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PERMEABILITY OF PEA CHLOROPLASTS TO ALCOHOLS AND ALDOSES AS MEASURED BY REFLECTION COEFFICIENTS

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SUMMARY

1. Intact chloroplasts were isolated in 2 min from *Pisum sativum* and then placed in external solutions varying in composition and in osmotic pressure. The relative osmotic changes in chloroplast volume caused by the impermeable solute sucrose compared with equal concentrations of various alcohols and aldoses were determined. From such osmotic responses the reflection coefficients (σ_x) of the latter compounds were calculated using a version of the Boyle–Van 't Hoff relation derived from irreversible thermodynamics.

2. The reflection coefficients of pea chloroplasts for alcohols became progressively larger as hydroxymethyl groups were added to the molecule, *viz.* methanol ($\sigma_x = 0.00$), ethylene glycol (0.40), glycerol (0.63), *meso*-erythritol (0.90), and adonitol (1.00). This increase in σ_x (decrease in permeability) with increasing molecular size parallels the decreasing lipid–water partition coefficients of the solutes. The reflection coefficient of D-mannitol was 1.01, of D-sorbitol was 1.02, and of *meso*-inositol was 1.00, suggesting that these six-carbon polyhydroxy alcohols are impermeant and hence would be suitable osmotica in which to suspend chloroplasts.

3. Chloroplasts are more permeable to certain aldopentoses, *viz.* D-xylose ($\sigma_x = 0.43$), D-lyxose (0.47) and L-arabinose (0.53), than they are to the optical isomers of the same compounds, *i.e.* L-xylose (0.87), L-lyxose (0.93), and D-arabinose (0.85). Although all of these compounds predominantly occur in a chair form of the pyranose ring, the first three are in the C1 conformation, while the latter three are in the 1C conformation. The greater permeability of the C1 aldopentoses was even more apparent for D-ribose, which had a reflection coefficient of 0.00.

4. Based on osmotic responses from 14 to 50 mM, the aldohexoses tended to have higher reflection coefficients for the limiting membranes of pea chloroplasts than did the aldopentoses: α-D-glucose ($\sigma_x = 0.98$), β-D-glucose (0.99), 2-deoxy-D-glucose (1.00), D-galactose (0.92), 2-deoxy-D-galactose (0.91), 6-deoxy-D-galactose (0.89), D-mannose (0.85), L-mannose (1.00), and 6-deoxy-L-mannose (1.00).

5. Based on the correlation between the reflection coefficient and the particular sugar stereoisomer, it is proposed that carriers in the limiting membranes of pea chloroplasts are specific for aldopentoses in the C1 form of the chair conformation of the pyranose ring and, furthermore, are particularly efficient in

shuttling D-ribose into pea chloroplasts. At concentrations above 50 mM, the apparent reflection coefficient of the C1 aldopentoses increased, suggesting that such carriers were becoming saturated. Moreover, the osmotic responses when two sugars were present together indicated that D-xylose, D-lyxose, L-arabinose, and D-ribose all competed for the same carrier.

INTRODUCTION

Chloroplasts isolated from various plants such as Nitella[1], poke weed[2], spinach[2–5] and pea[6] behave as osmometers when suspended in sucrose solutions. This indicates that the limiting membranes of chloroplasts are considerably more permeable to water than to sucrose, as would indeed be expected. Osmotic responses of isolated spinach chloroplasts have also been observed with glucose, mannitol, sorbitol, or glycerol in the external solution[5]. Based on microscopic observations of the relative volume changes of chloroplasts in sucrose compared with glycerol solutions, HEBER[5] concluded that glycerol penetrates the chloroplast membranes more easily than does sucrose. Besides this, no other even qualitative description of the permeation of neutral sugars and alcohols into chloroplasts apparently is available. In the present study, the relative permeability of pea chloroplasts for such compounds was determined using osmotic responses in various solutions.

The behavior of the volume of cells or organelles in response to changes in the osmotic pressure of external solutions can be described using the Boyle–Van 't Hoff relation[6–8]:

$$\pi^0(V - b) = RT \sum_{\mathrm{j}} \varphi_{\mathrm{j}} n_{\mathrm{j}}^{\mathrm{i}} \qquad (1)$$

where π^0 is the osmotic pressure of the external solution, V is the volume of the chloroplast or other membrane-bounded body, b is the nonaqueous volume within V, φ_{j} is a correction factor referred to as the osmotic coefficient, and $n_{\mathrm{j}}^{\mathrm{i}}$ is the number of moles of species j within $V-b$ (R and T are the gas constant and absolute temperature, respectively). Eqn. 1 only applies to the osmotic responses to impermeable solutes. To include the more usual case of penetrating solutes, NOBEL[7] has rederived the conventional Boyle–Van 't Hoff relation using irreversible thermodynamics and obtained the following expression:

$$\sum \sigma_{\mathrm{j}} \pi_{\mathrm{j}}^0 = RT \frac{\sum_{\mathrm{j}} \sigma_{\mathrm{j}} \gamma_{\mathrm{j}}^{\mathrm{i}} n_{\mathrm{j}}^{\mathrm{i}}}{\overline{V}_{\mathrm{w}} n_{\mathrm{w}}^{\mathrm{i}}} \qquad (2)$$

where σ_{j} is the reflection coefficient of species j, π_{j}^0 is the osmotic pressure contributed by external species j, $\gamma_{\mathrm{j}}^{\mathrm{i}}$ is the activity coefficient of internal species j, $\overline{V}_{\mathrm{w}}$ is the partial molal volume of water, and $n_{\mathrm{w}}^{\mathrm{i}}$ is the number of moles of water within the membrane-surrounded body. To help reconcile Eqn. 2 with the conventional Boyle–Van 't Hoff relation, it is useful to identify the volume per mole of water times the number of moles of water contained within the membrane bounded body, $\bar{V}_w n_w^i$, with $V-b$ in Eqn. 1, *i.e.* the total volume (V) *minus* the nonaqueous volume (b) equals the internal aqueous volume ($\bar{V}_w n_w^i$).

Before proceeding, some of the properties of reflection coefficients will be briefly mentioned. A reflection coefficient is a parameter that is introduced by irreversible or nonequilibrium thermodynamics and which is related to the permeability of some membrane for a particular solute[7-9]. For instance, a species j which cannot cross a certain membrane has a σ_j of unity for that barrier. On the other hand, if the membrane does not distinguish or select between water and some solute, then its σ_j is zero for that species, which is the case for solutes moving across very coarse barriers or for molecules very similar to water itself. It is often useful to consider a reflection coefficient of species j to be the osmotic pressure of that species which is effective in causing water movement divided by its theoretical osmotic pressure π_j, the latter being obtained from the Van 't Hoff relation ($\pi_j = RT\,c_j$). In fact, this latter definition characterizes the experimental approach employed in the present study.

Using Eqn. 2, the reflection coefficient for a particular solute can be determined from the osmotic responses of chloroplasts in external solutions containing that compound. The analysis can be considerably simplified if a nonpermeant solute is also available, since only relative osmotic responses would then need to be measured in order to determine unknown reflection coefficients. In this regard, NOBEL[6] has found that σ_{sucrose} for pea chloroplasts is unity. Using sucrose as a reference for osmotically-induced volume changes, NOBEL AND WANG[10] measured the reflection coefficients of pea chloroplasts for various amino acids. The reflection coefficients for the following amino acids were all 0.05 or less: glycine, alanine, serine, threonine, phenylalanine, methionine, and proline. Such extremely low σ_j's for amino acids means that chloroplasts are readily permeable to these compounds, in agreement with various metabolic studies *in vivo*[11-14] and *in vitro*[14-18].

The external solutions used in both the present and the previous studies[10] contained 0.2 M sucrose, 5 mM Tris–HCl (pH 7.9), and various concentrations (c_x) of a substance with an unknown reflection coefficient (σ_x). To analyze the resulting osmotic responses, Eqn. 2 can be reexpressed in the following convenient form[10]:

$$\sigma_x c_x + \frac{\alpha}{RT} = \frac{\beta}{V-b} \qquad (3)$$

where α is a constant representing the reflection coefficients times the osmotic pressures for 0.2 M sucrose and 5 mM Tris–HCl (pH 7.9), while β is the factor $\sum_j \sigma_j \gamma_j^i n_j^i$ in Eqn. 2. Eqn. 3 indicates that the reciprocal of the aqueous volume within the chloroplast, $1/(V-b)$, should vary linearly with the solute concentration in the external solution, c_x. Moreover, since the addition of solutes to the external solution would cause water to flow out of the chloroplasts, the reflection coefficients measured in these studies apply to the pair of membranes surrounding a chloroplast, this being the barrier to solute entry or exit encountered under physiological conditions in the plant. By comparing the osmotically induced volume changes of chloroplasts in the test solutions with those obtained in the sucrose solutions ($\sigma_{\text{sucrose}} = 1.00$), the σ_x of pea chloroplasts for the test solute can be readily determined. Using this approach, the reflection coefficients of the limiting membranes of pea chloroplasts for aldoses and alcohols were measured.

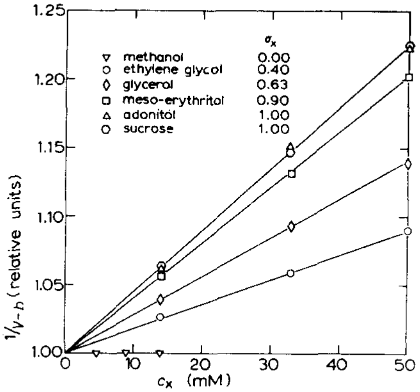
MATERIALS AND METHODS

Seeds of *Pisum sativum* L., cv. Blue Bantam (W. Atlee Burpee Co., Riverside, Calif.,) were soaked for 24 h and then grown at 20° in moist vermiculite under daylight fluorescent tubes, which provided 2000 lux for 12 h each day. On the 14th day, illuminated plants were harvested and 15 g of leaves and stems were used for chloroplast isolation. A gentle 2 min isolation technique[19] which yields 95 % intact chloroplasts[20] was employed. The isolation medium was 10 ml of 0.2 M sucrose buffered with 5 mM Tris-HCl (pH 7.9). After isolation, chloroplast volumes in various external solutions were determined. All such solutions contained 0.2 M sucrose, 5 mM Tris-HCl (pH 7.9) *plus* various concentrations (c_x) of alcohols, aldoses or additional sucrose (purchased from Calbiochem, Los Angeles, Calif. or Sigma Chemical Co., St. Louis, Mo.). Because of the great experimental precision of packed weight measurements[10], the weight of various chloroplast pellets following a 3 min centrifugation at 10000 × g was first determined and then the values were converted to volumes using measured densities[10,21]. After using [^{14}C]dextran to correct for interstitial fluid trapped in the pellet[10,20], the volume of the chloroplasts per mg chlorophyll (V) was calculated (chlorophyll was determined spectrophotometrically[22,23]). The magnitude of the nonaqueous volume of pea chloroplasts (b in Eqns. 1 and 3) is already known[6], and so $V-b$ is readily found. When $1/(V-b)$ is plotted *versus* c_x, the slope of the line obtained should be proportional to σ_x by Eqn. 3. The values of σ_x can be obtained from the relative slope obtained when the unknown is added to the external solution compared with that observed in the presence of added sucrose ($\sigma_{\text{sucrose}} = 1.00$). Each data point in the figures represents the average of three or more experiments.

RESULTS

The osmotic responses of pea chloroplasts to various concentrations of alcohols and aldoses in the external solution are presented in Figs. 1–5. The abscissa, c_x, is the concentration of a particular compound added to chloroplasts suspended in 0.2 M sucrose, 5 mM Tris-HCl (pH 7.9). The quantity $V-b$ represents the volume of osmotically responding water in chloroplasts of volume V, and thus an increase in the ordinate, $1/(V-b)$, indicates an efflux of water from the chloroplasts in response to an increase in the external osmotic pressure. In each case, the osmotic response to the impermeant solute sucrose ($\sigma_{\text{sucrose}} = 1.00$) is given as a control. When a substance penetrates more readily than does sucrose (*i.e.* a lower reflection coefficient), the effectiveness of the external osmotic pressure in causing a volume change is reduced, and thus the slope of a plot of $1/(V-b)$ *versus* c_x is less in that case.

Fig. 1 shows the osmotic responses of pea chloroplasts to a series of polyhydroxy alcohols of increasing chain length. Methanol is extremely permeable and has a reflection coefficient indistinguishable from 0.00. (The highest methanol concentration employed was only 14 mM (0.045 %), since higher concentrations tend to extract lipids from the membrane and consequently cause a disruption of the chloroplasts.) The next member of the series, ethylene glycol, has a much larger reflection coefficient, *viz.* 0.40 (Fig. 1), while the σ_x of glycerol is 0.63. The four-carbon polyhydroxy alcohol, *meso*-erythritol, is even less permeable ($\sigma_X = 0.90$), while adonitol is essentially impermeable ($\sigma_X = 1.00$). Not shown in Fig. 1 are results with six-carbon polyhydroxy alcohols. In particular, the reflection coefficient of D-mannitol was 1.01 and that of D-sorbitol was 1.02. Pea chloroplasts are also impermeable to the six-carbon cyclic polyhydroxy alcohol, *meso*-inositol ($\sigma_X = 1.00$).



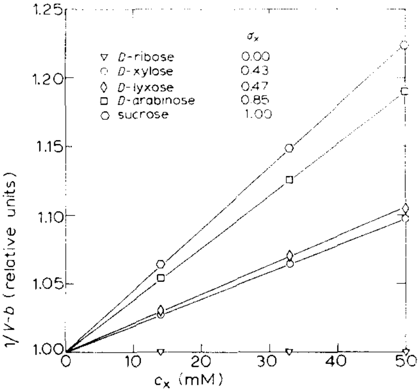
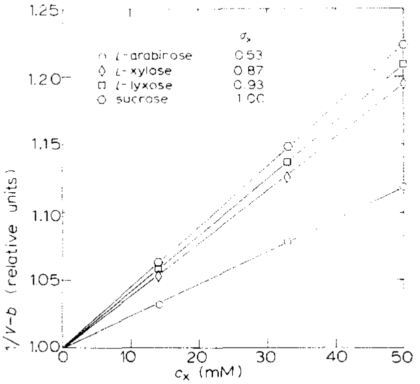


Fig. 1. Osmotic responses of pea chloroplasts to polyhydroxy alcohols. The reflection coefficients, σ_X, are calculated from the relative slopes.

Fig. 2. Osmotic responses of pea chloroplasts to D-aldopentoses.

Next, the reflection coefficients of pea chloroplasts for both the D- and the L-forms of five-carbon polyhydroxy aldehydes (the so-called aldopentoses) were determined (Figs. 2 and 3). It was found that D-ribose is extremely permeable, having a σ_X of 0.00 (Fig. 2). The reflection coefficient of D-xylose was 0.43, that of D-lyxose was 0.47, and the σ_X of D-arabinose was 0.85 (Fig. 2). Fig. 3 indicates that the σ_X's of L-xylose (0.87) and L-lyxose (0.93) are similar, being considerably higher than that of L-arabinose (0.53). The reflection coefficient of L-ribose, which is not commercially available, was not determined. Except for D-ribose, the



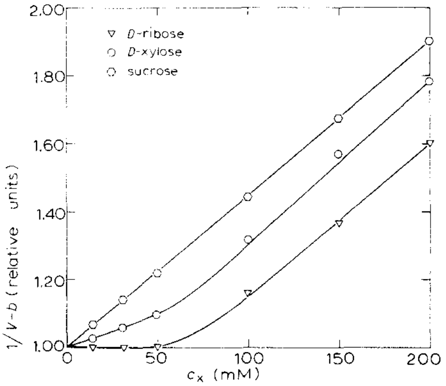


Fig. 3. Osmotic responses of pea chloroplasts to L-aldopentoses.

Fig. 4. Osmotic responses of pea chloroplasts to D-ribose and D-xylose at concentrations up to 200 mM.

reflection coefficients of the aldopentoses were grouped either near 0.5 or near 0.9, a matter that will be returned to below.

To help understand the differences in σ_X for the aldopentoses and also the unexpectedly low values for some of them, the osmotic responses of pea chloroplasts at even higher concentrations of D-ribose ($\sigma_X = 0.00$) and D-xylose (0.43) were tested (Fig. 4). As the concentration of either of these aldopentoses increased above 50 mM, the slope of the c_X *versus* $1/(V-b)$ plot increased and in fact became essentially parallel to the osmotic response to sucrose. In other words, the apparent reflection coefficient in the range of external concentrations from 100 to 200 mM was near unity, in marked contrast to the value for concentrations under 50 mM (Fig. 2). As will be discussed below, such a change of slope is consistent with saturation effects that can accompany a carrier-mediated uptake. Another characteristic of carriers is the phenomenon of competition, and so various pairs of aldopentoses were added to the external solution. As Fig. 4 indicates, 50 mM D-ribose leads to no water efflux, while $1/(V-b)$ is 1.09 for 50 mM D-xylose. If these two sugars acted independently as far as osmotic volume changes of pea chloroplasts are concerned, then $1/(V-b)$ should also be 1.09 for 50 mM D-ribose *plus* 50 mM D-xylose. However, the measured value under such conditions was 1.25. In analogous manner the effects of other pairs of aldopentoses on chloroplast volume were also studied. For 50 mM D-ribose *plus* 50 mM D-lyxose, $1/(V-b)$ was 1.26, while for 50 mM D-ribose *plus* 50 mM L-arabinose it was 1.28. Such nonadditivity of osmotic responses of pea chloroplasts for these aldopentoses strongly suggests that such sugars are competing for the same carrier, as will be discussed later.

The reflection coefficients of certain aldohexoses were also measured using the osmotic responses of pea chloroplasts. For instance, Fig. 5 suggests that optical isomerization affects the reflection coefficients of six-carbon sugars, since the σ_X of L-mannose (1.00) is somewhat higher than that of D-mannose (0.85). To help

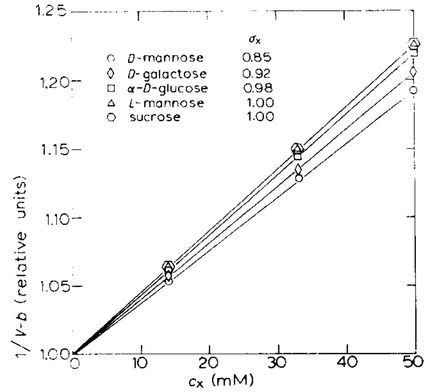


Fig. 5. Osmotic responses of pea chloroplasts to aldohexoses.

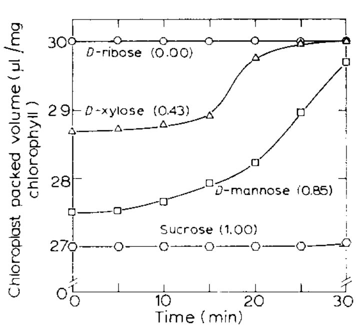


Fig. 6. The effect of storage time in the presence of sugars with various reflection coefficients on the mean volumes of pea chloroplasts. The external solutions contained 0.2 M sucrose, 5 mM Tris–HCl (pH 7.9), and 50 mM of the indicated sugar (σ_X given in parentheses). After storage at 0° for different periods of time, the chloroplast suspensions were centrifuged for 3 min at 10000 × g as usual and the mean chloroplast volumes were calculated as described previously[10].

see whether hydroxy groups affect the reflection coefficients of aldohexoses entering pea chloroplasts, the σ_X's of 2-deoxy- and 6-deoxyhexoses were determined. The reflection coefficients for deoxy aldohexoses were found to be quite similar to the σ_X's of their hydroxylated counterparts (Fig. 5), as can be seen from the following pairs: 2-deoxy-D-glucose (1.00) and α-D-glucose (0.98); 2-deoxy-D-galactose (0.91), 6-deoxy-D-galactose (D-fucose) (0.89), and D-galactose (0.92); and 6-deoxy-L-mannose (L-rhamnose) (1.00) and L-mannose (1.00). For completeness, it should be mentioned that σ_X for β-D-glucose was 0.99, similar to that of α-D-glucose (0.98).

In the derivation of Eqn. 2 a "stationary state" condition of no net volume flow across the limiting membranes was assumed[7,8]. This means that the volume of the cell or organelle should not be changing with time (after the initial rapid flux of water, which occurs when the osmotic pressure of the external solution is varied and is over within about 1 sec for pea chloroplasts). However, if the reflection coefficient is between 0 and 1, the external solute can enter the cell or organelle and thereby change the internal osmotic pressure, and so the volume must also be changing with time. To investigate the magnitude of such volume changes, chloroplasts were stored for various periods before their packed weights were determined. Fig. 6 summarizes such observations which were carried out using a c_X of 50 mM for the following sugars: D-ribose ($\sigma_X = 0.00$), D-xylose (0.43), D-mannose (0.85) and sucrose (1.00). D-Ribose has a reflection coefficient of 0 (Fig. 2) and thus would be expected to exert no effective osmotic pressure difference across the chloroplast membranes and consequently to cause no volume change with increasing storage times, as was indeed the case (Fig. 6). Sucrose, on the other hand, is judged to be impermeant[6] and thus the chloroplast volume also should not change in time, although it would have a different value than for D-ribose; this was the case for 30 min of storage in an external solution containing 50 mM sucrose (Fig. 6). The mean chloroplast volumes changed very little for approx. 10 min of storage in the D-xylose and the D-mannose solutions, indicating that the stationary state assumption and the experimental technique used in the present studies is suitable for determining reflection coefficients between 0 and 1. Finally it should be pointed out that eventually the permeant neutral solute will achieve the same concentration inside as outside, at which time it would exert no osmotic pressure difference across the membranes; consequently, the asymptote for the chloroplast volume is the same as the V obtained in the absence of the permeant solute (which is also the volume in the presence of 50 mM D-ribose, a solute whose reflection coefficient is 0, see Fig. 6).

DISCUSSION

Permeation of alcohols into chloroplasts

The rates of permeation of most nonelectrolytes through biological membranes are proportional to the partition coefficients of the solutes[8,24–26]. This situation arises because the concentration difference or "force" actually determining the diffusion of molecules across a membrane is the concentration just inside one side of the membrane minus that just within the other side, whereas the concentrations that are normally considered are those in the aqueous phases adjacent to the membrane. Consequently, a correction factor is needed to give the actual concentration gradient existing in the membrane. This factor, which is known as the partition coefficient of the solute, is essentially the solubility of the solute in the membrane phase divided by the solubility in the adjacent aqueous phase. Numerous experimental results have shown that the membrane-water partition coefficient of a solute can be approximated by the ratio of the equilibrium concentration of the solute in a lipid phase (such as ether) divided by the concentration in an adjacent and immiscible aqueous phase. In this regard, WRIGHT and coworkers[24,26] have shown that as such ether-water partition coefficients decrease from high values to low ones, the reflection coefficients for the same nonelectrolytes (obtained using epithelial cells of rabbit gallbladder and frog choroid plexus) go from 0 up to 1. In other words an inverse relationship exists between reflection coefficients and partition coefficients. The partition coefficients of polyhydroxy alcohols decrease as hydroxymethyl groups are added[27], and this should be accompanied by an increase in σ_X. Since the reflection coefficients of pea chloroplasts increase in going from methanol ($\sigma_X = 0.00$) to ethylene glycol (0.40) to glycerol (0.63) to erythritol (0.90) to adonitol (1.00), the most reasonable interpretation is that this is simply due to the concomitant decrease in the partition coefficients of the alcohols.

Next, some of the implications of the values for the reflection coefficients of polyhydroxy alcohols determined for pea chloroplasts will be briefly considered. For instance, the finding that the reflection coefficient of glycerol is 0.63 and that of sucrose is unity[6] supports the microscopic observation of HEBER[5] that the chloroplast membranes are more permeable to glycerol than to sucrose. In place of sucrose, sorbitol[28] and mannitol[29] have also been used in chloroplast isolation media. The values found for the reflection coefficients of pea chloroplasts for sorbitol (1.02) and mannitol (1.01) strongly suggest that both sugar alcohols do not enter the chloroplasts easily, and thus such polyhydroxy alcohols can function as osmotica in suspending media for chloroplasts just as well as sucrose can ($\sigma_{\text{sucrose}} = 1.00$). In other words, the study of osmotic responses can be used to evaluate the suitability of various osmotica for the isolation and suspension of cells or organelles. The reflection coefficient of *Nitella translucens*[30] for methanol is 0.27 while that of *Chara australis*[30] for methanol is 0.30 and for ethylene glycol is 1.0. Since the σ_X's of pea chloroplasts for methanol ($\sigma_X = 0.00$) and ethylene glycol (0.40) are considerably lower, it is clear that the reflection coefficient of a given solute depends on the particular membrane being considered.

Permeation of aldoses into chloroplasts

In the case of the aldoses the inverse relationship between reflection coefficients and partition coefficients did not seem to be valid. For instance, pea chloroplasts are apparently more permeable to certain aldopentoses like D-ribose ($\sigma_X = 0.00$), D-xylose (0.43), D-lyxose (0.47) and L-arabinose (0.53) than they are to 5- and 6-carbon polyhydroxy alcohols ($\sigma_X \geqslant 1.00$ for those tested) having even higher partition coefficients. Also, the reflection coefficients of pea chloroplasts are different for the D- and L-forms of the same aldose (Figs. 2, 3, and 5), which means that chloroplasts can differentiate between optical isomers of the sugars. Since it is generally assumed that partition coefficients do not depend on optical isomerization, the permeation of these sugars into pea chloroplasts must not be governed solely by the partition of aldoses between membrane lipids and water. Thus something more than the usual physicochemical factors may be involved in the movement of sugars into or out of chloroplasts. For instance, carriers may help shuttle specific aldoses across the limiting membranes of pea chloroplasts and thereby lower the observed reflection coefficient of a certain sugar isomer compared with that of the other optical form.

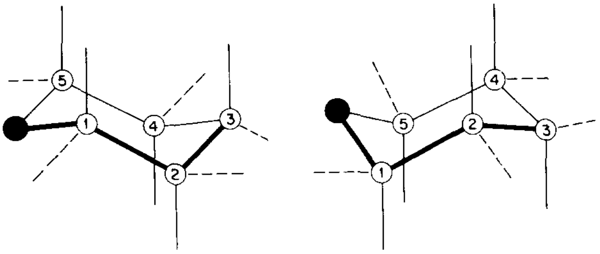
Three features that are generally observed for carriers are[8]: (1) specificity, such as being able to distinguish between the D- and the L-isomers; (2) saturation, *i.e.* the rate of entry asymptotically approaches an upper level as the solute concentration is increased; and (3) most carriers exhibit competition effects when presented with structurally similar solutes. It was observed in Fig. 4 that the osmotic volume response of pea chloroplasts was linear with the concentration of either D-ribose or D-xylose up to about 50 mM, but as either concentration was raised to 200 mM, the volume change for a given Δc_x became much greater. In fact, the increment in water efflux as either D-ribose or D-xylose was increased from 100 to 200 mM was essentially the same as for the impermeant solute sucrose. Stated another way, the carriers which had facilitated the entry of D-ribose or D-xylose at the lower concentrations appeared to become saturated at the higher concentrations. The osmotic responses of pea chloroplasts to the pairwise addition of 50 mM D-ribose *plus* 50 mM of various other aldopentoses indicate competition for common carriers, as will be considered below. Thus, evidence for carriers which can transport aldopentoses across the limiting membranes of pea chloroplasts is based on specificity, saturation, and competition phenomena. A carrier-mediated uptake occurring without an energy supply such as is proposed for the entry of certain aldoses into pea chloroplasts is known as facilitated diffusion[8,31]. The use of carriers as shuttles for such a passive process facilitates the rate of entry of a solute and thereby lowers its reflection coefficient.

The effect of optical isomerization on the reflection coefficients of pea chloroplasts for aldoses led to a re-investigation of certain of the amino acids tested previously. It had been found that the reflection coefficient of DL-valine was 0.35, of L-leucine was 0.56, and of L-isoleucine was 0.33 (ref. 10). Using the same technique, the reflection coefficients of the other optical isomers and racemic mixtures of these amino acids were measured with the following results: D-valine ($\sigma_x = 0.17$), L-valine (0.53), D-leucine (0.19), DL-leucine (0.34), D-isoleucine (0.13), and DL-isoleucine (0.25) (C.-T. WANG AND P. S. NOBEL, unpublished observations). Since the D- and the L-forms act as independent species, σ_{DL} should equal $(\sigma_D + \sigma_L)/2$, as the data for all three of these amino acids clearly indicate. Moreover, pea chloroplasts do differentiate between the D- and the L-isomers of valine, leucine, and isoleucine, suggesting that carriers for amino acids may also exist in the limiting membranes of pea chloroplasts.

Correlation between reflection coefficients and the structures of aldoses

As is the case for enzymes, carriers are known to exhibit specificity as to the particular solute transported. For instance, whether a hydroxy group on an aldopentose is in the axial or the equatorial position of the pyranose ring may well affect its ease of entering pea chloroplasts. Consequently, the structure of the neutral sugars will next be considered in a little detail.

PIGMAN AND ISBELL[32] have found that the majority of aldopentoses and aldohexoses in aqueous solutions occur almost exclusively as the α and the β anomers of the pyranose ring, with only a small quantity (< 1 %) in the furanose and the acyclic forms. (A pyranose ring is a six-membered ring containing five carbon atoms and one oxygen atom.) The pyranose ring can exist in two Sache strainless forms, *viz.* the chair and the boat conformation[32]. REEVES[33,34] has demonstrated that pyranoses prefer the chair to the boat conformation. However, owing to the presence of an oxygen atom in the ring and different substituent groups on the carbon atoms, such sugars may exist in two different chair conformations, denoted C1 and 1C (refs. 33–38):



C1 1C

where the heavy lines in the ring mark the side facing outward, the solid lines emanating from the ring are axial bonds, the broken lines are equatorial bonds, the solid circle represents the oxygen atom, and the numbered circles are the conventionally numbered carbon atoms.

The relative stability of the C1 and the 1C conformations of the pyranose ring depends on the substituent groups attached to the carbon atoms of the ring[33–38]. It has been shown that D-ribose ($\sigma_X = 0.00$ for pea chloroplasts), D-xylose (0.43), D-lyxose (0.47) and L-arabinose (0.53) are more stable in the C1 conformation[33,34,37–42]. On the other hand, D-arabinose (0.85) has been shown to be more stable in the 1C form[35,37,38], while L-lyxose (0.87) and L-xylose (0.93) are also predicted to be in the 1C form[33,34]. In other words, the four aldopentoses having the lower reflection coefficients for pea chloroplasts are all in the C1 conformation, while their optical isomers are in the 1C form. Consequently, data for the reflection coefficients of pea chloroplasts for aldopentoses suggest that those sugars in the C1 conformation are far more permeable (σ_X of 0.00 or near 0.5) than is the case for the 1C conformation (σ_X near 0.9). Such a specificity for aldopentoses in the C1 conformation has already been indicated for the transport of these sugars across membranes of the human red blood cell[43,44] and the frog choroid plexus[38]. Moreover, many enzymes are also specific for aldoses in the C1 conformation of the chair form of the pyranose ring[31].

Next, the position of hydroxy groups on the pyranose ring will be related to possible effects on the values of reflection coefficients of aldopentoses for pea chloroplasts. The position of the hydroxy group on C-1 apparently has little effect on the permeability, as can be seen by considering D-ribose ($\sigma_X = 0.00$), which is an equilibrium mixture of the α and the β anomers. Since $\sigma_{\alpha\beta}$ equals the fraction of α anomer times σ_α *plus* the fraction of β anomer times σ_β, the reflection coefficients of both α-D-ribose and β-D-ribose must be indistinguishable from 0, which is to say that the position of the hydroxy group on C-1 does not markedly affect the permeability into pea chloroplasts. The insensitivity of the reflection coefficient to the hydroxy group on C-2 can be appreciated by considering D-xylose ($\sigma_x = 0.43$) and D-lyxose (0.47), which are epimers differing only at C-2. L-Arabinose (0.53) and D-xylose (0.43) are epimers differing in the configuration of the hydroxy group attached to the C-4 of the pyranose ring. Since the reflection coefficients of these two sugars are about the same, the hydroxy group on C-4 apparently has little if any effect on the permeability of pea chloroplast membranes to C1 aldopentoses. An aldopentose has no hydroxy group on C-5, and so attention will next be directed to C-3.

One of the most striking aspects of the present results is that pea chloroplasts were much more permeable to D-ribose ($\sigma_x = 0.00$) than to the other three aldopentoses stable in the C1 conformation (L-arabinose, D-lyxose, and D-xylose). D-Ribose has the same configuration at C-5 as these other three sugars, and it was just argued that the positions of hydroxy groups on C-1, C-2, and C-4 do not exert a primary influence on the ease of penetration of C1 aldopentoses into pea chloroplasts. Consequently, interest is focused on the hydroxy group on C-3 of the pyranose ring. In fact, the hydroxy group is in the axial position and the hydrogen atom is equatorial for D-ribose, while the opposite is true for L-arabinose, D-lyxose, and D-xylose. Consequently, the shifting of this one hydroxy group causes a marked change in the permeability of the C1 aldopentoses, since the reflection coefficient goes from about 0.5 for D-xylose, D-lyxose, and L-arabinose to 0.00 for D-ribose.

The correlation between sugar structures and reflection coefficients leads to the following speculation concerning the common features of carriers that can be used for moving aldopentoses across the limiting membranes of pea chloroplasts. The carriers recognize those sugars in the chair form of the pyranose ring, specifically the C1 conformation. The positions of hydroxy groups on C-1, C-2, and C-4 have little effect on the ability of the carrier to move the sugars across the chloroplast membranes. If the hydroxy group on C-3 is in the axial position (or perhaps is simply lacking from the equatorial one), then the carrier can greatly enhance the permeability of the C1 aldopentose into pea chloroplasts. Implicit in this discussion is that one and the same carrier is involved in transporting all the aldopentoses into pea chloroplasts. Based on such a premise one would predict that competition effects should be observable between the various sugars, *i.e.* the osmotic response of pea chloroplasts to say 50 mM of a certain aldopentose should depend on whether a similar sugar was also present. If the effects of sugars on $1/(V-b)$ were independent of each other, then the data in Figs. 2 and 3 indicate that $1/(V-b)$ would be 1.09–1.12 for 50 mM D-ribose *plus* 50 mM of either D-xylose, D-lyxose, or L-arabinose (50 mM D-ribose by itself leads to no water efflux). However, under such circumstances $1/(V-b)$ was found to be 1.25–1.28, indicating nonadditivity of the osmotic responses. Specifically, each of the other C1 aldopentoses apparently competed with D-ribose for the proposed carrier and as a consequence tended to saturate the capabilities of such a carrier system (see Fig. 4). Since L-arabinose, D-lyxose, and D-xylose all competed with D-ribose, a reasonable deduction is that all four of these C1 aldopentoses can use the same carrier.

The relatively large reflection coefficients of aldohexoses for pea chloroplasts will next be briefly commented upon. Studies with animal cells have indicated that the carrier systems for aldohexoses can saturate at much lower concentrations than for the analogous pentoses (*e.g.* see refs. 38, 43). If the same situation held for the limiting membranes of pea chloroplasts, then the carriers may have been nearly saturated at the hexose concentrations employed (14–50 mM) in the present studies, resulting in reflection coefficients from 0.9 to 1.0. Alternatively, the increase in σ_X in going from an aldopentose to an aldohexose with the same configuration around the pyranose ring may be due to a steric hindrance to passage across the membrane caused by the additional hydroxymethyl group. The following series of compounds indicates the effect of this group: D-glucose (σ_X = 0.98) *vs.* D-xylose (0.43); D-mannose (0.85) *vs.* D-lyxose (0.47); and D-galactose (0.92) *vs.* L-arabinose (0.53). Such a marked decrease in the ease of penetrating the limiting membranes of pea chloroplasts is also observed when a methyl group is added to C-5, *e.g.* L-arabinose has a σ_X of 0.53, while 6-deoxy-D-galactose (D-fucose) has a reflection coefficient of 0.89. (To make these comparisons valid, D-glucose, D-mannose, and D-galactose should all occur predominantly in the C1 conformation of the chair form of the pyranose ring, as is indeed indicated to be the case[33,34,35].) A similar increase in reflection coefficients for pea chloroplasts caused by methyl groups had previously been attributed to the branch methyl groups on amino acids and methylated derivatives of acetamide[10]. As another possibility, the addition of a methyl or hydroxymethyl group on C-5 may decrease the permeation of pea chloroplast membranes by affecting the binding to the carrier used for the C1 aldopentoses.

In conclusion, it has been shown that two different properties affect the penetration of alcohols and sugars into pea chloroplasts. First, there is the partitioning of molecules between membrane lipids and the aqueous phases—the higher the partition coefficient, the easier the penetration—which is the basis of the relative permeabilities of the alcohols. Second, carriers may specifically shuttle certain aldoses into chloroplasts. In particular, carriers in pea chloroplast membranes may select those aldopentoses in the C1 conformation of the pyranose ring and apparently account for the vanishingly small reflection coefficient of D-ribose.

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