was recorded.

Measurement of ^{14}C-Amino Acid Penetration across Chloroplast envelope. The penetration of ^{14}C-labelled amino acids across the chloroplast envelope was measured by the silicone layer centrifugation technique of Klingenberg and Pfaff (1967):

Uptake of ^{14}C-labelled amino acids was determined by counting aliquots of the supernatant and sediment after rapid centrifugation of the chloroplasts through an inert layer of silicone oil. The centrifugation was carried out with the Beckman minifuge 152. 0.5 ml centrifuge tubes contained 20 µl 10% $HClO_4$, 70 µl silicone oil (one part Type AR 100 plus 1 part Type AR 150, from Wacker, Munich) and 300 µl suspending medium with chloroplasts containing 20 µg of chlorophyll. Extrachloroplast and chloroplast spaces in the sediment were calculated from the amounts of tritiated water (water space) and of the non-permeant [^{14}C]sorbitol (sorbitol space) sedimenting with the chloroplasts: The water space minus the sorbitol space was taken to be the osmotic space.

Determination of Back Exchange. To investigate back exchange, chloroplasts were incubated with radioactive alanine, glycine or malate for 30 min at 4° and washed subsequently with isotonic, but unlabelled medium followed by repeated centrifugations. The back exchange was started by adding unlabelled amino acids or dicarboxylic acids (final concentration of 0.5 mM), and terminated by rapid centrifugation of the chloroplasts and taking aliquots of the supernatant to calculate the amounts of radioactivity released on addition of the unlabelled substances.

All experiments were carried out at room temperature (21–23°) (pH 7.6) and without illumination, if not specified otherwise.

Results and Discussion

1. Relation between Light Scattering of Chloroplast Suspensions and Osmotic Volume Changes

Intact chloroplasts behave as perfect osmometers in solutions of non-permeant compounds such as sucrose (Heldt and Sauer, 1970; Nobel and Wang, 1970; Wang and Nobel, 1971). Hypertonic solutions result in shrinkage of the chloroplasts, hypotonic solutions in swelling and finally rupturing of the chloroplast envelope. Chloroplast integrity as a function of the tonicity of the reaction mixture is shown in Fig. 1. Indicator of integrity was non-reactivity of added ferricyanide in the light (Heber and Santarius, 1970). Only chloroplasts with broken or damaged envelopes reduce ferricyanide upon illumination. Osmotic support for isolated spinach chloroplasts is usually provided by 0.3 M sorbitol or 0.4 M sucrose. However, as seen in Fig. 1, 0.2 M sorbitol is sufficient to prevent osmotic rupture. At 0.15 M sorbitol only slight damage is observed and at a concentration of 0.1 M sorbitol still half of the chloroplasts were intact as indicated by the ferricyanide assay. Lower sorbitol concentration caused more chloroplasts to rupture. Intact chloroplasts kept at 0.33 M sorbitol exhibited high rates of photosynthetic reactions. CO_2 reduction was comparable to that in intact leaves (Table 1). From this it appears that the chloroplast system is similar to an *in vivo* system. The chloroplasts were also capable of photoreducing added phosphoglycerate, oxaloacetate and nitrite. Reduction of the latter two substrates was increased by uncoupling. The chloroplasts were stable when stored at 0° and could be used for several hours.

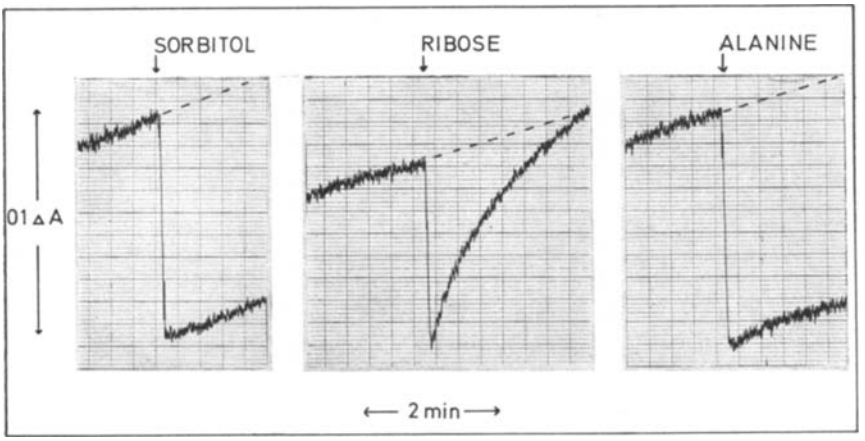

Fig. 2. Kinetics of the changes in apparent absorbance at 535 nm, introduced by the addition of sorbitol, ribose or alanine (↓) (final concentration 0.2 M) to intact chloroplasts. Osmolarity of the medium before additions 0.18. Integrity of the chloroplasts: 76%

Table 1. Photosynthetic activities of intact spinach chloroplasts as used in this investigation (A) and of osmotically shocked chloroplasts (B).

The integrity of the "intact" chloroplasts (A) ranged from 70 to 90%. Illumination with red light (half width 630–760 nm), light intensity 250 kergs cm^{-2} s^{-1}. Intact leaves of spinach assimilate CO_2 at rates around 100 μmol CO_2 mg^{-1} chlorophyll h^{-1}.

	Reactions	Rates (μmol mg^{-1} chlorophyll h^{-1})
A.	CO_2-reduction	80–140
	Reduction of phosphoglyceric acid	120–220
	Reduction of oxaloacetate	25— 60
B.	Ferricyanide reduction	400–600

Size changes of small particles in a suspension are accompanied by changes in light scattering. This has been used to measure changes in the volumes of thylakoids (Crofts *et al.*, 1967; Packer *et al.*, 1965), mitochondria (Chappel and Crofts, 1965) and erythrocytes (Jacobs, 1940). The same method is applicable to intact chloroplasts. Fig. 2 shows the kinetics of changes in apparent absorbance at 535 nm, which is a measure of light scattering, on addition of the non-permeant sorbitol and the permeant ribose to intact chloroplasts which were kept at an osmolarity of 0.18. Sorbitol caused a fast and irreversible increase in apparent

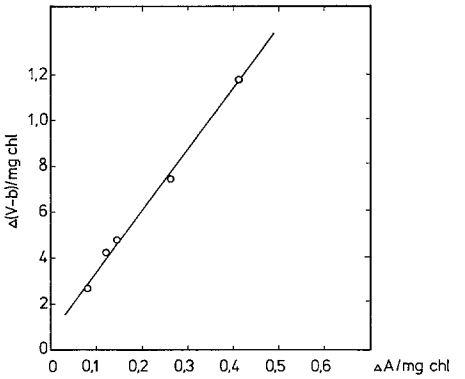

Fig. 3. Relation between the decrease in osmotically active volumes of intact chloroplasts (measured by micro volumetry) and the increase in apparent absorbance at 535 nm upon the addition of sorbitol (concentration range from 180 to 380 mosmol/l)

absorbance. The same concentration of ribose added to a suspension of chloroplasts first caused a fast increase in absorbance; this was followed by a slower decrease.The irreversible increase of absorbance upon addition of sorbitol indicates chloroplast shrinkage caused by the efflux of water from the chloroplasts. This is very fast as can be seen from the kinetics of the increase in light scattering. When ribose entered the chloroplasts, water flowed back and swelling occurred.

Results in principle similar to those observed with ribose were obtained with urea and glycerol. In agreement with Wang and Nobel (1971) this indicates that these compounds penetrate the chloroplast envelope rapidly. Results similar to those obtained with the non-permeant sorbitol were obtained with amino acids such as alanine (Fig. 2), indicating a very slow uptake or even impermeability of these compounds.

The relation between the increase in apparent absorbance at 535 nm upon addition of sorbitol and decrease of osmotically active chloroplast volumes as determined by hematokrit-type experiments is shown in Fig. 3 for the concentration range from 180 to 380 mosmoles/l. Within this range changes in apparent absorbance were linearly related to volume changes. These, in turn, obeyed the Boyle-Van'-t Hoff relationship.

To which extent envelope-free chloroplasts contaminating the chloroplast suspensions have contributed to the measured changes in apparent absorbance? Our chloroplast preparations contained between 70 and 90% intact chloroplasts. Fig. 4 compares absorbance changes produced

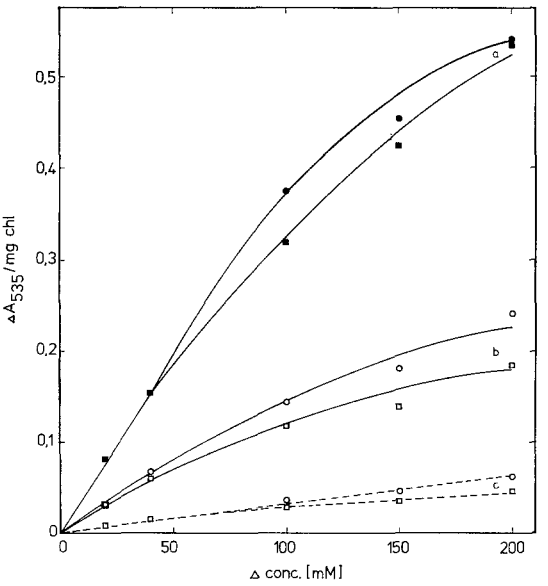

Fig. 4. Changes in apparent absorbance at 535 nm introduced by the addition of sorbitol (squares) or alanine (circles) to "intact" chloroplasts (solid symbols, a) or broken chloroplasts (open symbols, b). C reflects the contribution of broken chloroplasts to the absorbance changes in the "intact" chloroplasts

by additions of sorbitol and alanine to suspensions of "intact" chloroblasts (75% intact, 25% broken chloroplasts) and to chloroplasts, the envelopes of which had been completely ruptured by a brief osmotic shock. Both preparations were assayed under identical conditions. Ruptured chloroplasts (Fig. 4b) showed less than half the shrinkage response exhibited by the "intakt" chloroplasts (Fig. 4a). This indicates that the major part of the change in absorbance is caused by light scattering of the intact chloroplasts. Knowing the contamination of the "intact" chloroplast preparation with broken chloroplasts, we could calculate the contribution to the response of the "intact" chloroplasts on addition of sorbitol or alanine. It proved to be small (5–10%)[1] (Fig. 4c) and will be neglected in the following. From the data presented in Fig. 4 it is apparent that the absorbance changes producee by additions of alanine to chloroplasts are very similar to those caused by isoosmolar concentrations of sorbitol, which is a non-penetrating solute

1 It should be noted that this is true only for neutral solutes such as sugars and for neutral amino acids. In contrast, salts produce very significant light scattering changes also in broken chloroplasts, which are caused only in part by osmotic response.

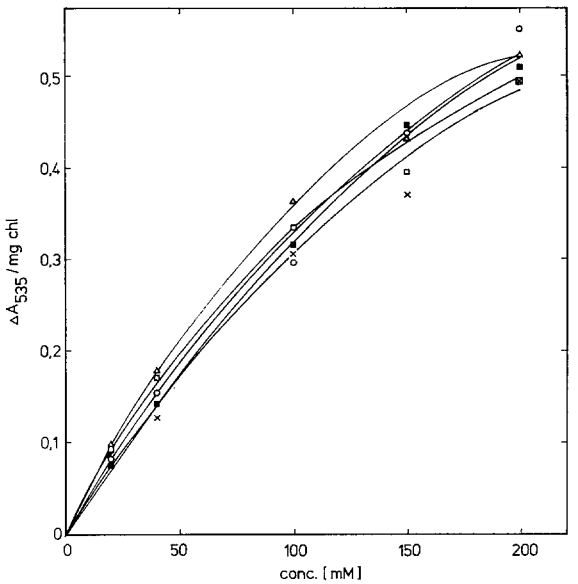

Fig. 5. Effect of different amino acids on the apparent absorbance at 535 nm of intact chloroplasts. Osmolarity of the medium before additions: 0.18 M. Sorbitol (■), alanine (○), serine (△), proline (×) and glycine (□). Additions of corresponding concentrations of ribose or glycerol (permeating compounds) yielded values close to zero or even negative values

(Fig. 2). The effect of alanine and sorbitol differs drastically from those of glycerol or ribose, which enter intact chloroplasts. As shown in Fig. 3 the absorbance changes reflect osmotic volume changes. The behaviour of the sorbitol- and alanine-dependent absorbance changes suggest that alanine in contrast to ribose does not or only very slowly penetrate the chloroplast envelope.

2. Osmotic Effects of Amino Acids on Intact Chloroplasts

Also several other amino acids behave like alanine, when added to suspensions of intact chloroplasts (Fig. 5). Glycine, serine and proline produce absorbance changes at 535 nm which are very similar to those caused by isoosmolar concentrations of sorbitol and alanine (Fig. 5). Similar responses were also observed with threonine and valine (not shown). The light scattering experiments presented in Figs. 4 and 5 are complemented by direct measurements of osmotic volume changes (Fig. 6). The precision of the latter measurements are not fully comparable to those of the optical method. Still it is clear that the water efflux

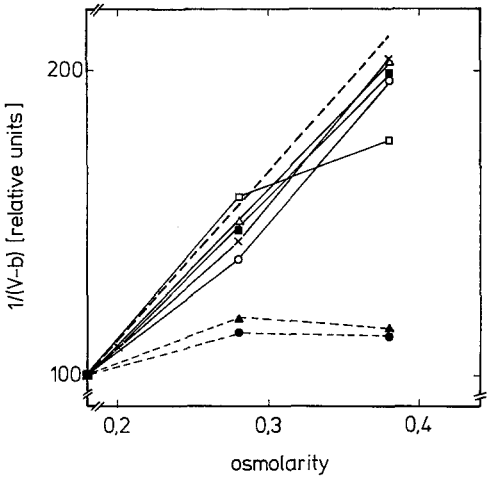

Fig. 6. Effect of different amino acids on osmotically active spaces of intact chloroplasts as measured by microvolumetry. Osmolarity of the medium before additions was 0.18 M. Averages of 10 experiments, the osmotical volume of the chloroplasts without additions was used as reference (100%). Sorbitol (■), alanine (○), serine (△), proline (×), glycine (■), ribose (●), glycerol (▲)

from the chloroplasts caused by amino acids is similar to that produced by the non-permeant sorbitol. Glycerol and ribose on the other hand do not support persistent chloroplast shrinkage. The conclusion that there is no fast net uptake of a number of neutral amino acids into the chloroplast interior was also tested by using neutral amino acids as the sole osmotic support for intact chloroplasts (Fig. 7). Chloroplasts were transferred from the original isolation medium, which contained sorbitol as the main solute to give osmotic support, into media where sorbitol was substituted by glycine, alanine, serine, proline, ribose, glycerol or urea. The contentration of these solutes was 0.15 M and thus just sufficient to prevent osmotic rupture (see Fig. 1) (total osmolarity of the solutions 0.18). Integrity of the chloroplasts was measured immediately, after 10 and after 60 min incubation at room temperature. Time dependent chloroplast rupture in the amino acid solutions did not exceed significantly that in the sorbitol medium, which reflects that normal chloroplast ageing was observed. In the experiment shown in Fig. 7. 76% of the chloroplasts were intact at the beginning of the experiment. After 1 h at room temperature 66% of the chloroplasts still had functional envelopes in the presence of sorbitol or the amino acids. In contrast to the amino acids, glycerol and urea were unable to provide osmotic support and caused fast chloroplast rupture. Chloroplast rupture

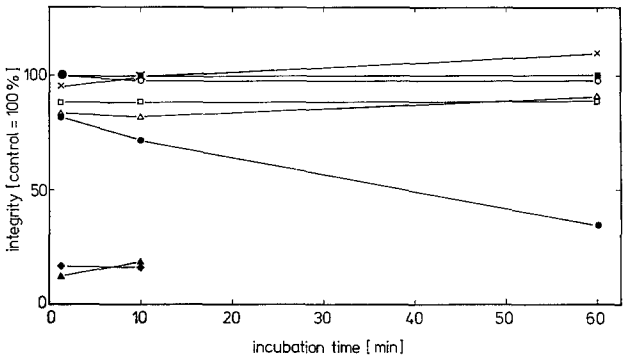

Fig. 7. Integrity of chloroplasts in media with different solutes for osmotic support. Integrity was tested by the ferricyanide reduction method (Heber and Santarius, 1970). The integrity of the chloroplasts in the original isolation medium (osmolarity of 0.38 sorbitol being the main compound) was 86%. In half concentrated medium 76% of the chloroplasts still had intact envelopes (Osmolarity of 0.18). Intact chloroplasts from this medium were transferred into other media where sorbitol was repalced by amino acids (symbols compare Fig. 5 or 6), ribose (•), glycerol (▼) and urea (◆). The integrity of the sorbitol containing control samples was used as reference (100%). The control samples contained 76% intact chloroplasts after 1 min and still 66% after 1 h. The samples were kept in the dark at 21°, ferricyanide was added immediately before the measurements

in the presence of ribose was slow indicating a slower uptake of ribose by the chloroplasts. It should be emphasized that the experiments presented in Figs. 4–7 do not exclude the possibility of very slow net uptake of amino acids or fast exchange. They merely establish that there is *no fast net uptake*. Chloroplasts would exhibit also full osmotic responses, if the uptake of neutral amino acids would occur in exchange against other solutes and would therefore not lead to osmotic water fluxes. The methods might also not be sufficiently sensitive to demonstrate very slow net uptake. Fig. 1 permits a very rough estimation of a maximum rate of net amino acid uptake; this rate would be compatible with the data shown in Figs. 4–7. Significant chloroplast rupture was observed on reducing the osmotic support from 0.2 to 0.1 M. Since the osmotic space attributed to 1 mg of chloroplasts is about 30 µl, linear uptake of a slowly permeating solute at a rate of about 3 µmol mg^{-1} chlorophyll h^{-1} would reduce osmotic support to such an extent as to cause an observable chloroplast rupture within 60 min, if sorbitol is the basis for osmotic support. In other words, the data of Fig. 7 merely show that net uptake of the neutral amino acids into the stroma of intact chloroplasts, if it occurs at all, is slower than about 3 µmol mg^{-1} chlorophyll h^{-1}.

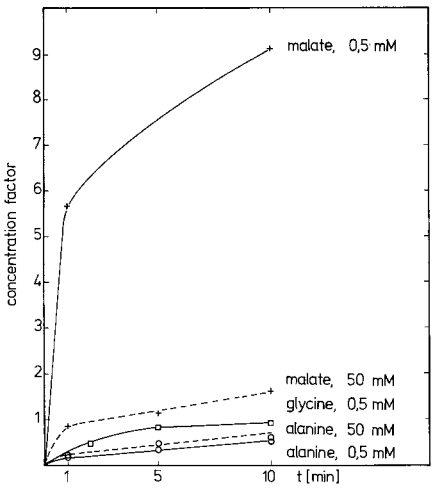

Fig. 8. Uptake of ^{14}C-labelled alanine (○), glycine (□) and malate (×) into the inner compartment of intact chloroplasts .The concentration factor is the ratio of the calculated concentration of the compound in the chloroplasts to the corresponding concentration in the external medium

3. Uptake of ^{14}C-labelled Amino Acids into Intact Chloroplasts

In order to investigate whether slow net uptake or a fast exchange transport of neutral amino acids via specific exchange carriers took place, the uptake of ^{14}C-labelled amino acids into intact chloroplasts was studied. In Fig. 8 the ratio of internal to external concentration of glycine, alanine and malate is plotted as a function of time. Uptake of labelled malate was measured as a reference, because it is known to be catalyzed by a specific exchange translocator and under appropriate conditions results in malate accumulation inside the chloroplasts (Heldt and Rapley, 1970). The ratio of internal to external concentration of labelled alanine or glycine never exceeded unity demonstrating that there is no accumulation of these compounds against the concentration gradient. Malate, however, accumulated in the chloroplasts, as should be expected from the experiments of Heldt and Rapley (1970). From the results pictured in Fig. 8, rates of uptake into the chloroplasts can be calculated. Obviously malate is rapidly transported into the chloroplasts, whereas the rates for amino acid uptake do not exceed 1 μmol amino acids mg^{-1} chlorophyll h^{-1}.

If this slow uptake is mediated by a specific translocator system, saturation of the rate of uptake should be expected at higher concentrations of substrates. However, as shown in Fig. 9, the increase of the

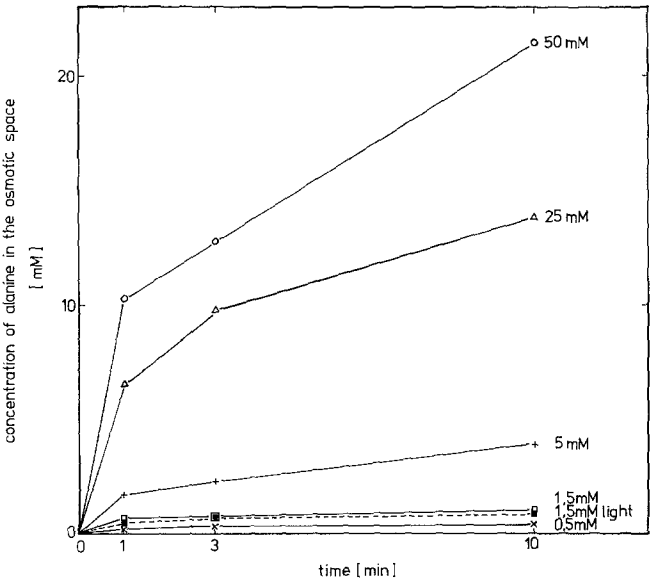

Fig. 9. Influence of alanine concentration in the external medium on the uptake of ^{14}C-labelled alanine into intact chloroplasts

concentration gradient with increasing concentrations of external amino acids (up to 50 mM) demonstrates non-saturation, as it should be expected from equilibration of external and internal concentration of the amino acids by unspecific diffusion.

As shown in Fig. 9, the change of the concentration gradient of alanine with time is not effected by light. It was also not inhibited by the addition of the electron transport inhibitor DCMU or the uncoupler FCCP.

4. Amino Acid Transport across the Chloroplast Envelope by Counter Exchange?

Intact chloroplasts preloaded with ^{14}C-labelled malate release labelled malate on addition of unlabelled malate, aspartate an glutamate (Table 2a, compare Heldt and Rapley, 1970). This demonstrates that the dicarboxylate translocator is functioning under our experimental conditions. The successful back-exchange with glutamine and asparagine shows that these compounds can also be transferred by this translocator and that there is no strict specificity for dicarboxylates. Similar results were obtained independently by Heldt (private communication). It may be of interest to note that major components of the free amino acid

Table 2. Release of radioactive malate (A) or alanine (B) from intact chloroplasts (Integrity 95%) upon addition of unlabelled malate and amino acids.

All compounds were added at zero time to give a final concentration of 0.5 mM, aliquots of the supernatant were measured after 3 min. Values were corrected for unspecific leaking of the labelled substances.

Preloaded with ^{14}C-labelled	Compound added	Radioactivity released (cpm mg^{-1} chlorophyll)
Malate	Malate	18300
(a)	Aspartate	16100
	Glutamate	8900
	Asparagine	3400
	Glutamine	2500
Alanine	Alanine	40
(b)	Glycine	0
	Serine	160
	Proline	0
	Threonine	0

pool of chloroplasts are glutamate, glutamine, aspartate and asparagine (not shown).

When chloroplasts were preloaded with ^{14}C-labelled alanine and the external radioactivity is removed by repeated washings, only little radioactivity was released on adding unlabelled alanine, glycine, serine or threonine (Table 2b). Similar negative results were obtained from experiments with chloroplasts preloaded with [^{14}C]glycine. The data give no evidence for transport of neutral free amino acids by counter exchange. We conclude that the very slow transport observed occurs by unspecific diffusion.

Concluding Remarks

The rapid appearance in the chloroplasts of labelled glycine and serine fed to intact leaves and light-dependent flow of carbon from these amino acids to sugars led Ongun and Stocking (1965) to conclude that glycine and serine are readily transferred across the chloroplast envelope. This view was strengthened by Nobel and Wang (1970) and Nobel and Cheung (1972), who suggested the existence of two different specific carriers in the chloroplast envelope mediating fast net uptake of neutral amino acids. Calculated reflection coefficients of amino acids, which were as low as 0.03 or 0.05, indicated a rate of amino acid transport, which was not much slower than the rate of water diffusion across the chloroplast envelope. The latter is very fast as can be seen from the fast decrease of chloroplast volumes on addition of osmotically active solutes. It may be noted that the chloroplast response to sorbitol or alanine as

shown in Fig. 2, which reflects water efflux, is limited by the response time of the measuring device and is in fact even faster than indicated by the negative deflection of the trace seen on addition of the solute.

In contrast to the fast catalyzed transfer of neutral amino acids as postulated by Nobel and Wang (1970) and Nobel and Cheung (1972), the results of this investigation fail to provide evidence for the existence of specific carriers for the uptake of neutral amino acids and rather suggest that transport takes place via slow diffusion. Observed rates of diffusion were more than sufficient to satisfy amino acid requirements of chloroplast protein synthesis which in expanded leaves are much below 0.1 μmol amino acid mg^{-1} chlorophyll h^{-1} as calculated from observed protein turnover (Hellebust and Bidwell, 1964).

It is been suggested that amino acids rather than phosphate esters play a role in the export of carbon from chloroplasts during photosynthesis (Roberts *et al.*, 1970). Photosynthesis rates of leaves are usually in the order of 100 μmol CO_2 reduced mg^{-1} chlorophyll h^{-1} (Table 1). Observed rates of triosephosphate or phosphoglycerate transfer across the chloroplast envelope approach 500 μmol mg^{-1} chlorophyll h^{-1} (Heber, 1974), while serine and glycine transfer at physiological amino acid concentrations is probably well below 1 μmol mg^{-1} chlorophyll h^{-1}. It does not appear possible therefore, to assign a major role to neutral amino acids in the transfer of carbon from the chloroplasts to the cytoplasm during photosynthesis.

The fast transfer of the amino dicarboxylates by the dicarboxylate translocator (Heldt and Rapley, 1970) may, on the other hand, be of considerable physiological importance and appears to link extra- and intrachloroplast pyridine nucleotide systems via a malate-oxaloacetate-aspartate shuttle (Heber and Krause, 1971). Such an indirect link is necessary since the chloroplast envelope is impermeable to pyridine nucleotides (Heber and Santarius, 1965).

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