The lactose carrier of *Escherichia coli* functionally incorporated in *Rhodopseudomonas sphaeroides* obeys the regulatory conditions of the phototrophic bacterium

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Rhodopseudomonas sphaeroides was provided with the ability to transport lactose via conjugation with a strain of *Escherichia coli* bearing a plasmid containing the lactose operon (including the lac Y gene, coding for the lactose carrier or M protein) and subsequent expression of the *lac* operon in *Rps. sphaeroides* (Nano, F.E. and Kaplan, S. submitted). The initial rate of lactose transport in *Rps. sphaeroides* was studied as a function of the light intensity and the magnitude of the proton-motive force. The results demonstrate that lactose transport is regulated by the rate of cyclic electron transfer in the same way as the endogenous transport systems.

Allosteric regulation *Conjugation* Escherichia coli *Lactose carrier* Rhodopseudomonas sphaeroides *Solute transport*

1. INTRODUCTION

In the phototrophic bacterium *Rhodopseudomonas sphaeroides*, in addition to a proton-motive force necessary for energization of solute transport, the rate of cyclic electron transfer regulates the rate of uptake of solutes like alanine [1], potassium [2] and succinate. In cells, incubated anaerobically in the dark, no uptake of alanine is measurable, in spite of the presence of a proton-motive force of about -100 mV. Illumination of such a suspension initiates cyclic electron transfer and allows solute uptake to proceed. The uptake rate of alanine increases with the light intensity [1].

Changes in the magnitude of the proton-motive force under these conditions (i.e., upon illumination) can occur in either direction: under conditions where most proton-motive force-consuming processes are functional a decrease occurs (see also section 4) whereas inhibition of these processes by treatment of the cells with dicyclohexylcarbodiimide and/or EDTA gives rise to a light-dependent increase in the magnitude of the proton-motive force.

Lactose transport via the M-protein in *Escherichia coli* has been studied extensively. It has been demonstrated that:

(i) Lactose is taken up in symport with 1 [3–5] or 2 [3,6] protons;
(ii) The presence of a potassium diffusion potential is sufficient to energize lactose accumulation not only in membrane vesicles [7] but also in liposomes in which the purified M-protein has been reconstituted [8], thus proving that a proton-motive force alone under these conditions is sufficient to allow lactose accumulation;
(iii) The rate of lactose uptake increases quadratically with the proton-motive force [9] and

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(iv) The redox state of essential dithiol groups in the carrier determines the functional activity of the carrier via a large change in the affinity of the carrier for its substrate [10].

Recently, a strain of *Rps. sphaeroides* has been constructed in which the lactose operon from *Escherichia coli* is expressed [11]. Such a mutant allowed us to study the properties of lactose transport in *Rps. sphaeroides* and to compare these properties with those of lactose transport in *E. coli*. Here we report that the lactose-transport carrier, when expressed in *Rps. sphaeroides* is subject to the same regulation as the endogenous solute transport systems in this organism.

Kinetic analysis of the changes in the initial rate of lactose uptake indicates that the regulation is due to a light-dependent change in the number of active carrier molecules in the membrane. These results are discussed in the light of similarities with the regulation of the activity of the chloroplast kinase that phosphorylates the light-harvesting complex II.

2. MATERIALS AND METHODS

2.1. *Construction of a* Rps. sphaeroides *strain expressing the lactose operon from* E. coli

Strain L39 is a *Rps. sphaeroides* 2.4.1 derivative harboring plasmid pUI31, and is unable to grow on glucose or lactose. Plasmid pUI31 is a recombinant R751::Mu dl(Ap^R, *lac*, Km^R) plasmid ([11] and Nano, F.E. and Kaplan, S., submitted). The *lac* operon expression on pUI31 is under the control of an unknown plasmid promoter.

2.2. *Harvesting and pretreatment of the cells*

Rps. sphaeroides L39 [2.4.1. glc° lac° (pUI31)] was grown anaerobically at a high light intensity at 30°C [12] in the medium described in [13], and containing kanamycin (20 μg/ml). After overnight growth the cells were harvested at an A_{660} of 2.3, washed twice with a buffer containing 50 mM potassium phosphate (pH 8), 5 mM $MgSO_4$ and 50 μg/ml chloramphenicol. The cells were stored at 4°C in the dark at 10 mg protein/ml. Protein was determined as in [14].

2.3. *Transport assays and measurements of the membrane potential*

Initial rates of solute uptake (lactose or alanine) and the transmembrane electrical potential ($\Delta\psi$) were measured simultaneously under anaerobic coditions at pH 8, in the buffer described above and at 30°C, in a thermostated vessel (maximal volume 5 ml) in which a tetraphenylphosphonium (TPP^+) sensitive electrode was inserted [1]. Maximal light intensity (100%) equals 2000 $J \cdot m^{-2} \cdot s^{-1}$. The cells were present at about 1 mg protein/ml.

2.4. *Calculations*

The magnitude of the membrane potential was calculated according to the Nernst equation. A correction for TPP^+ binding to the cells as described in [15,16] was applied. For determination of the amount of bound TPP^+, cells were de-energized by treatment with 1% (v/v) toluene for 1 h at 37°C.

2.5. *Materials*

[D-*glucose*-1-^{14}C]Lactose (8.5 Ci/mol) and L-[^{14}C]alanine (10 Ci/mol) were obtained from the Radiochemical Centre (Amersham). All other materials were of analytical grade.

3. RESULTS

Solute transport in *Rps. sphaeroides* requires in addition to a proton-motive force, (cyclic) electron transfer [1]. Such a requirement has not been observed in other bacteria like *E. coli*. It was therefore of interest to compare the properties of the *E. coli* transport system for lactose, incorporated in *Rps. sphaeroides* L39 with those of the endogenous transport system for alanine.

Fig.1 shows that the dependence on light intensity of the uptake of the two solutes is very similar. For lactose the uptake rate is very low in the dark and a significant increase in this rate is observed at the same light intensity at which the rate of alanine uptake increases significantly (13%).

To study the relation between the rate of lactose uptake and light intensity in more detail and to relate those results to changes in $\Delta\psi$ the experiment described in fig.2 was performed. At various light intensities (0–100%) the initial rate of lactose uptake was measured simultaneously with the $\Delta\psi$. A very low rate of lactose uptake is obtained in the dark (<0.01 nmol lactose·mg protein^{-1}·min^{-1}). This rate increases sigmoidally with light intensity. Saturation is observed at 66% of the maximal light

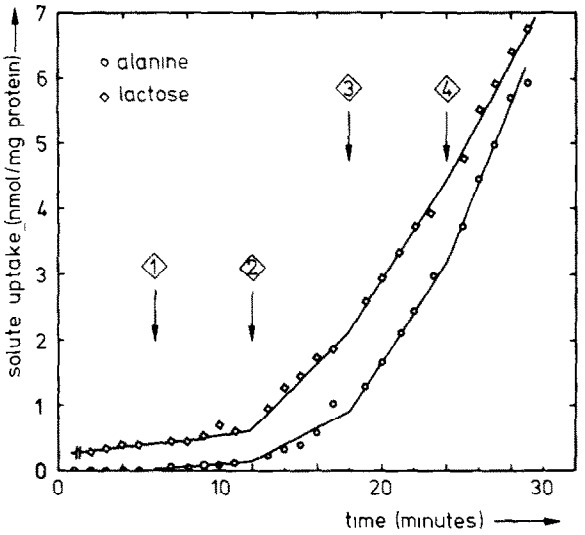

Fig.1. Comparison of lactose and alanine uptake at increasing light intensities in *Rps. sphaeroides* L39. Simultaneously, lactose or alanine uptake and $\Delta\psi$ were measured as described in section 2. Alanine, lactose and TPP^+ were used at a concentration of 50, 195 and 4 μM, respectively. At the arrows the light intensity was stepwise increased from 0 to 6 (1), 13 (2), 23 (3) and 34% (4) of the maximal light intensity.

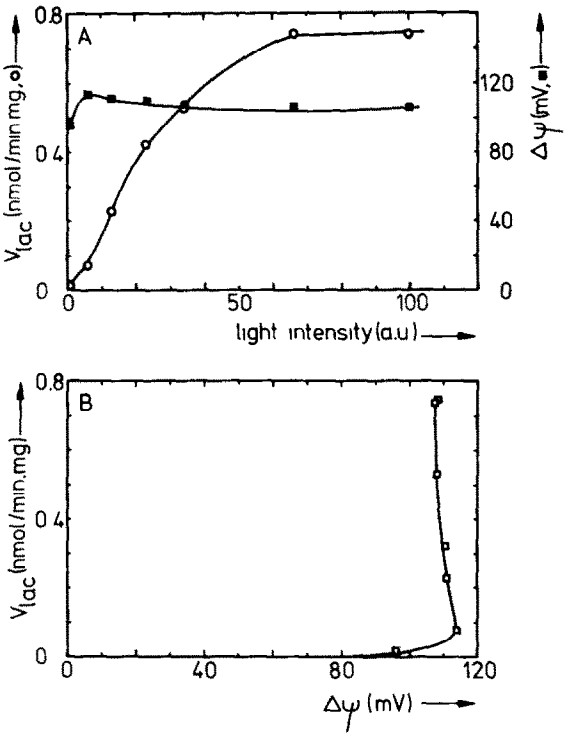

Fig.2. The initial rate of lactose uptake and the magnitude of $\Delta\psi$ as a function of the light intensity in *Rps. sphaeroides* L39. The conditions during the measurements were as described in fig.1, except that for each light intensity a separate sample was used to start the uptake measurements. (A) Initial rate of lactose uptake and $\Delta\psi$ as a function of the light intensity. (B) Replotting of the data in the form of a flow–force relation.

intensity. In the dark a $\Delta\psi$ of -96 mV exists, whereas the pH gradient is zero at this pH [17]. The increase in light intensity gives rise to only a very small (<20 mV) increase in $\Delta\psi$ at 6% of the maximal light intensity, with subsequently a decrease of about 10 mV until the maximal intensity of illumination is reached. The rate of lactose uptake as a function of the magnitude of the $\Delta\psi$ gives the flow-force relation, shown in fig.2B. Such a graph strongly suggests that in addition to the $\Delta\psi$ (an)other parameter(s) is(are) involved in the regulation of the rate of lactose transport. The mechanistic basis of this regulation can be investigated via an analysis of the kinetics of lactose uptake at different light intensities. At 13 and 66% of the maximal light intensity the magnitude of the $\Delta\psi$ was -110 mV. Therefore changes in the kinetics of lactose uptake between these two light intensities will reflect the regulation of the rate of lactose transport by light (or: cyclic electron transfer). Fig.3 shows that the affinity of the M-protein for lactose is not affected by light intensity: at both intensities a K_m of 550 μM is obtained. However, the maximal rate of lactose uptake does depend on the light intensity. At the highest intensity (66%, an intensity at which the rate of lactose uptake is saturated with respect to light intensity, fig.2A) a V_{max} for lactose uptake of 2.4 nmol·mg protein^{-1}·min^{-1} is obtained. A decrease in light intensity to 13% of the maximum lowers the V_{max} to 0.8 nmol·mg protein^{-1}·min^{-1}, one-third of the maximal value. Parallel measurements of the kinetics of alanine uptake yielded similar results. The light intensity has no effect on the affinity for alanine but the V_{max} is significantly lowered at decreasing intensities.

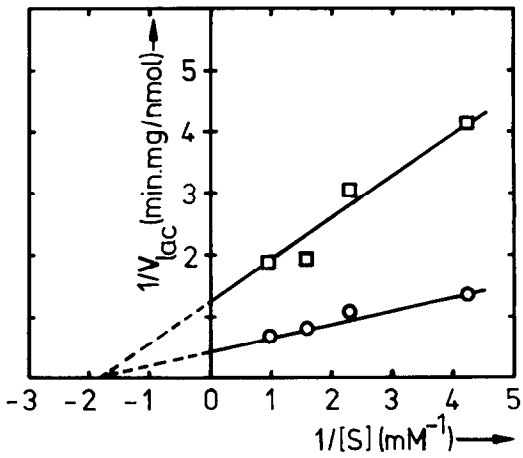

Fig.3. Kinetics of lactose uptake in *Rps. sphaeroides* L39 at constant driving force ($\Delta\psi$) but different light intensities. The initial rate of lactose uptake was measured at the indicated concentrations parallel with $\Delta\psi$, as described in fig.1. Two different light intensities were used: (□) 13% and (○) 66% of the maximal light intensity. Under both conditions the magnitude of the $\Delta\psi$ was −110 mV.

4. DISCUSSION

4.1. *Generation of a membrane potential anaerobically in the dark*

As we have reported before [1] our results show that *Rps. sphaeroides* can maintain a significant membrane potential in the dark. Without externally added energy (i.e., storing the cells anaerobically in the dark) and at 4°C this potential can be maintained for at least several days.

4.2. *Kinetics of lactose transport in* Rps. sphaeroides

The V_{max} of lactose transport in *Rps. sphaeroides* is significantly lower than in *E. coli*. For instance, for *E. coli* ML308-225 a V_{max} for lactose uptake of 140 nmol·mg protein^{-1} has been reported [18]. This probably reflects the smaller degree of expression of the lactose operon in the phototrophic bacterium since β-galactosidase activity was also low in cells of *Rps. sphaeroides*: 75 units/mg protein. In *E. coli* cells grown on lactose the β-galactosidase activity can reach values of 3000 units/mg protein. The degree of expression of the lactose operon in *Rps. sphaeroides* L39 could not be increased via addition of either lactose or cAMP to the growth medium.

The affinity of *Rps. sphaeroides* for lactose in these experiments is 550 μM. This differs slightly from the 200 μM, measured for the K_m for lactose uptake in *E. coli* in [9] and in [10]. One explanation may be that the affinity of the carrier is affected by its phospholipid environment but it should be noted that a K_m of 500 μM [18] has been reported for lactose uptake in *E. coli*.

At this moment we cannot exclude that a second (much higher) K_m can be detected at very high lactose concentrations.

4.3. *Interrelation of the different forms of regulation of the rate of lactose transport*

Two forms of regulation of the rate of lactose transport have previously been reported. Authors in [9,18] have reported the existence of two kinetic forms of the lactose carrier in *E. coli*, one with a high and one with a low affinity, such that the high-affinity form is generated by an increase in the magnitude of the proton-motive force. Subsequently, authors in [10] reported that the transition in the kinetics of the lactose carrier can also be brought about by SH-modifying reagents and redox mediators. A model was proposed [19] which explains the regulation of the rate of lactose uptake by the proton-motive force by a differential effect of the proton-motive force on two redox-sensitive dithiol-disulfide groups located on opposite sides of the hydrophobic barrier of the membrane. We consider it likely that in *Rps. sphaeroides* the redox state of the carrier is controlled by the redox state of one of the electron transfer intermediates.

4.4. *Similarities between regulation of secondary transport in* Rps. sphaeroides *and the regulation of a chloroplast kinase, phosphorylating light-harvesting complex II*

Since the discovery of the phosphorylation of the light-harvesting complex II by a chloroplast kinase [20], a number of observations, recently reviewed in [21], have indicated similarities with the regulation of solute transport in *Rps. sphaeroides*. In chloroplasts reduced plasto-

quinone directly reduces a membrane-bound kinase and subsequently a regulatory system is activated in which all components operate concertedly to provide the chloroplast with a rate of electron transfer that is optimally tuned to the need for photosynthesis [22]. This regulation gives rise to a large degree of homeostasis in the chloroplast bioenergetics.

Regulation of secondary transport in *Rps. sphaeroides* also shows significant homeostasis in the magnitude of the proton-motive force. Cells in the dark maintain a significant membrane potential. Upon illumination with very low light intensities this potential increases slightly (fig.2). Further increases in the light intensity induce depolarization, due to the activation of potential- or proton-motive force-dissipating processes.

Also on the molecular level some similarities exist between the *Rps. sphaeroides* and chloroplast systems. Secondary transport systems in general have disulfide/dithiol groups ([19], but cf. [23]), so presumably this is also true for alanine transport in *Rps. sphaeroides*, whereas it certainly is true for the M-protein [10,24,25]. These solute translocators therefore resemble the kinase in the chloroplast system. The regulatory component from the electron transfer chain in chloroplasts is plastoquinone, in *Rps. sphaeroides* it may well be a similar compound, like ubiquinone. Most likely it is one of the components of the cyclic electron transfer chain which is also part of the linear electron transfer chain from dehydrogenases to oxygen since alanine uptake is similarly regulated by electron transfer in cells aerobically in the dark (Elferink, M.G.L., Hellingwerf, K.J. and Konings, W.N., unpublished). In both systems all components involved in the regulation are membrane-bound, since regulation of cyclic electron transfer also is observed in membrane vesicles [26]. In this context it is important to elucidate the relation between the light intensity (via the rate of cyclic electron transfer) and the degree of reduction of the various redox carriers in the membrane of *Rps. sphaeroides*.

The relevance of this form of regulation of solute transport for other organisms remains to be established further. Our measurements on the regulation of alanine transport in *Rhodospirillum tenue* indicate that the same regulatory mechanisms exist in this organism.

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