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Essential Sulfhydryl Group in the Transport-catalyzing Protein of the Hexose-Proton Cotransport System of *Chlorella* [1]

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ABSTRACT

The polyene antibiotic nystatin transforms the sugar-proton cotransport system of *Chlorella* to a mere facilitated diffusion system. This experimental condition was used to test the sugar-translocating unit of the active uptake system for possible essential sulfhydryl groups. It could be shown that the catalyzed translocation of sugar is sensitive to the sulfhydryl-reactive compound N-ethylmaleimide. Sugar flow by passive leak as induced by the detergent Triton X-100 is not affected by sulfhydryl reagents. These results show that the sugar-translocating carrier protein possesses a sulfhydryl group, which is essential for its function.

The conclusion that nonelectrolyte uptake requires an essential —SH group (1–3, 5, 6, 12–15) has been based on the observation that —SH poisons block uptake. This is ambiguous, however, since for all cotransport systems the entire uptake process depends on the undisturbed function of both the energy-delivering machinery (like an ATPase) and the actual substrate-translocating device (*e.g.* the so-called "carrier"). A decrease in energy level, *e.g.* in the proton gradient, can reduce the rate of substrate translocation so that uptake even for concentration equilibration is slowed down (7, 8). Therefore, only in very few cases could the action of —SH poisons be clearly located at the substrate-translocating protein of the uptake system (5, 12).

In *Chlorella* the function of the hexose-translocating unit is strongly dependent on the electrochemical potential difference of protons (10, 11); the addition of energy poisons virtually halts sugar translocation so that neither influx nor efflux of sugar proceeds (8). When subjected to nystatin, cells which had accumulated 6-deoxyglucose lose the accumulated sugar down to concentration equilibrium. This efflux of 6-deoxyglucose does not occur by simple leakage through "holes" but is still mediated by the translocation system (9). This conclusion is based on the following observations: (*a*) the efflux of sugar follows the same zero order kinetic with the same K_m value as carrier-mediated efflux by untreated cells; (*b*) in the presence of nystatin influx of 6-deoxyglucose can be observed until concentration equilibrium is reached. This influx proceeds only in induced cells and is completely inhibited by glucose; (*c*) the addition of radioactive 6-deoxyglucose to nystatin-treated cells with high internal nonradioactive sugar causes a transient accumulation of labeled sugar. The phenomenon, known as overshoot, can be regarded as the most decisive evidence for facilitated diffusion (19).

The sugar translocation of nystatin-treated cells is no more sensitive to energy poisons such as uncouplers. The antibiotic nystatin thus apparently disconnects the strict coupling between sugar translocation and energy metabolism and hexoses then are transported by a mere facilitated diffusion system.

This experimental condition was used to test the sensitivity of the translocating unit of hexose transport toward the —SH reagent N-ethylmaleimide (16, 18).

MATERIALS AND METHODS

Growth and Induction of Uptake System. The green alga *Chlorella vulgaris* was grown autotrophically in inorganic medium (17). The cells were induced for hexose uptake by incubation in 25 mM Na-phosphate (pH 6) in the presence of 1.4 mg of glucose/ml. After 2 to 3 hr the glucose was used up and the cells stayed induced for several hr.

Preloading of Cells and Efflux of 6-Deoxyglucose. The induced cells were incubated in 1 mM [^{3}H] 6-deoxyglucose of specific radioactivity 0.09 μCi/μmol for 3 hr, then centrifuged, washed, and resuspended in 25 mM Na-phosphate buffer (pH 6). The efflux experiment was performed in the same buffer and at a cell density of about 20 μl of packed cells/ml. Nystatin or Triton X-100 was added in small volumes and the efflux was monitored as radioactivity appearing in the medium. At intervals aliquots were quickly centrifuged and part of the supernatant was counted by liquid scintillation technique.

Materials. 6-Deoxyglucose was a product of Koch-Light, Colnbrook (England) and was labeled by Radiochemical Centre Amersham (England). Nystatin (mycostatin), Triton X-100, and N-ethylmaleimide were delivered from Serva, Heidelberg (FRG).

RESULTS AND DISCUSSION

Cells of *Chlorella* were preloaded with radioactive 6-deoxyglucose up to an accumulation factor of about 20 Then the radioactive

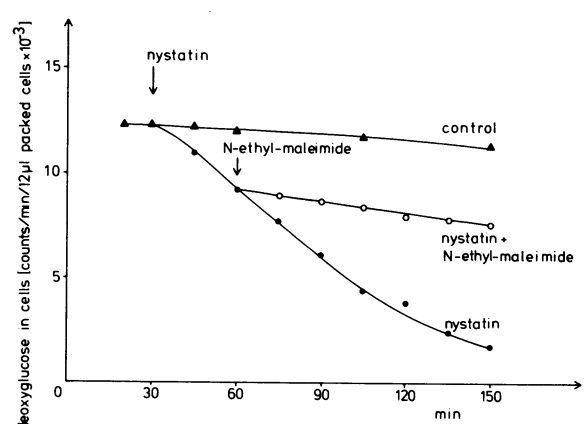

FIG. 1. Efflux of 6-deoxyglucose initiated by the addition of nystatin. The cells had been preloaded with 1 mM labeled 6-deoxyglucose (specific radioactivity 0.09 μCi/μmol) for 3 hr, the external medium was then removed and exchanged by buffer. Nystatin was added to a final concentration of 2.5 μg/ml, N-ethylmaleimide to a concentration of 50 μM.

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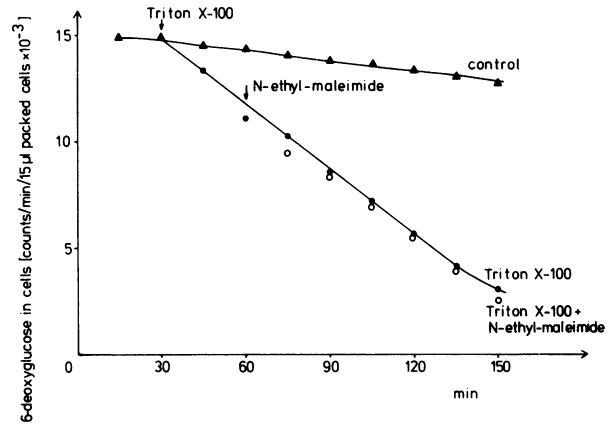

FIG. 2. Efflux of 6-deoxyglucose initiated by the addition of Triton X-100. The cells had been preloaded with 1 mM labeled 6-deoxyglucose (specific radioactivity 0.09 μCi/μmol) for 3 hr, the external medium was then removed and exchanged by buffer. Triton X-100 was added to give a final concentration of 0.06% and N-ethylmaleimide to a concentration of 50 μM.

medium was removed by centrifugation and replaced by buffer. After the addition of nystatin a considerable efflux of 6-deoxyglucose is observed, in contrast to the control with buffer alone. Energy poisons or uncouplers like trifluoromethoxycarbonyl cyanide phenyl hydrazone can slightly accelerate the nystatin-induced efflux (9). The addition of the —SH-reactive compound N-ethylmaleimide, however, virtually stops the efflux reaction (Fig. 1). This is evidence that the sugar translocating device of the cell needs a —SH group for undisturbed function.

On the other hand, this result can be considered as additional evidence that sugar translocation across the cell membrane in the presence of nystatin does require the carrier and does not proceed through holes. So far this has been mainly deduced from the countertransport behavior of nystatin-treated cells (9).

To prove that N-ethylmaleimide does not affect a true non-catalyzed substrate efflux—*e.g.* one caused by a general decrease of the cell membrane permeability—the following control experiment has been carried out. An amount of Triton X-100 was added to [^{3}H] 6-deoxyglucose-preloaded *Chlorella* cells sufficient to cause approximately the same rate of efflux as has been observed with nystatin. As can be seem from Figure 2 N-ethylmaleimide does not affect Triton X-100-induced 6-deoxyglucose efflux. Also, the efflux of hexose brought about by the action of other detergents such as SDS, deoxycholate, and others was not inhibited by N-ethylmaleimide. The conclusion is justified, therefore, that the inhibition of sugar translocation by N-ethylmaleimide in nystatin-treated cells is due to the blockage of —SH groups of the carrier protein.

The existence of a —SH group essential for the function of the sugar-translocating protein comprises a small step toward a more complete understanding of the chemistry of the transport-catalyzing protein. In addition this knowledge might serve, however, as a further tool to identify this protein in membrane extracts; the first isolation of a sugar transport-catalyzing protein has been performed with differential labeling with N-ethylmaleimide (5). In *Chlorella*, a plasma membrane-integrated protein, the synthesis of which is induced by hexoses, was isolated by a double labeling technique (4), and it has been assumed that the induction of this protein is the cause of the concomitant induction of the sugar uptake activity of the cells.

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