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Glucose Transport into Spinach Chloroplasts[1]

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ABSTRACT

The uptake of radioactively labeled hexoses and pentoses into the sorbitol-impermeable ^3H_2O space (the space surrounded by the inner envelope membrane) of spinach (*Spinacia oleracea* L.) chloroplasts has been studied using silicone layer filtering centrifugation. Of the compounds tested, D-xylose, D-mannose, L-arabinose, and D-glucose are transported most rapidly, followed by D-fructose and L-arabinose. The rate of L-glucose uptake is only about 5% of that of D-glucose.

The transport of D-glucose and D-fructose shows saturation characteristics, the Km for D-glucose was found to be about 20 mM. All sugars transport and phloretin inhibit D-glucose transport. The temperature dependency of D-glucose transport appears to have an activation energy of 17 kcal/mol.

With low external concentrations of D-glucose the transport into the chloroplasts proceeds until nearly the external concentration is reached inside the chloroplasts.

D-glucose transport is inhibited by high D-glucose concentrations in the medium. It is concluded that D-glucose and other hexoses are transported by carrier-mediated diffusion across the inner envelope membrane. This transport is similar to the transport of D-glucose into erythrocytes.

During CO_2 fixation by spinach chloroplasts the fixed carbon is exported from the plastid in the form of three-carbon compounds, namely triose-P and P-glycerate. The export is facilitated by a specific carrier, the phosphate translocator, located in the inner membrane of the envelope (6). This membrane is impermeable to hexose phosphates, but shows a slight permeability to ribose monophosphate (2). Osmotic measurements led to the conclusion that pea chloroplasts contain a specific carrier transporting aldopentoses (21). There are some indications that hexoses may be able to pass the chloroplast envelope. It has been observed that feeding of tobacco leaves (18) and of *Chlorella* (11) with 1-[^{14}C]glucose yielded starch, in which the glucose moiety was still primarily labeled in position 1. Since starch synthesis occurs in the chloroplasts, these findings indicated that most of the glucose passed the envelope without being transformed to trioses. With isolated chloroplasts, fructose caused a slight, but significant diminution of the initial induction period of CO_2 fixation (2), which suggested that this sugar permeated the envelope. Furthermore, glucose was found to be a minor product of starch degradation released by intact chloroplasts (7), whereas the major part of the mobilized starch was released in the form of triose-P and P-glycerate (7, 16). On the other hand, glucose has been successfully used as an osmoticant in the preparation of intact chloroplasts (20), which implies that the envelope is not permeable to this compound.

At present the evidence for a possible transport of glucose across the envelope is indirect and ambiguous. It is our aim to clarify this question by direct measurements of hexose uptake.

MATERIALS AND METHODS

Spinach (*Spinacia oleracea* L. cv. True Hybrid 102, Arthur Yates and Co., N.S.W., Australia) was grown in water culture according to Lilley and Walker (17). Chloroplasts with intact envelopes were prepared from fully grown leaves according to the method of Cockburn *et al.* (3) modified by Heldt and Sauer (8), or by a modified Jensen and Bassham (10) procedure (4).

The chloroplasts were suspended in a medium containing 0.33 M sorbitol, 50 mM HEPES (pH 7.6) neutralized with NaOH, 1 mM $MgCl_2$, 1 mM $MnCl_2$, and 2 mM EDTA (0.1 mg Chl/ml). Chlorophyll was assayed after the method of Whatley and Arnon (23). All experiments were carried out at 20 C (except Fig. 2) and in the dark. The uptake was initiated by adding ^{14}C-labeled sugars (0.3-1 Ci/mol) in a volume of 10 to 200 μl of chloroplast suspension. The uptake was terminated by rapid centrifugation of the chloroplasts through a layer of silicone oil into 20 μl of 1 M $HClO_4$. (For details of silicone layer filtering centrifugation and evaluation of the uptake into the sorbitol-impermeable ^3H_2O space, which is the space surrounded by the inner envelope membrane, see reference 22.)

The ^{14}C-labeled sugars were obtained from Amersham, England and New England Nuclear.

RESULTS

Rates of Hexose Uptake. Figure 1 shows the time course for the uptake of the two stereoisomers of glucose into the sorbitol-impermeable ^3H_2O space of spinach chloroplasts. The uptake of D-glucose is very rapid. The uptake rate is evaluated from the initial value measured after 10 sec. It may be noted that the uptake slows down before the external D-glucose concentration (2 mM) is reached in the chloroplasts. As the thylakoid space represents only about 1/8 of the sorbitol-impermeable H_2O space (9), the only partial uptake of D-glucose cannot be explained solely by an impermeability of the thylakoid membrane. As will be discussed later, this partial uptake is dependent on the concentration in the medium. The rate of L-glucose uptake is about 4% of the rate of D-glucose uptake.

Table I shows initial rates of uptake obtained for various sugars. These data demonstrate a strong specificity of the uptake. From these values a sequence for the rates of uptake can be

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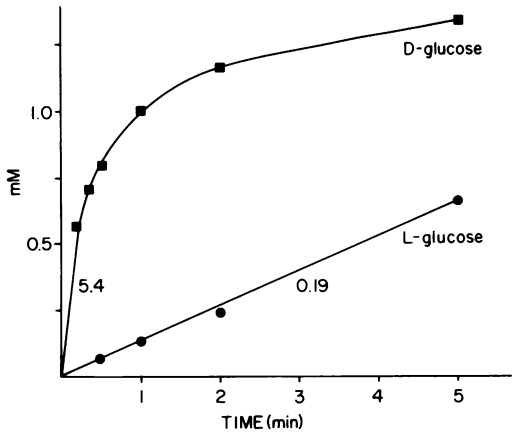

FIG. 1. Uptake of the two stereoisomers of glucose (2 mM) into the sorbitol-impermeable H_2O space of the chloroplasts. The size of this space was 26 μl (mg Chl)$^{-1}$. The numbers represent rates of uptake in μmol (mg Chl)$^{-1}$ hr^{-1}.

Table I

Rate of Sugar Uptake into Chloroplasts. The Measuring Time was 20 sec.

sugar, 5mM	rate of uptake
	μmol (mg Chl)$^{-1}$hr^{-1}
D-glucose	7.4
L-glucose	0.3
D-mannose	8.4
D-fructose	2.8
D-xylose	9.3
D-ribose	6.0
L-arabinose	7.7
D-arabinose	1.6

derived: D-xylose > D-mannose > L-arabinose > D-glucose > D-ribose > D-fructose > D-arabinose > L-glucose.

Temperature Dependency. The uptake of D-glucose is very dependent upon temperature. In Figure 2 the logarithm of the initial rate has been plotted *versus* the reciprocal of the temperature. The derived activation energy of the D-glucose uptake is 17 kcal/mol (10–25 C). This evaluation is based on the assumption that there is no discontinuity in the slope, as might have been caused by a temperature-dependent transition of membrane properties. A more detailed analysis of the temperature dependency would be required to verify this. However, extensive studies on the temperature dependency of dicarboxylate and phosphate transport (unpublished results) across the chloroplast envelope gave no evidence for the occurrence of such a discontinuity.

Concentration Dependence of Hexose Uptake. The rate of D-glucose uptake depends on the concentration in the medium (Fig. 3). The concentration dependence reveals hyperbolic saturation characteristics, indicating substrate saturation of the transport. A double reciprocal plot of the data yields a linear function, which enables the determination of *Km* (hexose concentration causing half-maximal rate of transport) and of V_{max} (maximal rate of transport). In a number of similar experiments the *Km* of D-glucose was found to be 10 to 30 mM, and for D-fructose 80 to 100 mM.

Competition between Hexoses for Transportation. Those sugars taken up by the chloroplasts, as shown in Table I, also inhibit the uptake of D-glucose (Table II). It seems that these sugars compete for transport into the chloroplasts. In order to compare the inhibition by the various sugars the concentration dependence of the transport has to be taken into account. With 5 mM D-glucose in the medium the rate of uptake is 71, and with 25 mM D-glucose, 178. In other words: if 20 mM unlabeled D-glucose is added to 5 mM labeled D-glucose, the rate for the labeled D-glucose is decreased to

$$\frac{178 \cdot 5}{25} = 36.6$$

as compared to the uninhibited rate of 71. The data show that D-

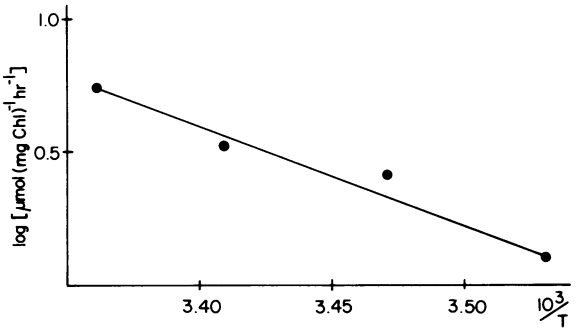

FIG. 2. Temperature dependence of D-glucose (2.5 mM) uptake into chloroplasts.

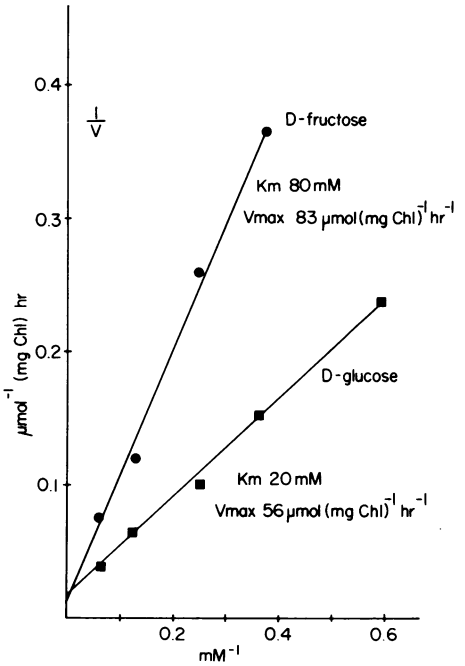

FIG. 3. Concentration dependence of the uptake of D-glucose and D-fructose into chloroplasts.

Table II

Inhibition of D-Glucose Uptake into Spinach Chloroplasts by Various Sugars.

Mean Values from three Experiments. The Measuring Time was 45 sec.

sugar, 20 mM	D-glucose conc.	D-glucose uptake	inhibition
	mM	nmol (mg Chl)$^{-1}$	%
-	25	178	
-	5	71	
D-mannose	5	47	34
D-galactose	5	52	27
D-fructose	5	64	10
D-xylose	5	39	45
D-ribose	5	65	8
L-arabinose	5	50	30
D-arabinose	5	69	3

xylose and D-mannose are the strongest inhibitors, followed by D-galactose and L-arabinose. D-Fructose, D-ribose, and especially D-arabinose are only weak inhibitors. It may be noted that the order of the sugars according to their inhibition is the same as the order according to the rates of uptake. This is a strong indication that all of these compounds are transported by the same carrier.

Inhibition of D-Glucose Uptake. Table III shows that phloretin, a known inhibitor of glucose transport in erythrocytes, also inhibits D-glucose uptake in chloroplasts. The phloretin concentration required for 50% inhibition of D-glucose transport in chloroplasts is higher than that required in erythrocytes (14), but very similar to the required concentration in rat liver cells (1).

Dependency of the Extent of D-Glucose Uptake on the Concentration in the Medium. From the properties of the hexose transport studied so far, one would expect that chloroplasts kept in a D-glucose medium should swell and rupture rapidly due to D-glucose uptake. This is illustrated with a model calculation (Fig. 4) for the uptake of 5 mM and 320 mM D-glucose, employing the kinetic constants from Figure 2. According to this calculation, with a D-glucose concentration of 320 mM in the medium (B), the concentration ratio between the medium and the stroma should be decreased by 80% within 12 min, and the chloroplasts should be broken by then.

In fact, D-glucose can be used as an osmoticant for chloroplast suspensions (20). In an experiment not shown here, chloroplasts had been suspended for 60 min at 20 C in media which contained 150 mM of various sugars, and the stability of the chloroplasts was determined by ferricyanide reduction (5). Of the intact chloroplasts initially present, 97% remained intact in the presence of sorbitol, and still about 80% in the presence of D-glucose, D-fructose, or D-ribose. To resolve these conflicting data, the time course for the uptake of D-glucose has been measured under the conditions of the calculated model in Figure 4. Especially with high D-glucose concentrations, the observed uptake is very different from the calculated curve. With 320 mM D-glucose, the concentration gradient is only decreased by 7% after 12 min incubation. Obviously, the relationship

$$V = \frac{V_{max}\,(S)}{(S) + Km}$$

does not hold for high D-glucose concentrations. The reason for this anomalous behavior of D-glucose transport is not known. It is feasible that the transport is inhibited by increasing D-glucose concentrations in the stroma. In this way, high D-glucose concentrations in the medium could be excluded from the stroma, whereas low D-glucose concentrations are able to equilibrate.

The question arises whether this anomalous behavior of the D-glucose uptake also applies to sorbitol, which is usually used as a marker substance for the solute space outside the inner envelope membrane. To check this, the chloroplasts were kept in a medium containing either 320 mM sorbitol + 5 mM D-glucose or 320 mM D-glucose + 5 mM sorbitol, and the corresponding solute spaces were measured with ^{14}C-labeled sugars and ^3H_2O according to Heldt and Sauer (8) (Table IV).

It appears from the data that the sorbitol space is independent of the sorbitol concentration. Similar results have been also observed with sucrose (not shown here). These results clearly indicate that the inner envelope membrane is impermeable to sorbitol and sucrose irrespective of their concentration. The D-glucose space obtained with 5 mM of this sugar is almost as high as the ^3H_2O space, which shows that the D-glucose concentration in the ^3H_2O-sorbitol space (the space surrounded by the inner envelope membrane [8]) approaches the concentration in the medium (see also Fig. 5). At high concentrations (320 mM) the D-glucose space is similar to the sorbitol space. Furthermore, the ^3H_2O-sorbitol space is not significantly altered. Since the ^3H_2O-

Table III

Inhibition of the Uptake of D-Glucose by Phloretin. The D-Glucose Concentration was 5 mM.

conc. of phloretin	uptake rate	inhibition
μM	μmol (mg Chl)$^{-1}$hr^{-1}	%
-	3.56	
250	1.63	54
150	2.84	20
50	2.96	17

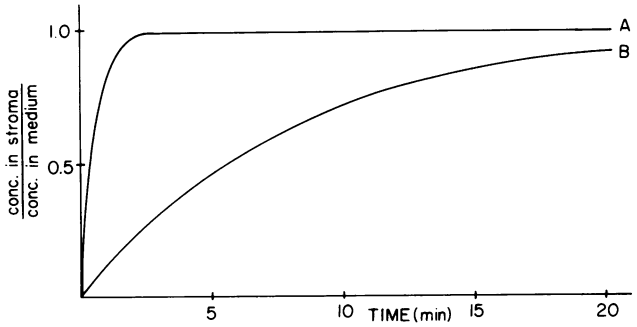

FIG. 4. Model calculation of D-glucose uptake into the stroma from the kinetic constants in Table II. Concentration of D-glucose in medium: A: 5 mM; B: 320 mM. The calculation is based on the assumption that the glucose carrier catalyzes a facilitated diffusion into either direction with a Michaelis-Menten characteristic. Concentration in the stroma/concentration in the medium = $n/n\infty$. n is the amount of D-glucose present in the stroma space (22 μl H_2O/mg Chl) at the time t (min):

$$n = n\infty \cdot e^{-(V \cdot t)/(n\infty)}$$

n stands for the final amount of D-glucose in the stroma if the concentration in the medium (C) is reached there. Velocity of transport:

$$V = \frac{V_{max} \cdot C}{Km + C}$$

Table IV. Size of the Glucose and Sorbitol Permeable Spaces of Spinach Chloroplasts Depending on the Corresponding Concentrations in the Medium.

Incubation was 15 min. The reaction was started by the addition of chloroplasts to the medium containing D-glucose and sorbitol.

Concentration in medium		Spaces			
D-glucose	sorbitol	D-glucose	sorbitol	^3H_2O	^3H_2O-sorbitol
mM		μl (mg Chl)$^{-1}$			
5	320	67.4	42.4	70.0	27.6
320	5	47.2	45.0	73.6	28.6

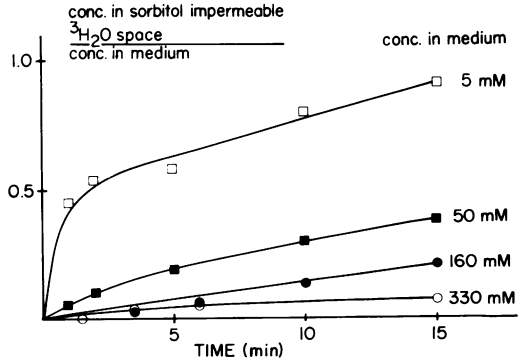

FIG. 5. Uptake of D-glucose into chloroplasts depending on the concentration in the medium. The reaction was started by the addition of chloroplasts to the medium containing D-glucose. In all experiments the sum of the concentrations of D-glucose and sorbitol was 330 mM.

sorbitol space includes the osmotic space of the chloroplasts, these data clearly indicate that 320 mM D-glucose provides osmotic support for intact chloroplasts.

DISCUSSION

The steric specifity, saturation characteristic, competition of substrate molecules, and the sensitivity to specific inhibitors of glucose transport clearly demonstrate that glucose and other hexoses and also pentoses pass the inner membrane of the chloroplast envelope by a specific carrier. The present results suggest that the carrier mediates a facilitated diffusion. Preliminary studies with illuminated chloroplasts showed no evidence for any active uptake.

The specificity of the glucose transport in spinach chloroplasts shows striking similarities to the specificity of the glucose transport in human erythrocytes (15) and in rat liver cells (1). Also, the *Km* and the activation energy in erythrocytes (8 mM [15], 20 kcal/mol [19]) and in liver cells (30 mM, 22 kcal/mol [1]) are very similar to the values reported here.

It appears that glucose transport in spinach chloroplasts follows a mechanism which seems to be ubiquitous in nature. A sugar carrier has been described by Kotyk and Höfer (13) in yeast, involved in active transport. Komor and Tanner (12) showed that hexose uptake in *Chlorella* is facilitated by a specific carrier, which also catalyzes active transport.

Wang and Nobel (21) postulated for pea chloroplasts a carrier transporting the pentoses D-xylose, L-arabinose, and D-ribose. The authors drew attention to the fact that the concentration range of their investigations (14–50 mM) was so high that a possible uptake of hexoses by the carrier might have been almost saturated, and for these reasons, the high reflection coefficients (>0.8) for hexoses were obtained. Our data make it likely that these authors could not detect hexose uptake because of the high concentrations employed.

The activity of the glucose carrier (about 3 μmol [mg Chl]$^{-1}$ hr^{-1} with 1 mM D-glucose) does not compare to the activity of the phosphate translocator (about 200 μmol of dihydroxyacetone phosphate transported [mg Chl]$^{-1}$ hr^{-1}) (6). It has been shown with isolated chloroplasts that during starch mobilization most of the carbon is released as triose-P and P-glycerate, but some also as glucose (7). It may be one function of the glucose carrier in chloroplasts to enable the release of glucose formed during starch hydrolysis in chloroplasts.

It still remains to be elucidated if and how the D-glucose taken up by intact chloroplasts may be metabolized there. One might have expected that added D-glucose may be incorporated into starch. However, when spinach chloroplasts performing high rates of starch synthesis were incubated in the light with ^{14}C-labeled D-glucose, there was no or very little incorporation of the label into starch observed (7) (Gibbs, M., personal communication). Further investigations will be required to clarify the matter.

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