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A Constitutive Enzyme System for Glucose Transport by *Chlorella sorokiniana*[1]

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ABSTRACT

It was found that the transport system for glucose (as measured by deoxyglucose uptake) in the high temperature strain of *Chlorella* (strain 07-11-05 or *C. sorokiniana*) was constitutive and the rate of uptake did not increase upon incubation of autotrophically grown cells with either deoxyglucose or glucose. The uptake obeyed Michaelis-Menten type kinetics with a concentration of 200 micromolar for half-saturation. The maximum rate of uptake was nearly 10 times faster per cell (at 38 C) than that reported for any other *Chlorella*. This rapid accumulation of deoxyglucose causes the passive efflux to become significant compared to the pump-driven influx and nonlinear uptake appears even after only 3 to 4 minutes.

Tanner, Komor, and others (3, 4, 6, 12) have demonstrated that the glucose transport system in several species of *Chlorella* requires at least a 1-h incubation in glucose for induction (12). The system utilizes a permease which manifests Michaelis-Menten kinetics (4), requires an energy source (6), and cotransports a proton during the transport of glucose (3).

We found a constitutive glucose transport system in the high temperature strain of *Chlorella* (07-11-05 or *C. sorokiniana*). A high specific rate of glucose transport exists in these autotrophically grown cells, which is not further stimulated by the incubation of the cells in glucose.

MATERIALS AND METHODS

C. sorokiniana were grown autotrophically at 38 C in 3% CO_2 in air, as previously described (1). Washed cells (2×10^8 cells/ml) were suspended in a medium consisting of K-phosphate buffer (5 mM, pH 6.5), 1 mM $CaCl_2$, 1 mM $MgCl_2$, to which glucose or deoxyglucose (5 mM) had been added. Cells were preincubated with glucose under room illumination or in the dark at 38 C for various times. Preincubated cells were then washed by rapid centrifugation, resuspended in fresh phosphate buffer, and the number of cells determined by Coulter Counter. The rate of transport at 25 C was assayed using a virtually unmetabolized glucose analog, 2-deoxyglucose (6, 11) tagged with [1-^{14}C]deoxyglucose (0.1 μCi/ml). At various times, 0.2-ml aliquots were removed and the cells were washed free of the supernatant by Millipore (0.2-μm pore diameter) filtration using 25 volumes of medium without tracer (15- to 20-s wash). The cells and Millipore were counted in a POPOP-PPO-toluene-Triton X-100 cocktail (2) in a Beckman LS-100 scintillation counter. At no time was more than 1% of the supernatant deoxyglucose taken up by the cells. All ± values reported in this paper represent standard deviations.

RESULTS AND DISCUSSION

The amounts of deoxyglucose found within the cells after varied preincubation periods (0, 1 or 2 h) are shown in Figure 1 and are nearly identical. The amount of uptake is not linear (Fig. 1A), but appears to be exponential (Fig. 1B). This differs from the linear uptake observed by Tanner and Komor (6, 12). The uptake appears to be completed after 8 to 10 min. Longer time periods were not measured in order to avoid the possibility of induction by deoxyglucose.

As shown in Table I, the kinetic coefficients for an exponential fit of the uptake do not vary with the time of preincubation with glucose. Thus, the system for taking up glucose in this species of *Chlorella* appears to be fully functional at all times and is not inducible. This table also demonstrates that the amount of uptake at 7 min is nearly 90% complete (last column).

The nonlinear kinetics can be understood by examining the initial rate of uptake and the total amount of deoxyglucose taken up after 7 to 10 min. The initial rate is $5.9 \pm 0.9 \times 10^{-16}$ mol cell^{-1} min^{-1} (Table I). The rate for other *Chlorella* can be calculated to be 4.5×10^{-17} mol cell^{-1} min^{-1} from Tanner's data (4, 6, 12) or only 10% of the rate reported here. Further, the amount of deoxyglucose accumulated is $1.4 \pm 0.1 \times 10^{-15}$ mol cell^{-1}. For an average cell volume of 2×10^{-14} l (2), the internal concentration is 70 mM, much higher than the amount of glucose present within the cells. (Using fluorometric and enzymic assays for glucose [7], it was found that autotrophically grown cells contained $5.2 \pm 2.4 \times 10^{-18}$ mol glucose cell^{-1} or 2.6×10^{-4} M, similar to that measured in other *Chlorella* species [13].) Thus, if an inward pump, operating 10 times faster than in other *Chlorella*, rapidly builds up the internal concentration of deoxyglucose to a level where a passive efflux becomes significant, such exponential-like kinetics would be observed (8).

The net rate of deoxyglucose uptake would be (8):

$$\frac{1}{a} \cdot \frac{d[D]_i}{dt} = \frac{V_m\,[D]_o}{a(K_m+[D]_o)} - P\,\frac{[D]_i}{v_c} - 10^{-3}\,[D]_o \qquad [1]$$

where a = the cell surface area in cm^2 cell^{-1}, $[D]_i$ = internal concentration of deoxyglucose in mol cell^{-1}, t = time in min, V_m = maximum rate of the pump in mol cell^{-1} min^{-1}, $[D]_o$ = external concentration of deoxyglucose in M, K_m = pump affinity constant in M, P = permeability coefficient in cm min^{-1}, and v_c = internal cellular volume in cm^3 cell^{-1}. The solution to this equation is:

$$[D]_i = \left\{\frac{V_m\,[D]_o}{K_m+[D]_o} + 10^{-3}\,Pa\,[D]_o\right\}\left\{\frac{v_c}{Pa}\right\}\cdot\left\{1-\exp(-Pta/v_c)\right\} \qquad [2]$$

For a volume of 2×10^{-11} cm^3 cell^{-1} and an area of 3.56×10^{-7} cm^2, the values for the exponential coefficient (0.41 min^{-1} in Table

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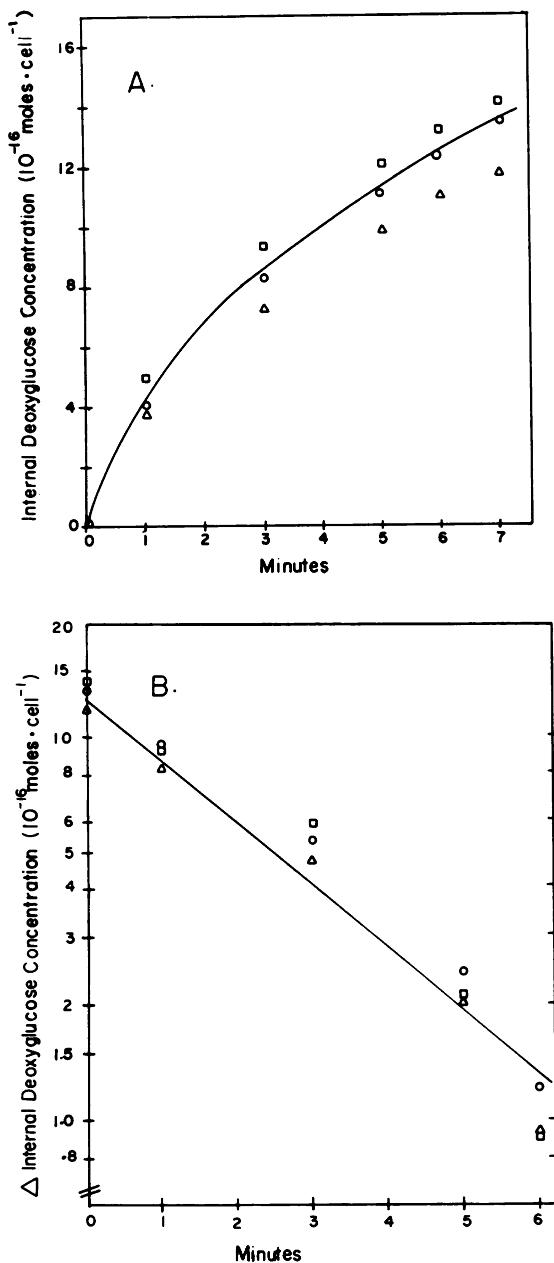

FIG. 1. Uptake of deoxyglucose by *Chlorella* for various times of glucose incubation. Cells were preincubated for various times in the presence of 5 mM glucose (no preincubation [○], 1 h [□], 2 h [Δ]) and then assayed for the pump activity by deoxyglucose uptake as described under "Materials and Methods." A: uptake with time; B: exponential plot of uptake with time. Maximum amount of uptake was assumed to be reached at 7 min and used to calculate the Δ internal concentration = concentration at 7 min—concentration at t min.

I) yield a permeability value of 2.3×10^{-5} cm min^{-1} or 3.8×10^{-7} cm s^{-1}, which agrees with the passive permeability coefficient previously measured for mannitol penetration in these cells (2).

The reproducibility of the initial rate uptake and the lack of induction during shorter time intervals are shown in Table II. There is a small variation of 15 to 20% in the initial rate from culture to culture, which may represent a variation in culture density at the time of harvesting. Among cell samples incubated for various times, the variation in initial uptake rates is never greater than 13% (SD). This is not nearly enough to constitute an "induction" (in refs. 4 and 12 the initial rate of deoxyglucose increases by nearly 2 orders of magnitude after induction).

The plot of initial rate of uptake as a function of external deoxyglucose concentration fits Michaelis-Menten kinetics (Fig. 2). Again, there is little difference (10%) for cells preincubated in glucose compared with cells taken from an autotrophic culture, except that the rate seems to be slightly lower after a 3-h preincubation. An Eadie-Scatchard (V/S *versus* V) plot (9) shows that the K_m is 110 μM, and 183 μM for 0 and 3-h preincubated cells, respectively, similar to that for other *Chlorella* (4, 6, 12). The V_m is 5.6×10^{-16} mol cell^{-1} min^{-1} and 4.5×10^{-16} mol cell^{-1} min^{-1} for 0 and 3-h preincubated cells, respectively. The exponential coefficient for the fit of these data to equation 2 (Fig. 2) is 0.40 ± 0.04 min^{-1}, which is identical to that calculated in Table I.

From the data of Figure 2, we can calculate the total expected uptake, which is the time-independent factor in equation 2, to be 1.44×10^{-15} mol cell^{-1} and 1.16×10^{-15} for 0 and 3-h preincubations, respectively.

The efflux of deoxyglucose out of preloaded cells is shown in Figure 3. The half-time of the efflux is about 2 to 2.5 min, similar to that measured for the influx (Fig. 1). The initial rates for the two experiments shown here were used to calculate the permea-

Table I. *Calculated Kinetic Coefficients for Uptake of Deoxyglucose by C. sorokiniana*

Uptake was measured as described under "Materials and Methods." Cell number was determined after preincubation in glucose since the medium allowed a small amount of cell growth. Rate constants were calculated by a least-squares fit of the kinetics of the uptake of deoxyglucose, similar to Figure 1. Maximum uptake is extrapolated intercept of ordinate of Figure 1B, which corresponds to the maximum uptake of deoxyglucose. Square of regression coefficient for the five data points in each time period was always greater than 0.96. Initial rate is given by the product of maximum uptake and exponential coefficient.

Preincubation Time	Maximum Uptake	Exponential Coefficient	Initial Rate	At 7 min, % of maximum uptake[1]
h	*10^{-16} mol/cell*	*min*	*10^{-16} mol/cell·min*	
0	14.5	−0.39	5.6	86
0.5	16.2	−0.45	7.3	91
1	15.6	−0.43	6.7	90
1.5	12.6	−0.40	5.0	95
2	13.0	−0.41	5.3	96
2.5	14.1	−0.39	5.5	98
Average ± SD	14.3 ± 1.4	−0.41 ± 0.02	5.9 ± 0.9	92 ± 4

[1] Approximation used in Figure 1 is shown to be adequate since uptake at 7 min is within 8 to 14% of maximum calculated uptake.

Table II. *Reproducibility of the Uptake of Deoxyglucose by C. Sorokiniana*

A separate batch culture of the algae was used for each experiment. Individual initial rates were calculated from extrapolated uptake and the rate constant as in Figure 1 and Table I. Cells were suspended in standard medium (see under "Materials and Methods") but were bubbled with pure O_2. Grand average of all 26 data points was 4.3 ± 1.2 (SD) × 10^{-16} mol/cell·min. Averages of extrapolated uptake and rate constants were 12.2 ± 1.9 × 10^{-16} mol/cell and 0.35 ± 0.08/min, respectively.

Time of Incubation	Initial Rate				
	Experiment no.				
	1	2	3	4	5
min	*10^{-16} mol/cell·min*				
0	3.78	4.00	2.70	3.45	5.17
10	4.50	4.22	3.17	3.63	6.12
20	4.10	5.26	3.05	4.23	6.14
30	3.28	4.41	2.80	4.40	7.45
40	2.94	4.76	3.39		6.25
50		4.55	3.83		

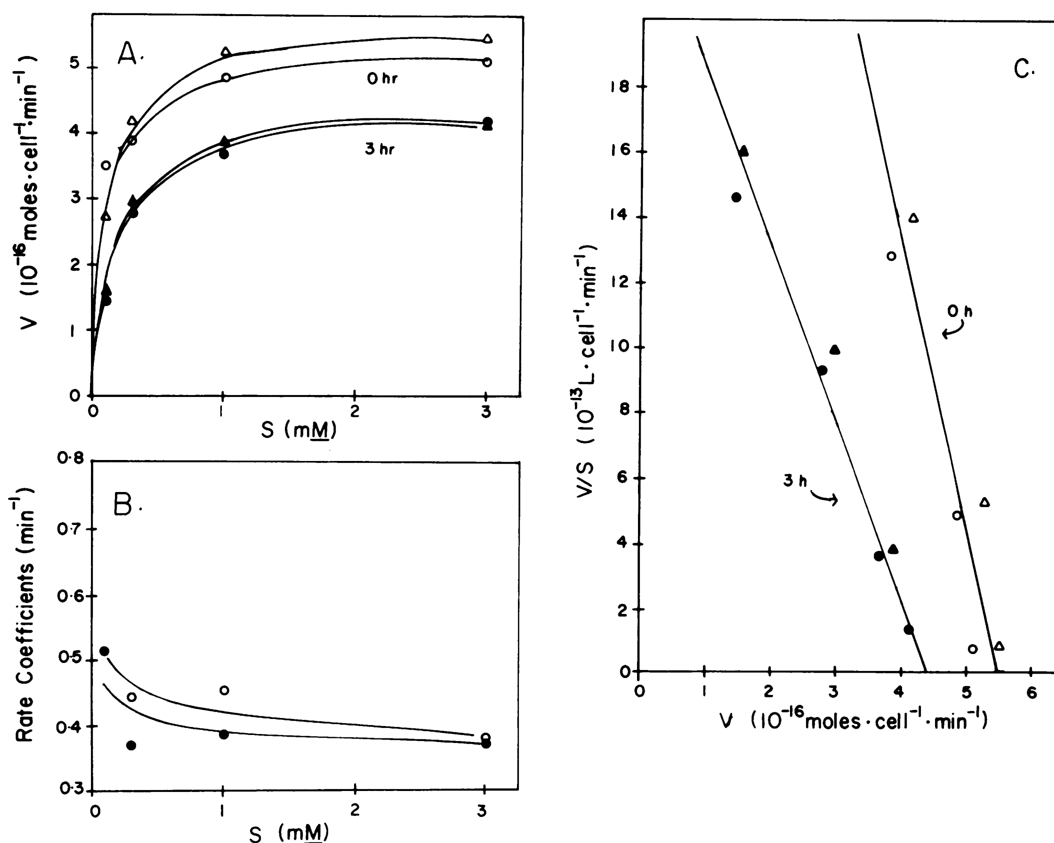

FIG. 2. Dependence of deoxyglucose uptake on external concentration of deoxyglucose. Initial rate (0–4 min) of deoxyglucose uptake for various external concentrations, for no preincubation (○, △) and 180-min preincubation (●, ▲) with glucose. Rates were calculated according to equation 2 as in Table I. Circles were based on uptake at 7 min; triangles were based on maximum uptake. S = external concentration (in M). V = initial rate in 10^{-16} mol cell^{-1} min^{-1}. A: linear plot of initial rate; B: plot of exponential coefficient. Calculations were carried out as described in Table I and in text. C: Eadie-Scatchard plot of initial rate (9).

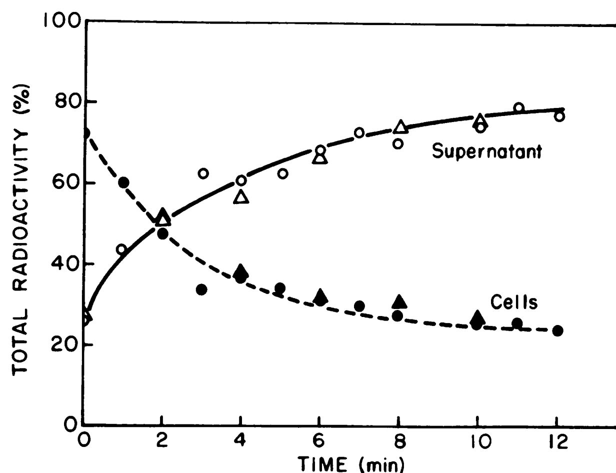

FIG. 3. Efflux of deoxyglucose from *Chlorella*. *Chlorella* cells were prepared and incubated for 8 min in 3 mM deoxyglucose with 0.14 μCi ml^{-1} [^{14}C]deoxyglucose in phosphate buffer, as under "Materials and Methods." Cells were then rapidly washed twice by centrifugation to remove radioactive supernatant (total time, 10 min). After suspension in the same medium as above without tracer, the label was assayed in both cells and supernatant as described under "Materials and Methods." Two experiments (○, △) are shown in the figure. Total amounts of label present are 3.4 and 2.6 × 10^{-7} mol deoxyglucose ml^{-1}, respectively. Radioactivity is found in the supernatant even at zero time due to the efflux during the last stages of washing.

bility coefficients of 1.6 and 1.8 × 10^{-7} cm s^{-1} according to Fick's law. This value of P is about one-half the value measured from the influx experiment.

There are several possible explanations for this low value of P: (*a*) the uptake and efflux of deoxyglucose might be more complex than the model shown by equations 1 and 2; (*b*) there might be a real difference in the permeability coefficient with direction of flow; (*c*) the internal concentration of deoxyglucose may be calculated too high due to the existence of metabolically modified deoxyglucose, multiple pools, or a smaller cell volume; (*d*) the unstirred layer near the plasma membrane might be larger inside the cell than outside; (*e*) the uptake of the label may be more significant than calculated (about 7–10% at zero time) if the cell pumps in the deoxyglucose molecules which have just le the cell. At this stage of investigation it is very difficult to evalu e any of the above explanations. For this paper the values of P seem to be near enough to suggest that the simple model is fairly accurate.

CONCLUSION

The high temperature strain of *Chlorella* grows at a rate nearly 3.5 times faster than the Emerson strain (10) at the respective temperature optima (38 C *versus* 26 C). Since the respiration rate of *C. sorokiniana* is likewise nearly 3 times higher (10), the high activity of its glucose pump (per cell at 38 C) is not totally unexpected (Figs. 1 and 2) although its constitutive nature seems unusual. The high pump rate and small size of these cells are the major causes of the pronounced deviation from linear kinetics. Within minutes, the internal concentration of deoxyglucose rises rapidly to a level at which the outward passive leak becomes significant (Fig. 3) and the net gain in internal deoxyglucose per unit time declines.

Another possible interpretation of the nonlinear kinetics is that there is a lack of ATP to drive the pump. The work of Komor and Tanner (5) showed nonlinear kinetics when the levels of ATP

within the cells were lowered by anaerobiosis. The cells in the present report are incubated in the absence of an energy source except for endogenous metabolite pools and thus, after accumulating nearly 100 mM internal deoxyglucose, the energy derived from pools could be depleted to such an extent that further accumulation could not occur. However, the same type of exponential kinetics is obtained when the cells have been preincubated for 3 h in deoxyglucose (Fig. 2) and when the cells are preloaded with [^{14}C]deoxyglucose, washed free of the supernatant and allowed to lose the radioactive deoxyglucose to medium with unlabeled deoxyglucose (Fig. 3). Thus, the coefficients of the passive and active component are nearly the same, indicating that energy must not be limiting.

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