PROTON-COUPLED HEXOSE TRANSPORT IN *CHLORELLA VULGARIS*

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1. Introduction

Mitchell [1] has proposed that active transport of non-electrolytes is directly energized by a proton gradient which is maintained by cell metabolism. The permease is supposed to catalyze the transport of a sugar molecule together with a proton, 'proton symport', whereby the sugar is accumulated depending on the extent of the proton gradient or membrane potential. For the β-galactoside transport by *Escherichia coli* evidence sustaining this theory exists [2,3].

Experiments have now been made to see if this concept also applies to eucariotic organisms. When 6-deoxyglucose, a sugar actively transported, but not metabolized by *Chlorella vulgaris*, was added to a suspension of algae, an immediate alkalinization of the external medium was observed. This sudden pH-change shows the same characteristics as the transport of the sugar with respect to specificity, saturation behaviour and energy demand.

2. Methods

The green alga *Chlorella vulgaris* was grown and adapted as described previously [4]. For pH-measurements 600 μl packed cells of washed induced algae in 5.5 ml destilled water were aerobically incubated and stirred in a glass beaker with a pH-electrode (Lot–205 M 5, Ingold) therein. The solution was connected with a reference electrode by a salt bridge with 10^{-3} M KCl. The pH of the suspension of algae was adjusted to pH 6.5 by sodium hydroxide and then the experiment was started by addition of 20 μl 1 M sugar. For anaerobic conditions the algae were incubated in glass cuvettes with a sinter glass bottom through which N_2 was supplied continuously [5].

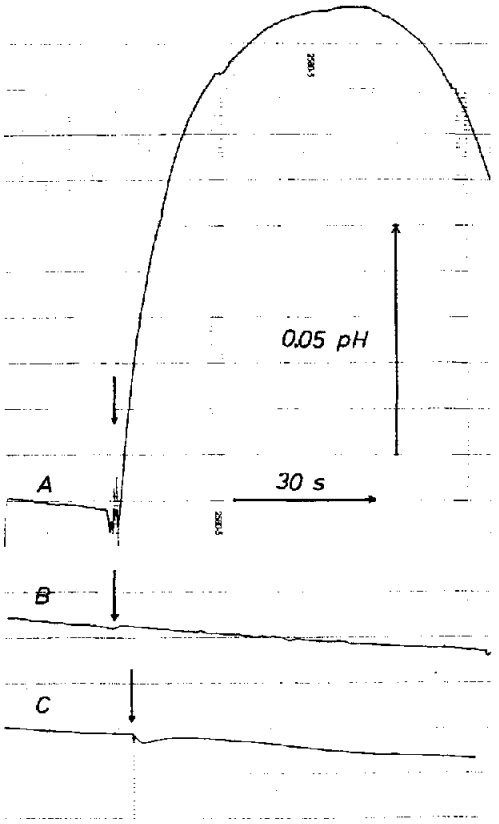

Fig. 1. pH-change of the external medium by addition of 6-deoxyglucose (arrow) to a suspension of *Chlorella* cells under aerobic condition. (A) induced algae; (B) not induced algae; (C) induced algae, but α-methylglucoside instead of 6-deoxyglucose added.

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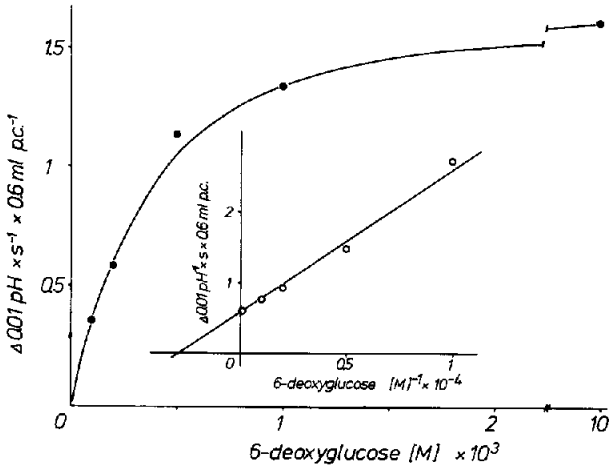

Fig. 2. K_M of the velocity of the initial pH-change with increasing 6-deoxyglucose concentrations (p.c. stands for packed cells).

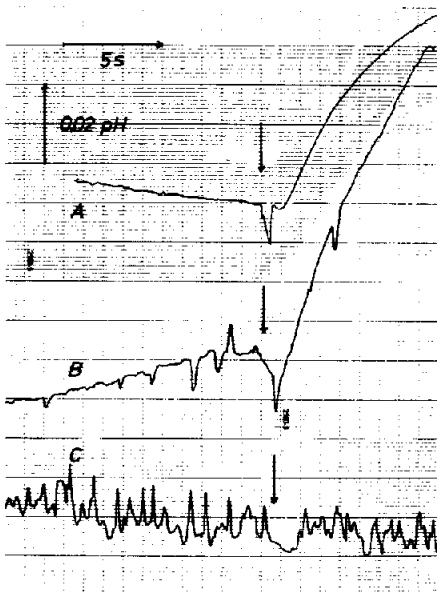

Fig. 3. pH-change of the external medium by addition of 6-deoxyglucose (arrow). (A) aerobic condition, dark; (B) anaerobic condition plus light (9000 erg·cm^{-2}·sec^{-1} at 712 nm); (C) anaerobic condition, dark. In samples B and C, DCMU 2 × 10^{-6} M was present.

3. Results

When 6-deoxyglucose is added to the aerobic cell suspension an immediate transient pH rise of more than 0.1 pH-unit is observed; the transient lasts about 60 sec (fig. 1A). This alkalinization of the external medium can be obtained only with algae induced for the hexose uptake system, but not however with non induced algae (fig. 1B). Furthermore only transportable sugars gave the pH-change, and not sugars such as α-methylglucoside [6], which are not transported (fig. 1C). The velocity of the pH-change was dependent on the concentration of 6-deoxyglucose added to the algae and showed saturation behaviour with respect to sugar concentration (fig. 2). A K_M of the initial speed of the pH-change could be determined for 6-deoxyglucose (3.1 × 10^{-4} M) and this is identical to the K_M for uptake of this sugar (3 × 10^{-4} M) [6]. Thus the sugar stimulated uptake of protons under aerobic conditions correlates well with the uptake of hexoses [6, 7].

When the buffer capacity is considered the protons are transported with a stoichiometry of about 1 proton per 6-deoxyglucose. The sugar stimulated uptake of protons, i.e. alkalinization of the external medium, should be transport-specific but independent of the energy driving the transport. Thus 6-deoxyglucose taken up under anaerobic conditions in the light [5] should also be accompanied by proton uptake. In fig. 3B it can be seen that this is the case; the same velocity of pH-change is observed under these conditions as under aerobic ones (fig. 3A). It can be excluded that oxygen, produced by photosynthesis is responsible for the effect, since the algae were irradated with far-red light (712 nm). In addition DCMU (2 × 10^{-6} M), which completely inhibits photosynthetic O_2-evolution was present. Under anaerobic conditions in the dark no pH-change is observed, which is expected, since the rate of sugar uptake under these conditions is only one tenth of that under aerobiosis. A small pH-change might be hidden by the scattering of the recorded line (fig. 3C).

4. Discussion

The results presented here are most easily explained by the assumption that protons are co-transported (or hydroxyl ions counter-transported) with

6-deoxyglucose, such corroborating Mitchell's theory. The immediate sugar-specific alkalinization of the external medium was observed when respiration or when photosynthesis was driving sugar uptake. It is improbably, therefore, that changes of the pH-gradient brought about by organelles (mitochondria or chloroplast) and induced by the energy demand for sugar transport, has caused the apparent proton uptake from the medium, since pH-change generated by the chloroplast membrane is opposite to that of the mitochondrion [8, 9]. The stoichiometry of protons taken up per sugar taken up seems equal under respiratory and photosynthetic conditions.

An apparent sugar-induced proton uptake might also occur when sugar uptake is energized by cation antiport or anion symport, which in turn would induce proton uptake for charge equilibration. However no effects, at least of potassium or sodium ions, on sugar uptake activity have been observed so far in *Chlorella* [7]. If the accumulation of sugar is due to proton symport, a proton gradient must be maintained through the cytoplasmic membrane. This could be achieved by a membrane ATPase, which is pumping protons out of the cells. In *Chlorella* inhibitor studies indicate however that ATP might not be involved in sugar uptake and another proton pump has been postulated therefore [10]. During light driven uptake a different mechanism of proton gradient generation would have to operate [10].

This communication also indicates that in an eucariotic cell the transport of non-electrolytes can be coupled to proton movement.

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