Plant Physiol. (1974) 53, 14-20

Regulation of Hexose Transport in *Chlorella vulgaris*

CHARACTERISTICS OF INDUCTION AND TURNOVER[1]

Received for publication April 17, 1973 and in revised form August 22, 1973

D. HAASS AND W. TANNER
Fachbereich Biologie der Universität Regensburg, 8400 Regensburg, Universitätsstr. 31, Germany

ABSTRACT

Of nine species of unicellular algae tested, *Chlorella vulgaris* showed the highest inducibility for an active hexose transport system. Whereas the rate of uptake in all other species was increased by induction less than 5-fold, it was increased more than 400-fold in one strain of *C. vulgaris*. With glucose as inducer, the minimum time necessary to synthesize inducible proteins of the transport system was 15 minutes. The Km for induction with glucose is 5 μM and with 6-deoxyglucose 1 mM. The inducing sugars have to penetrate the cells to be effective.

Evidence indicating that regulation of induction occurs at the transcriptional level was obtained. The induction was inhibited by 6-methylpurine. When cells were exposed to induce in the presence of actidione no increase in transport activity could be measured. After removal of actidione as well as the inducer, an increased uptake activity was observed after 30 to 60 minutes. The induced uptake system showed a turnover with a half-life of 4 to 6 hours at 26 C under nongrowing conditions; at 0 C turnover was negligible. Turnover was partly inhibited by anaerobic condition and by actidione; it was accelerated under growing conditions.

Chlorella vulgaris possesses an inducible active hexose transport system (21, 23). The induction occurs in the presence of glucose or can be brought about by glucose analogues such as 3-OMG[2] or 6-dG, which are not metabolized (13, 21). The induction is energy-dependent and probably involves the synthesis of one or several proteins (23).

In this paper the occurrence of the inducible hexose transport system among unicellular algae and the characteristics of induction and turnover in *C. vulgaris* have been examined in greater detail. It has been found that the total process of induction takes about 15 min and that the induced activity shows a turnover with a half-life of 4 to 6 hr. Evidence for an induction from within the cells and for a regulation at the transcriptional level has been obtained.

MATERIALS AND METHODS

The strain of *Chlorella vulgaris* used and the conditions of autotrophic culture were the same as previously described (23). The same nutrient medium was also used for the following algae, which were obtained from the collection in Göttingen (as well as those with a number given below): *C. vulgaris* 211-11h/P; *Chlorella pyrenoidosa* 211-8b; *C. vulgaris* 211-11h/9; *C. vulgaris* 211-11h/20. To the media of the last two (colorless mutants) 2% glucose was added.

The nutrient medium of Kessler *et al.* (10) was used for: *Scenedesmus quadricauda* 276-4c; *Scenedesmus obliquus* D_3; *Scenedesmus dimorphus* 276-10; *Chlorella saccharophila* 211-9a; *Ankistrodesmus braunii* 202-7c. *Chlorella protothecoides* 211-7a was grown on the medium of Shihira-Ishikawa and Hase (20) and *Bumilleriopsis filiformis* on the medium of Böger (1).

3-OMG was obtained from Calbiochem; 3-*O*-methyl-^{14}C-glucose from New England Nuclear. 6-dG was purchased from Koch-Light Laboratories, Colnbrook, England and it was tritiated by the Radiochemical Centre, Amersham; ^{3}H-6-dG was purified subsequently by paper chromatography (butanol-pyridine-water-acetic acid, 60:70:30:3, v/v). Cycloheximide (Actidione) was obtained from Fluka, and membrane filters of 0.8-μm pore size from Sartorius GmbH, Göttingen.

Adaptation and Uptake Experiments. To induce sugar uptake, approximately 300 μl of autotrophically grown packed cells were incubated in 10 ml of 32 mM sodium phosphate buffer, pH 6.5, with 7.8 mM glucose present and shaken in the dark in air at 26 C in a 100-ml Erlenmeyer flask while the control was shaken without sugar. The glucose was completely consumed in about 2 to 3 hr. All strains, which showed a constitutive uptake system, were tested once more for inducibility after being starved in 32 mM sodium phosphate buffer, pH 6.5, for 24 or 48 hr. To measure sugar uptake, the algae were incubated with the sugar analogue (0.2 mM ^{14}C-3-OMG or ^{3}H-6-dG) in 32 mM sodium phosphate buffer, pH 6.5. The reaction mixture was shaken in air in an Erlenmeyer flask in the dark at 26 C; where not otherwise indicated samples were withdrawn at 1-min intervals for 4 min and filtered rapidly through membrane filters. The filters were washed twice with 2 ml of 32 mM ice-cold sodium phosphate buffer and extracted by boiling the filters with the cells in 1 ml of 10 mM HCl for 10 min. The radioactivity of the extract was measured directly by pipetting an aliquot into the scintillation fluid. The rate of uptake was taken as the slope of the line drawn through the four points.

Turnover Experiments. The turnover of the inducible hexose uptake system was followed by measuring the uptake velocity of 3-OMG or 6-dG immediately after induction and at various times thereafter. The algae were shaken aerobically in the dark at 26 C in sodium phosphate buffer, 32 mM, pH 6.5; aliquots were withdrawn; and the rate of uptake was determined. To measure the turnover under anaerobic conditions in the dark, water-saturated N_2 was bubbled through the algal suspension; the rate of uptake was determined as usual under aerobic conditions.

[1] This work was supported by Deutsche Forschungsgemeinschaft.

[2] Abbreviations: 6-dG: 6-deoxyglucose; 3-OMG: 3-*O*-methylglucose; p.c.: packed cells.

RESULTS

Occurrence of Inducible Hexose Uptake Systems in Unicellular Algae. It had been shown previously (22) that an inducible hexose uptake system is present in *C. vulgaris* and to some extent in *C. pyrenoidosa* but that such a system is constitutive in *Scenedesmus obliquus* and *Ankistrodesmus braunii*. In Table I the results obtained with a broader range of organisms, all grown autotrophically in the absence of any organic compounds, have been summarized. Cells, either pretreated with glucose (induced) or not pretreated (control), were incubated with 3-*O*-methyl-^{14}C-glucose for 1 hr. The uptake of the radioactive sugar was linear up to 20 min; the velocities were determined from this initial uptake. Only *C. vulgaris* showed a high increase in the rate of transport due to induction. With the other species investigated, including three species of *Chlorella*, the transport system to a large extent seems to be constitutive. Similar results were obtained when the cells were starved for 24 hr prior to the uptake experiment. Since the two colorless mutants of *C. vulgaris* had to be grown on glucose, they were starved for 48 hr or more; they did not, however, lose their uptake activity to the levels of the wild types (Fig. 5).

Kinetics of Induction. To see how long it takes for a functioning protein to be synthesized in *Chlorella*, a time course of induction was studied in detail (Fig. 1). From the time the aliquot of induced algae was withdrawn until the rate of uptake could be measured it took 2 min; during that time the algae were at 0 C. After centrifugation and decantation of the supernatant the algae were resuspended directly in the uptake medium containing the radioactive sugar. In Figure 1 the rates of uptake are plotted against the time of induction. The data show that the processes of induction and protein synthesis together take 15 min. The experiment was repeated four times with almost identical results; in no experiment did it take less than 10 or more than 18 min to induce the system.

When the concentration of the inducer glucose was varied, the rate of induction (measured past the initial lag as an increase in the rate of 3-OMG uptake) was concentration-dependent below 50 μM. Figure 2 shows that induction is half-saturated with glucose at 5 μM and with 6-dG at 1 mM (Fig. 3). This reflects to some extent the difference in the K*m* values for the uptake of these two sugars by fully induced cells; they were found to be 10 μM for glucose and 0.3 mM for 6-dG (4).

Table I. *Occurrence of Inducible Hexose Transport Systems in Unicellular Algae*

The algae were preincubated in buffer plus glucose (induced) or in buffer only (noninduced) for 3 hr. Then the rate of uptake of 0.2 mM 3-OMG specific radioactivity 1 μc/6.25 μmoles) was determined.

Alga	Rate of uptake		Rate Induced Cells / Rate Noninduced Cells
	Noninduced	Induced	
	μmoles/ml p.c.·hr		
C. vulgaris K	0.07	28.9	412
C. vulgaris 211-11h/P	0.31	19.3	62
C. vulgaris 211-11h/9[1]	29.35	78.0	2.6
C. vulgaris 211-11h/20[1]	6.83	28.6	4.2
C. saccharophila	2.83	12.5	4.4
C. pyrenoidosa (fusca)	5.65	19.7	3.5
C. protothecoides	41.15	78.0	1.9
Scenedesmus quadricauda	1.84	3.23	1.8
S. obliquus	5.13	8.32	1.6
S. dimorphus	27.5	28.4	1.0
Ankistrodesmus braunii	10.7	12.0	1.1
Bumilleriopsis filiformis	2.58	4.9	1.9

[1] These mutants grown on glucose were kept without sugar for 48 hr; then the rate of uptake of these cells and of a batch which had been reinduced was measured.

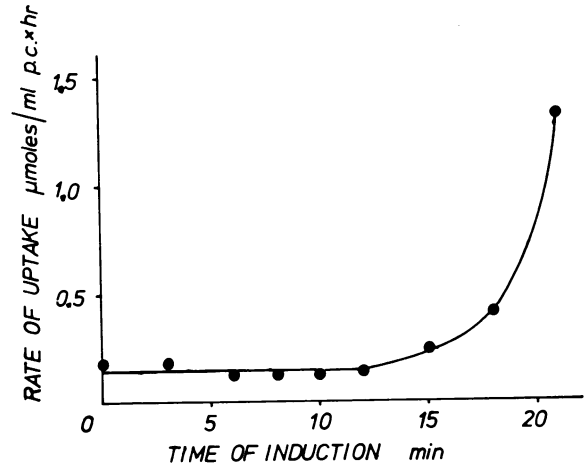

FIG. 1. Time course of induction with glucose. 540 μl of p.c. were shaken in 15 ml of 32 mM sodium phosphate buffer (pH 6.5). At time zero, the inducer was added (7.8 mM glucose). Samples of 1.5 ml were withdrawn at times indicated, diluted 6.6-fold with ice-cold water, centrifuged in the cold and uptake of 0.2 mM ^{3}H-6-dG was measured.

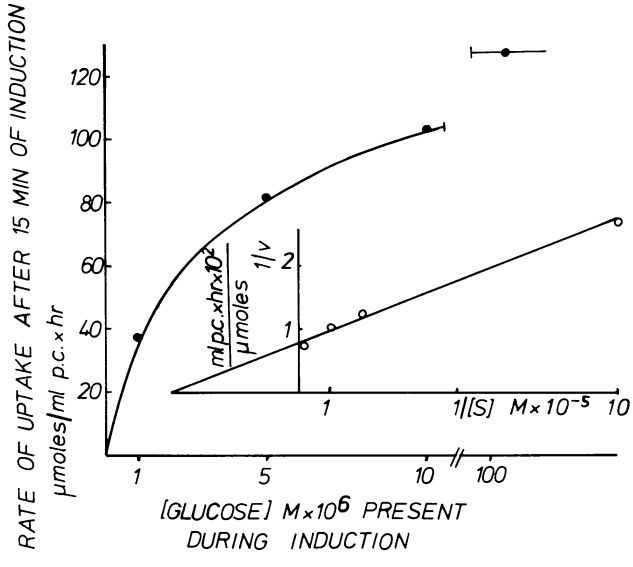

FIG. 2. Induction by glucose:concentration-dependence of the rate of induction. 48 μl p.c. were shaken in 10 ml of 32 mM sodium phosphate buffer with glucose (concentrations as indicated) at 26 C. After 15 min incubation, the cells were diluted with 100 ml of ice-cold buffer, centrifuged, washed with 10 ml of buffer, and resuspended in 2 ml of 32 mM sodium phosphate at 26 C. After 30 min the rate of uptake was determined as in Fig. 1.

Evidence for an Induction of RNA Synthesis. Protein synthesis can be regulated at the transcriptional as well as at the translational level. Previous experiments to distinguish between these possibilities with actinomycin D have been negative, possibly because the poison did not penetrate *Chlorella* (23).

6-Methylpurine, an inhibitor of RNA synthesis in *Chlorella*

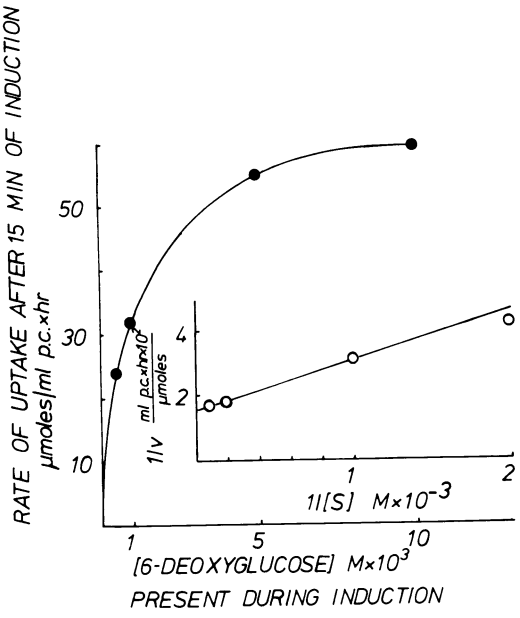

FIG. 3. Induction by 6-dG:concentration-dependence of the rate of induction. The experiment was carried out as described in Fig. 2.

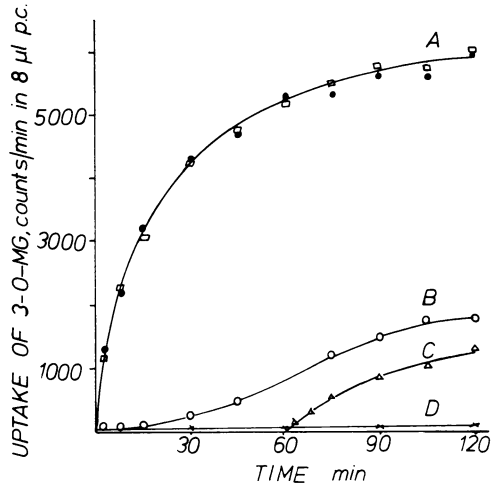

FIG. 4. Influence of actidione on induction. Algae were incubated in 32 mM sodium phosphate buffer, pH 6.5, with glucose (1.4 mg/ml) or with glucose (1.4 mg/ml) plus actidione (4.2 μg/ml = 15 μM). After induction (3 hr) both samples were washed and the cells resuspended in the same volume of 32 mM sodium phosphate buffer. At time 0, ^{14}C-3-OMG was added to two parallel samples induced with glucose in the absence of actidione, and the uptake of radioactivity was followed (curve A, with (□) and without (●) actidione present during uptake). Curve B was obtained when algae induced in the presence of actidione were used. Curve C corresponds to experiment B except that ^{14}C-3-OMG was added 60 min past time 0. Curve D is a control with noninduced algae to which ^{14}C-3-OMG was added at time 0.

(15), at a concentration of 1 mM, inhibited the induction of the hexose uptake system severely. The time required for uptake to become linear was doubled, and the maximal rate of uptake was inhibited by 84%. To observe these inhibitory effects, the cells had to be incubated with the poison for several hours before the inducing sugar was added. (This preincubation did not change the rate of respiration of the cells.)

Evidence has been obtained from another type of experiment which shows that the induction of the synthesis of protein(s) for hexose uptake is probably preceded by RNA synthesis. The experiment is based on the fact that RNA synthesis is not inhibited by low concentrations of actidione. Induction should, therefore, be possible in the presence of actidione, although it should only be expressed after the removal of the inhibitor. Figure 4 presents the results of such an experiment; curve A gives the uptake of 3-OMG by fully induced cells in the presence and absence of actidione. The uptake is not affected by actidione. Curve B was obtained with algae induced in the presence of actidione, but the actidione, and the inducer were removed after induction and uptake was measured in the absence of the poison. Curve D shows the uptake of a noninduced sample. It is obvious that the rate of uptake increases much more in the sample induced in the presence of actidione than in the noninduced sample. In the latter it took more than 5 hr until an increase in uptake due to an induction by 0.2 mM 3-OMG, the substrate of the uptake measurement, could be observed. Thus, an induction by 3-OMG cannot explain the increase in the rate of uptake of curve B. In an additional control it has also been excluded that preincubation with actidione alone is responsible for the effect. In that control measurable uptake takes place neither immediately after removal of actidione nor 60 min thereafter. An obvious explanation is that RNA is synthesized in the presence of inducer although actidione is present. After the removal of actidione RNA is translated into the protein(s) required for uptake. That this interpretation is correct is strengthened by experiment C. In this case the uptake has been measured 60 min after the removal of actidione and inducer. During this time the translation should have occurred, and the rate of uptake should be faster right away. This is indeed the case (compare B and C). The induction under these conditions is, however, less effective than in a normal control (A). When increasing times are allowed between the removal of actidione plus inducer and the measurement of uptake activity, it should even be possible to see the "instability" of the messenger. Figure 5 shows this clearly: when more than 90 min are allowed to pass before uptake is measured, the rate decreases again. The messenger must be turning over. No exact half-life can be given, however, since the turnover of the transport protein itself (see below) will contribute to the effect.

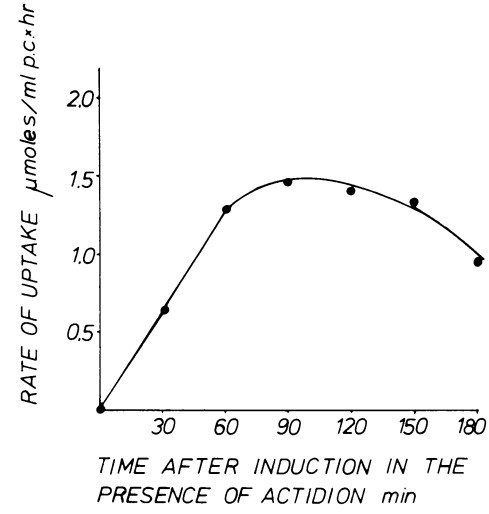

FIG. 5. Change in uptake activity with time following induction in the presence of actidione plus glucose. The experiment was carried out as in Fig. 4. The time given on the abscissa corresponds to the time interval between the removal of actidione plus glucose and the start of the uptake measurement. The rate of uptake was determined as described in "Materials and Methods," except that uptake was followed up to 15 min.

Turnover of the Induced Uptake System. Figure 6 shows that the fully induced uptake activity decays with time after removal of the inducing substrate. This decay has a half-life of 4 to 6 hr at 26 C with nongrowing cells. The individual experimental points of Figure 6 were obtained from six separate experiments carried out over several weeks. It is obvious from the data that the half-life differs by a factor of more than 2 with different batches of algae. The reason is not known, but it seems likely that the differences might be smaller with synchronized cultures.

At 0 C the activity decreases extremely slowly (Fig. 6). This observation has been technically useful. By holding the cells at 0 C, it is possible to perform several experiments, *e.g.*, when studying active transport, on one batch of induced algae.

The experiments in Table II were carried out to study whether the turnover (*a*) can be inhibited by actidione, (*b*) is an energy-requiring process, or (*c*) is changed under growing conditions. The results show that actidione has only a minor inhibiting effect on turnover. Anaerobic conditions, on the other hand, significantly decrease the decay of the uptake system, whereas growing conditions clearly increase it. The latter is also true, when correction is made for the increase in cell number. Thus, there was no increase in cell number in 2.5 hr and an increase of only 20% in 6 hr. The difference after 23 hr, on the other hand, between the control and the experimental sample vanishes, since the packed cell volume is four times greater in the treated sample after this time.

Does the Inducer Act from within the Cells? Evidence with *Escherichia coli* suggests that the induction of the inducible hexose-6-P transport system results from a stimulus outside the cells (6, 25), and this also seems to be the case for the induction of the C_4-dicarboxylic acid anion translocator of *Azotobacter vinelandii* (18). The lac operon, on the other hand, is induced from within the cells (7, 8). One approach to the problem in *Chlorella* has been to follow the decrease in uptake activity of induced cells, when inducer was present either inside only or inside *and* outside the cells.

Fully induced *Chlorella* cells were allowed to take up 6-dG

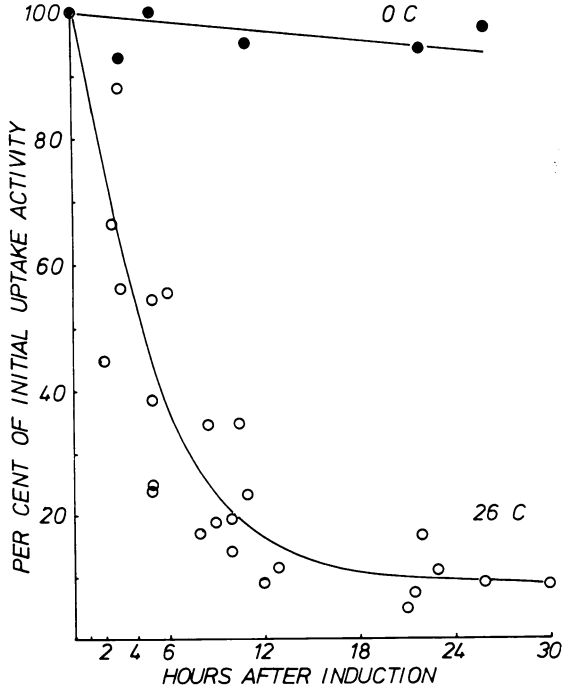

FIG. 6. Decay of induced uptake activity. For experimental details see "Materials and Methods." 100% uptake activity corresponds to the activity of freshly induced cells.

Table II. *Effect of Actidione, N_2, and Growth on the Turnover of the Uptake System*

Algae induced for 3 hr were shaken in the dark in phosphate buffer for various times (controls). During the same time a parallel sample was treated as indicated. Aliquots were withdrawn and washed, and the rate of uptake of 0.2 mM 3-*O*-methylglucose was determined.

Expt.	Time after induction	Treatment after Induction	Rate of 3-OMG Uptake	
			Control	Treated sample
	hr		*μmoles/ml p.c.·hr*	
I	0		35.6	35.6
	8.5	Actidione, 7.5 μM	12.3	17.7
	12		3.2	7.2
II	0		24.9	24.9
	5.5	N_2	13.6	23.7
	10.5		8.6	16.9
III	0		69.3	69.3
	2.5	Medium + light	46.1	31.4
	6		38.6	9.0
	23		7.6	1.7

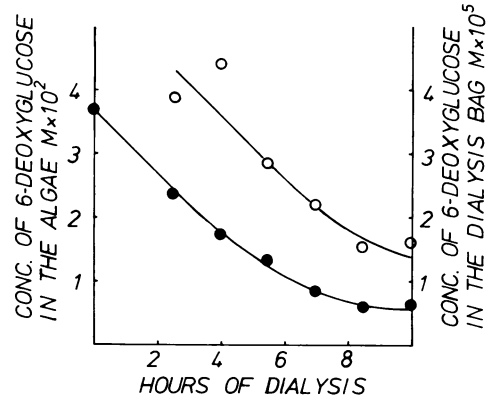

FIG. 7. Change of 6-dG concentration in *Chlorella* cells dialyzed against a continuously changing buffer. For details see text. ●: Concentration of 6-dG in the cells; ○: concentration of 6-dG in the bathing medium of the dialysis bag.

until a steady state condition with an internal concentration of 30 to 50 mM had been reached. Then the cells were centrifuged and washed once to remove the external sugar. They were resuspended in 4 ml of 32 mM sodium phosphate buffer, placed in a dialysis bag, and dialyzed for several hours against 1 liter of the same buffer, which was changed continuously such that the complete dialyzing volume was replaced in 1 hr. Individual dialysis bags were taken at intervals, and the radioactivity was determined in the algae and in the buffer of the dialysis bag. The concentration of sugar within the cells decreased from 37 to 6 mM within 10 hr (Fig. 7). In the time interval from 2.5 to 10 hr the concentration in the dialysis bag decreased from 40 to 15 μM. A concentration difference of more than 500-fold is thus maintained between the inside and the outside of the cells. The efflux out of the cells at the very low concentrations of sugar in the outside medium has a rate of 5.1 μmoles/ml p.c. hr.[3]

[3] Under the conditions of the experiment this rate is approximately 75 times slower than the corresponding efflux rate under steady state conditions (*i.e.*, sugar outside and inside). This reflects the extremely large positive transmembrane effect for efflux described and discussed elsewhere (12).

Table III. *Effect of Inducer Present Inside, or Inside and Outside, the Cells on Their State of Induction*

Cells were induced by glucose for 3 hr. Uptake of 6-dG was measured as described in "Material and Methods."

Expt.	Treatment	Activity of Uptake System
		μmoles/ml p.c.·hr
I	a. Induced cells at time 0 (inside concn of 6-dG = 0)	93.5
	b. Induced cells at time 10 hr (inside concn of 6-dG = 32 to 3.5 mM)	80.4
	c. Induced cells at time 10 hr (inside concn of 6-dG = 0)	17.8
	d. Induced cells at time 90 min (inside concn of 6-dG = 4.1 mM)	94.0
	e. Noninduced cells after 10 hr of induction with 50 μM 6-dG	13.6
II	a. as a above	145.0
	c. as c above	22.8
	d. as d above (inside concn, however, 37 mM)	220.0
	f. Induced cells after 10 hr of steady state 6-dG uptake in the presence of 15 μM actidione (inside concn of 6-dG = 37 mM; outside 0.56 mM)	77.5

Cells treated in this way for 10 hr were now tested for residual uptake activity and compared with induced cells which had been kept without any sugar for 10 hr. The results in Table III compare the rates of uptake immediately after induction (a), after preloading with nonradioactive 6-dG followed by 10 hr of dialysis (b), and after 10 hr of dialysis without any sugar treatment past the first induction (c). The uptake activity in the sample with sugar neither inside nor outside (c) dropped to 17.8 μmoles/ml p.c.·hr, which corresponds to 19% of the initial activity. It fell to 86% only when the cells contained sugar in the inside, plus traces (<40 μM) in the outside during dialysis. In interpreting this result, two further points have to be considered.

The rate of uptake into preloaded cells generally is larger than the rate of uptake into empty cells (positive transmembrane effect). In *Chlorella* this can amount to a factor larger than 2 for 6-dG (4). Therefore, a control in which cells were preloaded with a concentration as close as possible to that of the experimental sample after dialysis had to be included. The rate of 94.0 μmoles/ml p.c.·hr (d) given in Table III represents the rate of uptake into these cells. Thus, it is obvious that with an inside concentration of 4.1 mM no transmembrane effect is observed.[4]

The other point to be considered is the following: although the outside concentration is kept fairly low (40–15 μM), it could still suffice to keep the system fully or almost fully induced. A further control was, therefore, carried out with noninduced cells, which were treated for 10 hr with the low concentration of 50 μM 6-dG in the outside medium. The rate of uptake of these cells after 10 hr was only 15% of that of fully induced cells and again far below the value of treatment b. This result agrees with the apparent K*m* of 1 mM for induction with 6-dG (Fig. 3); the high K*m* value explains the poor induction with 50 μM 6-dG. We concluded, therefore, that the outside concentration does not contribute significantly to the effect of keeping the system induced. The possibility that the concentration measured in the dialysis bag might be considerably lower than that in the immediate neighborhood of the cell (*e.g.*, due to the cell wall layer) seems unlikely; at least no indication for the existence of a "second diffusion barrier" has been obtained (12), in contrast to results with *E. coli* (19).

Although the results presented are consistent with an induction from within the cells, they do not really prove it. The lack of a decrease in uptake activity when an inducing sugar analogue is inside the cells could also mean that the sugar merely protects the induced system from breakdown or inactivation. The data of experiment II in Table III show, however, that this is not the case. Even in the presence of 6-dG in the cells, protein of the transport system has to be synthesized continuously since the addition of actidione to such cells leads to a considerable decrease in uptake activity within 10 hr (compare d and f of experiment II). The conditions a, c, and d are analogous to those of experiment I; since the inside concentration of the cells (d and f) is about 10 times that in experiment I, the rate of uptake into loaded cells (d) as expected is considerably larger than that into empty cells (a). Thus, this experiment shows that the uptake activity of the cells containing sugar is maintained because of the steady synthesis of transport protein which counteracts the continuous breakdown or inactivation of this protein.

Two other types of experiments previously used to solve the problem of the topology of induction in the case of hexose-6-P and β-galactoside transport systems in *E. coli* (3, 7, 26) were also undertaken with *Chlorella*. The results obtained are consistent with an induction of the uptake system from within the cells. In the first type of experiment the time course of induction is followed at low inducer concentrations. Since the cells do not increase in number during induction, the uptake activity should increase linearly with time, if induced from the outside.

On the other hand, if induction took place from the inside, a nonlinear relationship would be expected, since a time-dependent uptake step now precedes induction. Only from the time when saturating amounts of inducer are in the cells will the induction proceed linearly with time. The result of Fig. 8 is in agreement with an induction from within the cells.

The observation made in the second type of experiment is known as the "maintenance" phenomenon. It can be described briefly as follows: cells which accumulated inducing substrate for some time will continue with a high rate of induced protein synthesis when they are transferred into a medium with low inducer concentration. This is not the case when induction occurs from outside (26). Such an experiment was carried out with *C. vulgaris* (Fig. 9). After 20 min of incubation in 20 mM 6-dG an aliquot of cells was transferred to a medium containing only 0.5 mM inducer. The increase of uptake activity was followed and compared with a sample induced with 0.5 mM 6-dG only. As can be seen in Figure 9, the rate of "permease" formation of the sample transferred to low inducer concentration equals the rate of the sample maintained at high concentration for at least 40 min after the change. Also the rate of production of the uptake system thereafter remains considerably faster than the rate in cells at the low inducer concentration from the start. Although the interpretation of this experiment depends on the unsolved problem, how fast a new steady state level of messenger RNA will be established in the cells in relation to the lower concentration of inducer, the result

[4] This agrees well with the high K*m* of 21 mM (11) measured for efflux, since the transmembrane effect for influx is a function of the saturation of carrier with substrate at the inside (14).

obtained at least agrees with an induction of the hexose uptake system of *C. vulgaris* from within the cells.

DISCUSSION

All nine species of unicellular algae tested were able to take up the glucose analogue 3-OMG and to accumulate it against a considerable concentration gradient. However, except for *Chlorella* the inducibility of uptake was small, and even with the four species of *Chlorella* a dramatic increase was observed only in the two strains of *C. vulgaris*. The inducibility of the glucose transport system in *C. vulgaris* was exceptionally high, as compared with other organisms. Bacteria, for example, with inducible glucose transport generally can be stimulated by a factor less than 10 by induction (Lengeler, personal communication).

Although the inducible uptake system of *C. vulgaris* might be a good system to study regulation of protein synthesis in a eucaryotic cell, its major drawback is that hitherto it has not been possible to show directly and characterize the protein(s) involved. Furthermore this is the main reason it has been impossible to decide whether the "turnover" in uptake activity is caused by an inactivation of the transport system or a breakdown of protein(s). The inhibitory effect of actidione on the turnover observed here is much smaller than, for example, on isocitritase (24), another inducible protein in *Chlorella*. Thus, if a protease is required for the decrease in hexose uptake activity, it probably doesn't have to be synthesized *de novo*.

The question of how long it takes a eucaryotic cell to synthesize a protein has repeatedly been asked (*e.g.*, Ref. 16). The data available for nitrate reductase in plants indicate that it takes about 30 to 60 min (5, 17). In contrast, the synthesis of a bacterial functional protein takes 2.5 min (2). The time of 15 min required by *Chlorella* compares favorably with the velocity in bacterial cells, if one considers the large difference in doubling time (8 hr compared to 20 min).

The question of the topology of induction has been extensively investigated in bacteria (7, 18, 26). To our knowledge eucaryotic cells have not been examined for this phenomenon so far. Heimer and Filner (5) have shown that a large nitrate pool within cultured tobacco cells is not responsible for induction of the nitrate uptake system and the nitrate reductase. They postulate the existence of separate substrate and inducing pools; from their data it cannot be excluded that the latter is exogenous nitrate.

Protein synthesis that is regulated by exocellular molecules is not restricted to the induction of bacterial transport systems. An analogous situation can be seen in the action of hormones which regulate their target cells and tissues from outside the cells (9). The results presented for the induction of hexose transport in *Chlorella* show, however, that the inducer acts from within the cells as in the case for β-galactoside transport in *E. coli* (7 ,8).

Glucose is taken up by induced cells with a K*m* about 30 times lower than that for 6-dG (4). Since there is a similarly large difference between the K*m* values for these two sugars for induction (Figs. 2 and 3), this might indicate that the uptake of inducer into noninduced cells proceeds *via* constitutive carrier molecules present.

The fact that induction can be brought about by glucose analogues, which are not metabolized at all (13, 21), suggests that the transport system of *C. vulgaris* is induced directly by glucose. It has to be pointed out, however, that in the analogous situation in *E. coli*, the inducer for the lac operon is not lactose, but allo-lactose (8). Thus, it still is possible that the physiologically active inducer in *C. vulgaris* is a metabolite derived from glucose.

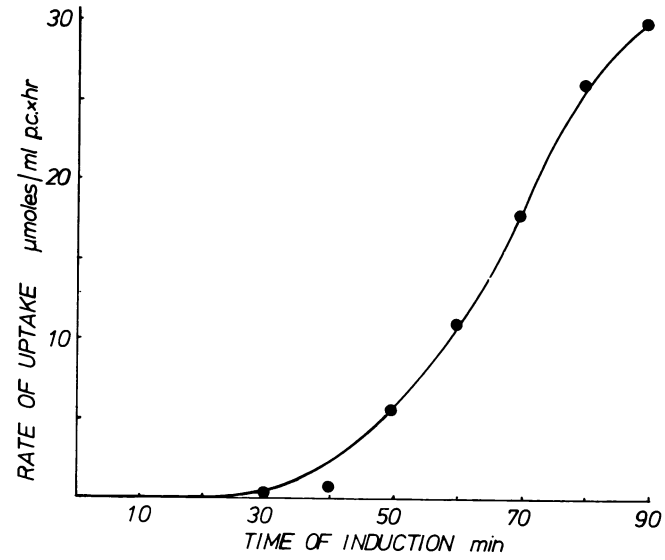

FIG. 8. Time course of induction with 1 mM 6-dG as inducer. 460 μl p.c. were shaken in 16.5 ml of 32 mM sodium phosphate buffer in the presence of 6-dG. At the times indicated, aliquots were withdrawn and their uptake activity determined with 6-dG after washing. For details see Fig. 1 and "Materials and Methods."

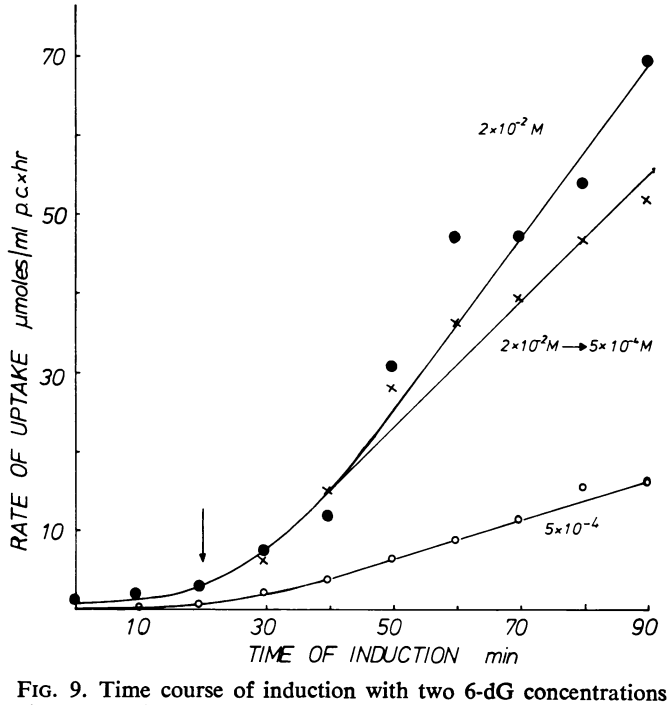

FIG. 9. Time course of induction with two 6-dG concentrations (maintenance phenomenon). 576 μl p.c. were shaken in 24 ml of 32 mM sodium phosphate buffer with 20 mM 6-dG present. After 20 min of incubation, two samples of 12 ml were centrifuged. One sample (●) was resuspended in 20 mM 6-dG, while the other was transferred to a medium with only 0.5 mM 6-dG (×). Uptake rates were determined as described in Fig. 1. Control (○), uptake rates of cells incubated all the time in 0.5 mM 6-dG.

Acknowledgments—Thanks are due Z. Kohlhund for skillful technical assistance and A. Oaks for a helpful discussion during the preparation of the manuscript.

LITERATURE CITED

1. BÖGER, P. 1969. Photophosphorylierung mit Chloroplasten aus *Bumilleriopsis filiformis* Vischer. Z. Pflanzenphysiol. 61: 85–97.

2. BRANSCOMB, E. W. AND R. N. STUART. 1968. Induction lag as a function of induction level. Biochem. Biophys. Res. Commun. 32: 731–738.
3. COHN, M. 1957. Contributions of studies on the β-galactosidase of *Escherichia coli* to our understanding of enzyme synthesis. Bacteriol. Rev. 21: 140–168.
4. DECKER, M. AND W. TANNER. 1972. Respiratory increase and active hexose uptake of *Chlorella vulgaris*. Biochim. Biophys. Acta 266: 661–669.
5. HEIMER, Y. M. AND P. FILNER. 1971. Regulation of the nitrate assimilation pathway in cultured tobacco cells. III. The nitrate uptake system. Biochim. Biophys. Acta 230: 362–372.
6. HEPPEL, L. A. 1969. The effect of osmotic shock on release of bacterial proteins and on active transport. J. Gen. Physiol. 54: 955–1135.
7. HERZENBERG, L. A. 1959. Studies on the induction of β-galactosidase in a cryptic strain of *E. coli*. Biochim. Biophys. Acta 31: 525–538.
8. JOBE, A. AND S. BOURGEOIS. 1972. Lac repressor-operator interaction. VI. The natural inducer of the lac operon. J. Mol. Biol. 69: 397–408.
9. JOST, J.-P. AND H. V. RICKENBERG. 1971. Cyclic AMP. Annu. Rev. Biochem. 40: 741–774.
10. KESSLER, E., I. LUDEWIG, AND H. WIECHMANN. 1963. Bildung von Sekundär-Carotinoiden bei Stickstoffmangel und Hydrogenase-Aktivität als taxonomische Merkmale in der Gattung *Chlorella*. *In:* Studies on Microalgae and Photosynthetic Bacteria. The University of Tokyo Press, Tokyo. pp. 7–20.
11. KOMOR, E., D. HAASS, B. KOMOR, AND W. TANNER. 1973. The active hexose uptake system of *Chlorella vulgaris*. Km values for 6-deoxyglucose influx and efflux and their contribution to sugar accumulation. Eur. J. Biochem. In press.
12. KOMOR, E., D. HAASS, AND W. TANNER. 1972. Unusual features of the active hexose uptake system of *Chlorella vulgaris*. Biochim. Biophys. Acta 266: 649–660.
13. KOMOR, E. AND W. TANNER. 1971. Characterization of the active hexose transport system of *Chlorella vulgaris*. Biochim. Biophys. Acta 241: 170–179.
14. KOTYK, A. AND K. JANÁCEK. 1970. Cell Membrane Transport. Plenum Press, New York-London.
15. MCCULLOUGH, W. AND P. C. L. JOHN. 1972. The inhibition of functional RNA synthesis in *Chlorella pyrenoidosa* by 6-methylpurine. New Phytol. 71: 829–837.
16. NISSL, D. AND M. H. ZENK. 1969. Evidence against induction of protein synthesis during auxin-induced initial elongation of *Avena* coleoptiles. Planta 89: 323–341.
17. OAKS, A., W. WALLACE, AND D. STEVENS. 1972. Synthesis and turnover of nitrate reductase in corn roots. Plant Physiol. 50: 649–654.
18. REUSER, A. J. J. AND P. W. POSTMA. 1973. The induction of translocators for di- and tricarboxylic acid anions in *Azotobacter vinelandii*. Eur. J. Biochem. 33: 584–592.
19. ROBBIE, J. P. AND T. H. WILSON. 1969. Transmembrane effects of β-galactosides on thiomethyl-β-galactoside transport in *Escherichia coli*. Biochim. Biophys. Acta 173: 234–244.
20. SHIHIRA-ISHIKAWA, I. AND E. HASE. 1964. Nutritional control of cell pigmentation in *Chlorella protothecoides* with special reference to the degeneration of chloroplast induced by glucose. Plant Cell Physiol. 5: 227–240.
21. TANNER. W. 1969. Light-driven active uptake of 3-O-methylglucose via an inducible hexose uptake system of *Chlorella*. Biochem. Biophys. Res. Commun. 36: 278–183.
22. TANNER, W., R. GRÜNES, AND O. KANDLER. 1970. Spezifität und Turnover des induzierbaren Hexose-Aufnahmesystems von *Chlorella*. Z. Pflanzenphysiol. 62: 376–386.
23. TANNER, W. AND O. KANDLER. 1967. Die Abhängigkeit der Adaption der Glucose-Aufnahme von der oxydativen und der photosynthetischen Phosphorylierung *Chlorella vulgaris*. Z. Pflanzenphysiol. 58: 24–32.
24. THURSION, C. F., P. C. L. JOHN, AND P. J. SYRETT. 1973. The effect of metabolic inhibitors on the loss of isocitrate lyase activity from *Chlorella*. Arch. Mikrobiol. 88: 135–145.
25. WINKLER, H. H. 1970. Compartmentation in the induction of the hexose-6-phosphate transport system of *Escherichia coli*. J. Bacteriol. 101: 470–475.
26. WINKLER, H. H. 1971. Kinetics of exogenous induction of the hexose-6-phosphate transport system of *Escherichia coli*. J. Bacteriol. 107: 74–78.