A POSSIBLE MECHANISTIC ROLE OF THE MEMBRANE POTENTIAL IN PROTON–SUGAR COTRANSPORT OF *CHLORELLA*

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1. Introduction

The electrochemical potential gradient of ions such as Na^+ or H^+ can drive active uptake of sugars in a variety of cells. Na^+ coupled non-electrolyte transport mainly occurs in animal cells [1], whereas H^+ coupled transport is predominant in bacteria and plant cells [2]. The unicellular green alga *Chlorella vulgaris* takes up hexoses such as glucose or 6-deoxyglucose together with protons in 1:1 stoichiometry [3]. The translocation of hexose plus proton by means of the transport catalyst (carrier) is electrogenic, i.e., without charge compensation, as revealed by a rapid transient depolarization of the membrane potential [4]. The more than 1000-fold accumulation of the non-metabolizable sugar 6-deoxyglucose can be thermodynamically explained by the electrochemical potential gradient of protons, composed of a pH gradient and an electrical membrane potential [4]. Not understood, however, is the question how the pH gradient and electric potential act mechanistically to bring about the sugar accumulation observed. A possible role for the pH gradient has been suggested for *Chlorella,* since a more than 100-fold decrease of K_m value for 6-deoxyglucose uptake was observed by protonation of the transport system, whereas the V_{max} value stayed comparably constant [5]. The more alkaline pH of the cell interior will shift the K_m value for 6-deoxyglucose at the inside in a way which reduces the exit reaction, resulting in a rise of internal sugar concentration until efflux balances influx [5].

The mechanistic role of the electric potential, which generally comprises the major part of the electrochemical potential difference for protons, is unknown. In artificial lipid films increased voltage across the membrane accelerates the transfer rate of charged compounds [6]; the same can occur with ionophore-catalyzed cation transport in artificial membranes [7]. Therefore, it is generally assumed that the maximal velocity of solute uptake should be sensitive to changes in membrane potential. Model calculations are based on this assumption and revealed a complex interference of electric potential with V_{max} and K_m values [8] and experiments with pigeon red cells seem to prove it [9]. In contrast the maximal rate of hexose uptake at pH 6.0 in *Chlorella* exhibited no dependance on electric membrane potential over a considerable range. But an increase of membrane potential, inside negative, apparently facilitates the protonation of the transport catalyst, since lower H^+ concentrations are required for maximal sugar uptake activity when measured between pH 6–8. This result can be quantitatively explained by a membrane model where the proton-accepting group of the 'carrier' is at the bottom of a proton-conducting pore, which spans approximately half of the membrane.

2. Materials and methods

6-Deoxyglucose was delivered by Koch-Light, Colnbrook (England) and tritiated by Radiochemical Center, Amersham. The strain of *Chlorella vulgaris* and its growth conditions were the same as in [10].

The hexose uptake system was induced in 25 mM sodium phosphate pH 6.0 and with 1.4 mg glucose/ml at a cell density of 25 µl packed cells/ml. After an

incubation period of 2–3 h the cells were induced and glucose had been used up.

The hexose uptake activity was tested in 25 mM sodium phosphate buffer of appropriate pH value at a cell density of 12 μl packed cells/ml with 1 mM 6-deoxy[^{3}H]glucose for the determination of the pH dependance of uptake, and with 10 mM for determining maximal velocity (spec. act. 0.022 μCi/μmol). Samples were withdrawn in 30 s intervals, rapidly filtered and extracted; part of the extract was then counted by liquid scintillation technique. For manipulation of the membrane potential the cells were incubated with different concentrations of potassium citrate, and for osmotic control with glucose-free mannitol. The electric membrane potential was determined via the tetraphenylphosphonium chloride distribution in the same medium composition as the sugar uptake tests as in [4].

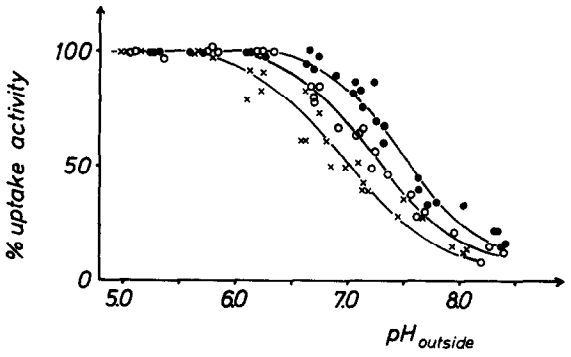

Fig.2. pH Dependance of 6-deoxyglucose influx at three different membrane potentials. The uptake of 6-deoxyglucose was tested at conc. 1 mM. The 3 titration curves were obtained: without potassium citrate (membrane potential −135 mV, midpoint pH 7.54) (•——•); with 30 mM potassium citrate (membrane potential −105 mV, midpoint pH value 7.30) (○——○); with 200 mM potassium citrate (membrane potential −74 mV, midpoint pH 7.01 (×——×).

3. Results

The electric potential at the cytoplasmic membrane (inside negative) can be imagined as a driving force for the sugar–proton complex of the transport catalyst. A reduction of the electric potential would be expected to reduce the translocation rate. The experiments, however, reveal no dependance of the rate of initial 6-deoxyglucose influx on membrane potential between −130 mV and −130 mV (fig.1).

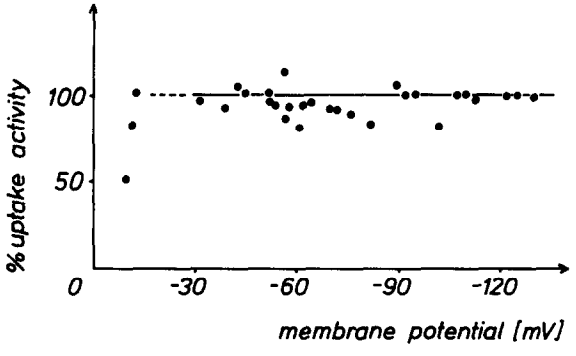

Fig.1. Maximal uptake rate for 6-deoxyglucose at pH 6.0 and at different membrane potentials. The uptake rate was tested with 10 mM 6-deoxyglucose in the presence of up to 200 mM potassium citrate for manipulation of the membrane potential. The rates were corrected for the osmotic effects of the salt added.

When high concentrations of potassium citrate were used to depolarize the membrane potential (e.g., 0.2 M) about 30% inhibition was observed which could be simulated however by an iso-osmolar amount of mannitol; this inhibition thus appears to result from osmotic effects and not from depolarization. Mannitol itself does not change the membrane potential. Therefore the uptake rates were always compared with control cells with mannitol present. (The failure of proper osmotic controls led formerly to the wrong interpretation of an influx depression caused by salt [3].) Since the maximal velocity of sugar uptake seems to be insensitive to changes in membrane potential in a range, where membrane potential certainly has to contribute to sugar accumulation, mechanistically the electric force must act in an other way. It seemed possible that the electric force facilitates the protonation of the transport catalyst at the outer side of the cytoplasmic membrane.

The sugar transport catalyst of *Chlorella* can function in either the protonated form with low K_m value or in the deprotonated form with high K_m value(s). The titration curve for protonation of the 'carrier' seems similar to the general pH spectrum of sugar transport activity (at least at the alkaline side) when tested at sugar concentrations of 1 mM where only the low K_m system responds. Thus the pH dependance

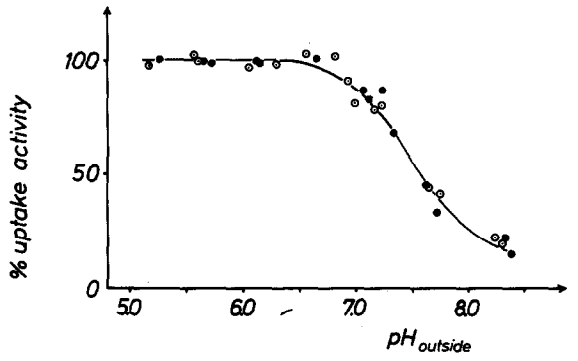

Fig.3. pH-Dependance of 6-deoxyglucose influx with 600 mM mannitol present. The sugar uptake was tested with 1 mM 6-deoxyglucose. The 2 experiments indicate: without mannitol (membrane potential −135 mV, midpoint pH 7.54) (•——•); with 600 mM mannitol (membrane potential −132 mV, midpoint pH 7.54) (⊙——⊙).

of initial 6-deoxyglucose uptake was tested at different membrane potentials, i.e., in the presence of different concentrations of potassium citrate. Indeed there is a significant shift in the pH spectrum to the acid region by reduction of the electric potential (fig.2). For example, the depolarization from −135 mV to −74 mV changes the midpoint pH value (i.e., the pH value where just 50% of activity is reached) from pH 7.54–7.01. It should be noted that the pH spectrum can vary for different batches of cells, therefore these experiments were performed always in parallel with the same batch of algae. The shift of the pH titration curve seems really due to the depolarization of the electric potential since the addition of mannitol with an osmotic strength identical to that of potassium citrate had no effect (fig.3).

4. Discussion

The effect of the membrane potential on the protonation of the 'carrier' molecule might be attributed to some conformational changes in the membrane. A more simple explanation, however, is offered by the assumption that the proton-accepting site of the 'carrier' molecule is located in the interior of the membrane having access to the outside by a proton-conducting channel. The electric field across the membrane will draw in the positively charged protons with the result of a more acid pH value at the carrier site than in the bulk phase of the medium. At depolarized conditions less protons will be concentrated in the pore and a more acidic pH value of the medium is needed to obtain a sufficiently high proton concentration at the 'carrier' site. The depth of such a proton channel can be roughly estimated: a depolarization of 61 mV causes the midpoint pH value to shift by 0.53 units. Since an electric potential of 60 mV corresponds to a chemical potential of 1 pH unit the protonated group of the carrier should be located at a place where the electric potential has fallen half of its value. Assuming a linear decrease of the electric field across the membrane this would correspond to half of the membrane diameter. The proposal of a proton-conducting channel has been brought up before, since the membrane-integrated part of the ATPase of mitochondria, chloroplasts and bacteria (the so-called F_0) has been suggested to be a proton well through which protons might be concentrated at the catalytic site of the ATPase [11].

The concept of a proton-conducting pore would also explain the observation that a significant sugar accumulation still takes place at alkaline pH values where apparently no pH gradient exists anymore [5]. The cells nevertheless possess a considerable electric potential under these circumstances [4].

The results are interpreted on the basis of transmembrane potential changes, which are measured by the method of lipophilic ion distribution. But since the depolarization could only be achieved by addition of high salt concentrations also the surface potential at the outside surface of the membrane might have changed. Increased salt concentrations will reduce the generally negative surface potential of biological membranes, so that the access of positively charged ions onto the membrane will be hindered [12]. This possibility cannot be excluded since presently there seems no reliable method at hand to measure surface charges on membranes of intact, cell wall enclosed cells.

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