The hexose uptake system of *Chlorella*: Is it a proton symport or a hydroxyl ion antiport system?

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Received 8 March 1983; revision received 31 March 1983

2-Amino-2-deoxyglucose is taken up by the hexose transport system of *Chlorella*. The uptake of the neutral sugar proceeds together with stoichiometric alkalization of the medium. The uptake of the positively charged sugar (i.e., with a protonated amino group) proceeds as uniport and is not accompanied by net proton uptake but by charge compensating exit of proton and potassium. Thus the proton at the amino group replaces the cotransported ion, which therefore has to be a proton and not a hydroxyl ion. A depolarization of the membrane from −130 mV to −70 mV increased the K_m-value 3-fold without change of V_{max}. It is concluded that neutral sugar and proton are bound in close vicinity at a site in the middle of the membrane and that transport of sugar and proton occurs simultaneously.

H^+-symport *Glucose transport* *2-Amino-2-deoxyglucose* *Membrane potential* Chlorella

1. INTRODUCTION

There is plenty of evidence that accumulative transport of organic material by bacteria and plant cells is driven by the protonmotive potential difference [1–4]. But it is unclear if the transport of for instance sugar is coupled to the transport of protons (proton symport) or the exchange of hydroxyl ions (hydroxyl ion antiport). Both possibilities can be imagined in analogy to Na^+-coupled transport [5] or anion exchange [6]. The question whether the sugar is taken up and coupled to proton symport or hydroxyl ion antiport is important for the devise of mechanistic models at the level of protein structure.

Since the fast equilibration of water does not allow the tracing of radioactively-labeled protons, another strategy was devised: It would be proof of proton symport, if it were possible to obtain efficient uptake of a positively charged hexose analogue, of which the positive charge replaces the cotransported (respectively, counter-transported) ion. Such an analogue was found, namely 2-amino-2-deoxyglucose, which can be taken up in the positively charged form by the hexose-transport system of *Chlorella*.

2. MATERIALS AND METHODS

The alga *Chlorella vulgaris* (Beijerinck) was grown autotrophically in inorganic salt medium with CO_2-enriched air in presence of light. The algae were harvested by centrifugation after 3 days of growth, washed with water and, at a cell density of 25 μl packed cells/ml, incubated for 3 h in 25 mM sodium phosphate (pH 6.0) and glucose (1.4 mg/ml) which caused the induction of the hexose-transport system.

The uptake of hexose was normally tested by incubation of 3 ml suspension of algae at 5 μl packed cells/ml in 25 mM sodium phosphate of the desired pH-value and the addition of a mixture of radioactive hexose and non-labeled hexose. Samples of 0.5 ml were withdrawn at 20 or 30 s intervals over 2 min, rapidly filtered on 0.8-μm pore size membrane filters, washed and put into scintillation vials and counted in dioxane–naphthalene–PPO solution.

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The purity of the hexoses was proved by paper chromatography in butanol–pyridine–water–acetic acid (108:72:54:5.4, by vol.) and butanol–ethyl acetate–acetic acid–water (40:30:25:40, by vol.) and by paper electrophoresis in 2 mM ammonium formiate, pH 3.7.

The net movement of ions was followed with electrodes inserted into the algal suspension in a stirred chamber. The output was amplified and continuously recorded. For pH-measurements a pH-microelectrode of Ingold (Zürich) and for K^+ measurements a plastic membrane electrode from Philips (Eindhoven) were used. The algal suspension was normally at a cell density of around 20 μl packed cells/ml in 5 mM NaCl. No buffer was present. The ion flux was followed before and after the addition of hexose.

The depolarization of the membrane potential was achieved with incubation in 200 mM KCl. The membrane potential was measured via the uptake rate for [^{3}H]tetraphenylphosphonium ion, which is a defined function of the membrane potential [7]. 6-Deoxyglucose was purchased by Koch-Light (Colnbrook), 2-amino-2-deoxyglucose by Sigma (München); the 6-deoxy[^{3}H]glucose had been prepared by the labelling service of Amersham, 2-amino-2-deoxy[^{14}C]glucose was obtained from New England Nuclear. [^{3}H]Tetraphenylphosphonium chloride was a gift from Dr Geck (Frankfurt).

3. RESULTS

The hexose transport system of *Chlorella* is stereo-specific for D-glucose [8]; L-hexoses, methyl-glucosides, negatively charged D-glucose analogues (e.g., glucuronic acid), and positively charged glucose analogues such as 6-amino-6-deoxyglucose virtually do not compete for the glucose transport site ([9] and Schobert et al., in preparation). Only 2-amino-2-deoxyglucose can efficiently compete for the glucose transport system (fig.1). The calculated inhibition constant K_i of 2-amino-2-deoxy-D-glucose of 0.7 mM agrees very well with the K_m-value for uptake of this sugar (0.65 mM). The question is: does the uptake of 2-amino-2-deoxyglucose occur in the protonated, i.e., positively charged, form or in the neutral form? Both forms exist at neutral pH-value since the pK of the amino groups is 7.3 and 7.7,

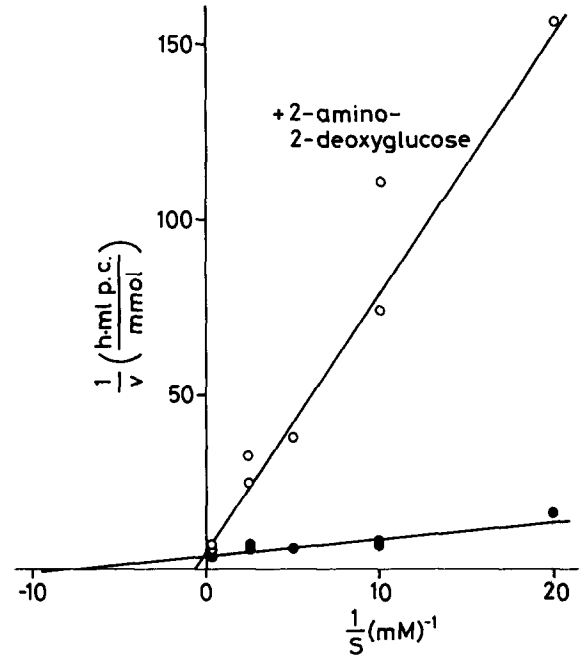

Fig.1. Lineweaver-Burk plot of 6-deoxyglucose uptake in presence and absence of 2-amino-2-deoxyglucose. The uptake of 6-deoxy[^{3}H]glucose (spec. act. 25–1500 kBq/μmol) was measured at pH 6.0; 2-amino-2-deoxyglucose was present at 10 mM.

respectively (depending on α- *vs* β-configuration [10]). If only the neutral form of 2-amino-2-deoxyglucose is taken up, the concentration giving half-maximal uptake rate should decrease drastically by increasing pH. If only the protonated form is taken up the concentration for half-maximal uptake rate should increase with increasing pH-value. As is shown in fig.2, the K_m-value decreases slightly with increasing pH-value, far less than would be predicted for the exclusive uptake of neutral glucosamine (broken line). The conclusion is that both forms of glucosamine can be taken up efficiently: at alkaline pH-value it is mostly the neutral form; at acid pH-value it is the protonated form. (There is some discrimination since the K_m-value for the neutral form is lower by about factor 3.)

The uptake of neutral 2-amino-2-deoxyglucose proceeds with the alkalization of the medium and a charge-compensating outflow of K^+ (fig.3A). The stoichiometry is about 0.6 H^+ taken up per 2-amino-2-deoxyglucose taken up. The intriguing

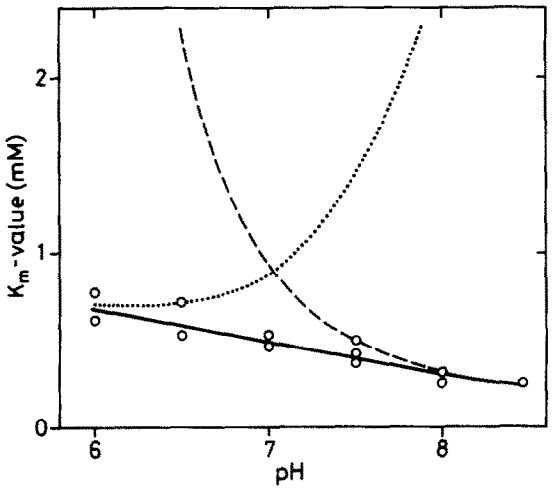

Fig.2. K_m-value for uptake of 2-amino-2-deoxyglucose at different pH-values. The broken line is the theoretically expected change of K_m-value if exclusive uptake of neutral 2-amino-2-deoxyglucose proceeds (taking the value at pH 8.5 as a fixed value). The dotted line is the theoretical curve for exclusive uptake of protonated 2-amino-2-deoxyglucose, calculated with the K_m-value at pH 6.

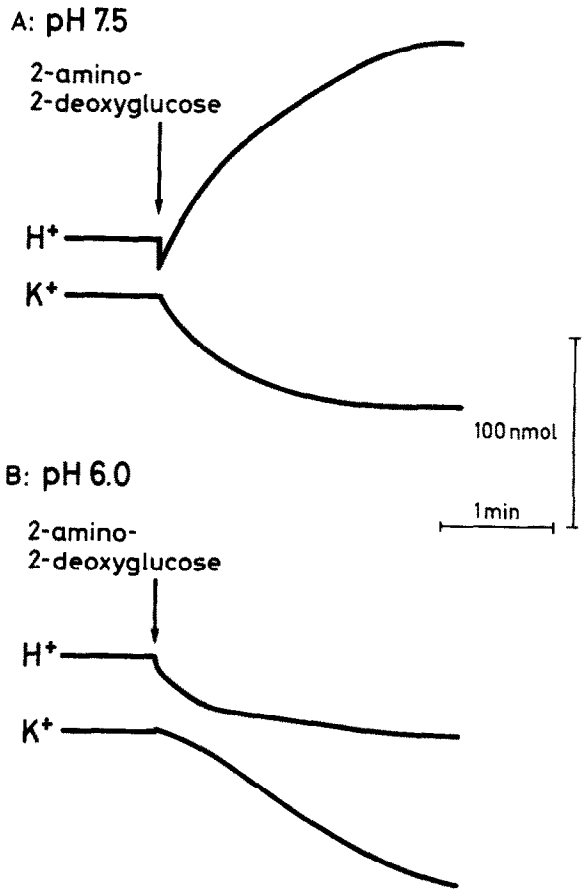

Fig.3. Change of H^+ and K^+ in the cell suspension after addition of 1 mM 2-amino-2-deoxyglucose. The cell density was 18 μl packed cells/ml in A and 16 μl packed cells/ml in B. Upward deflection means uptake, downward deflection means efflux of ions.

question now is: does the uptake of positively charged 2-amino-2-deoxyglucose proceed with alkalization as would be expected for an ion-coupled mechanism, or does the positively charged amino group of 2-amino-2-deoxyglucose substitute for the coupled transport of ion? The experiment clearly shows (fig.3B) that there is no alkalization at pH 6.0. In contrast there is short acidification and later a K^+ outflow, which together compensate for the electric charge of the protonated 2-amino-2-deoxyglucose. 0.9 cations (H^+ plus K^+) are leaving the cell per 2-amino-2-deoxyglucose taken up. A very similar response is seen if the uptake of positively charged amino acids (e.g., arginine) follows (Cho and Komor, in preparation). It must be concluded that the uptake of charged 2-amino-2-deoxyglucose proceeds as uniport.

The transport of the charged 2-amino-2-deoxyglucose is accompanied by a reduction of the membrane potential by 20–30 mV. Reduction of the membrane potential by KCl from −130 mV to −70 mV changes the K_m-value for uptake from 0.65 to 2.5 mM, but not the V_{max}-value (fig.4) for uptake. This result is very different from the result with 6-deoxyglucose uptake, where no change of K_m-value was observed ([11], 0.20 and 0.22 mM, respectively) but it is similar to the effect on K_m-value for proton-binding (0.028–0.089 μM, [12]).

4. DISCUSSION

2-Amino-2-deoxy-D-glucose can be transported in the charged form; i.e., with the amino group protonated. In this case no proton uptake is observed. The interpretation is that charged 2-amino-2-deoxyglucose binds at that site of the transport protein which is normally occupied by glucose plus proton. There are two conclusions:

(i) Since it is difficult to imagine that a site for hydroxyl ion binding can be substituted by a

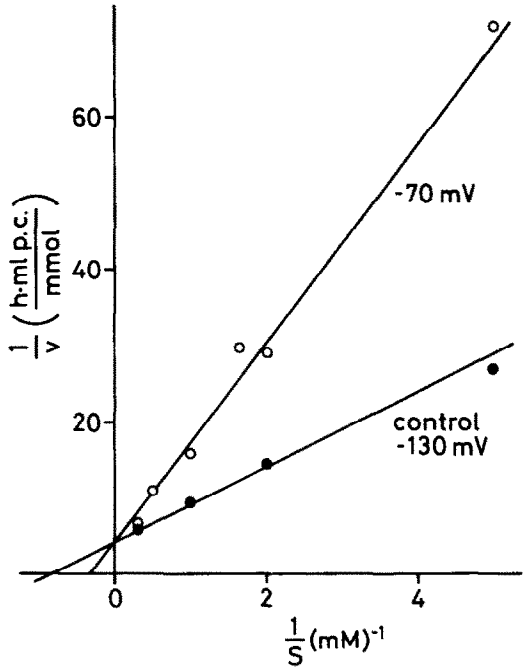

Fig.4. Lineweaver-Burk plot of 2-amino-2-deoxyglucose uptake at control conditions and at reduced membrane potential. The membrane potential of control cells is −130 mV, the reduced membrane potential is −70 mV.

positively charged amino group, hexose transport by *Chlorella* apparently works as proton symport and not as hydroxyl ion antiport. Perhaps it is a histidine which had been found previously at an essential site for hexose transport [1] that is the proton binding site.

(ii) Hexose and proton are transported in close vicinity, whereby the proton is bound very near to the C_2 of the pyranose ring. This close vicinity could easily allow coordinated stoichiometric flow of both molecules. Models which postulate independent, only indirectly connected transport of sugar and ion [14], are therefore unlikely for *Chlorella*. Thus glucose proton-symport resembles arginine uniport, where the guanidino group has to be directly protonated [15].

The effect of membrane potential on proton binding [12] had been interpreted previously on the basis of a proton-conducting path [16] along which the protons are accumulated at the binding site. The binding site for protons had been suggested in the middle of the membrane [11]. If the same interpretation is used for the effect of membrane potential on uptake of 2-amino-2-deoxyglucose, and considering the close vicinity of proton and hexose binding, the sugar binding site is also in the middle of the membrane. (Since a depolarization of the membrane by 60 mV causes a 3-fold change of K_m-value, the site where charged 2-amino-2-deoxyglucose is enriched and presumably bound is the one at which the transmembrane potential has declined to half of its value.)

The involvement of protons (and not hydroxyl ions) has not been proven for other systems which generate or rely on protonmotive potential difference. But the occurrence of a free carboxyl group as an essential part of the ion conducting path in F_o of mitochondrial and bacterial ATPase [17] is suggestive of proton to be conducted. For transport of organic solutes the hypothesis of close transport Na^+ and amino acid had been offered to explain amino acid-dependent Na^+-stoichiometries [18].

ACKNOWLEDGEMENTS

This work had been supported by Deutsche Forschungsgemeinschaft. The critical reading of the manuscript by G. Orlich is gratefully acknowledged.

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