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UNUSUAL FEATURES OF THE ACTIVE HEXOSE UPTAKE SYSTEM OF *CHLORELLA VULGARIS*

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SUMMARY

1. No indication for a co-transport of hexoses and Na^+ or K^+ in *Chlorella vulgaris* has been obtained.

2. Uncoupling agents added during the steady state of 3-*O*-methylglucose uptake do not cause any efflux, although net influx and steady-state influx of the sugar are strongly inhibited at the same poison concentration.

3. The rapid exchange efflux initiated by the addition of non-radioactive sugars to cells in the steady-state of 3-*O*-methyl [^{14}C]glucose uptake can be completely inhibited by uncouplers.

4. Net efflux achieved by high dilution is a very slow process. The rate of efflux is increased by a factor of 30–80 when the dilution medium contains hexoses (trans-stimulation).

5. Carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) inhibits uptake also below concentration equilibration.

6. The Q_{10} values of net influx, steady-state efflux and influx are 2.8, 2.4 and 2.8, respectively; for net efflux considerably higher values were observed.

7. Although a very high transmembrane effect for efflux and only a small one for influx was observed, no indication for an additional diffusion barrier was obtained.

8. Based on the results in this and the subsequent paper a transport model is proposed. Metabolism is thought to accelerate the translocation both of the carrier–substrate complex into and of free carrier out of the cells.

INTRODUCTION

Cells of the unicellular green alga *Chlorella vulgaris* possess an inducible active uptake system for hexoses[1]. Sugar accumulation in these cells can be described as a pump and leak system and it has been shown that no phosphorylation of the sugar has to take place during the actual translocation and accumulation[2].

In this paper influx, efflux and exchange transport mainly of 3-*O*-methylglucose have been studied in greater detail. From the effect of uncoupling agents on these various fluxes it has been concluded that *Chlorella* does not possess a facilitated diffusion system: sugar translocation is intimately linked to the energy dissipating

Abbreviation: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

system. A similar conclusion has been reached by Höfer and Kotyk[3] for sugar uptake in *Rhodotorula gracilis* and recently Koch[4] has also stated this for β-galactoside transport of *Escherichia coli*. However, the most unusual feature of the uptake system of *Chlorella* is the complete inhibitability of steady-state influx and efflux by uncoupling agents.

Experiments concerning the energetics of sugar uptake in *Chlorella* are reported in the subsequent paper[5].

MATERIALS AND METHODS

The strain of *Chlorella vulgaris* used and the conditions of culture were the same as previously described[6]. 3-*O*-methylglucose was obtained from Calbiochem, D-[^{14}C]glucose from the Radiochemical Centre Amersham, and 6-deoxyglucose from Koch–Light Laboratories, Colnbrook, England. Carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) was a generous gift of Dr P. Heytler, Dupont de Nemours. Membrane filters of 0.8 μm pore size were purchased from Sartorius GmbH, Göttingen.

Adaption and uptake experiments: The adaption of the *Chlorella* cells and the uptake experiments were conducted as previously described[2]. All measurements were carried out at 26 °C. All data are related to packed cell volume without the correction for extracellular water (about 33 %).

RESULTS

Effect of K^+ and Na^+ on 3-O-methylglucose uptake in Chlorella

It has been reported repeatedly that in various organisms amino acids as well as sugars are co-transported with sodium ions[7–9]. Therefore, it was of interest to check whether there exists an effect of sodium or potassium ions on hexose transport in *Chlorella*. For these experiments cells were grown either in the normal medium which contains only K^+ and no Na^+ or in a K^+-free medium containing all the K^+ salts in the sodium form. In the second medium the cells showed a considerably decreased growth rate. Uptake of 3-*O*-methylglucose was then measured into the two types of

TABLE I

EFFECT OF SODIUM OR POTASSIUM ON UPTAKE OF 3-*O*-METHYLGLUCOSE

Chlorella cells, grown as described in the text with potassium salts or sodium salts respectively were washed three times and were induced for uptake in deionized water. 28 μl packed cells were then incubated in 2 ml deionized water or 0.04 M phosphate buffer (pH 6.5) with 0.2 μCi 3-*O*-methylglucose (spec. act. 2 μCi/μmole). Samples of 0.5 ml were withdrawn after 3 and 6 min.

Incubation conditions	*Rate of sugar uptake (nmoles/min per 3.5 μl packed cells)*	
	Cells grown on potassium salts	*Cells grown on sodium salts*
Deionized water	0.11	0.030
Potassium phosphate buffer	0.11	0.023
Sodium phosphate buffer	0.092	0.020

cells; during the experiment the cells were incubated either in sodium or potassium phosphate buffer or in deionized water. From the data of Table I it can be seen that the cells grown in the normal K^+ medium did not show significant differences in the uptake rates in the presence and absence of ions. Also the cells grown in the Na^+ medium were not stimulated by the addition of ions to the incubation medium. The lower uptake rates of the Na^+ cells is most likely an expression of the fact that these cells grow rather slowly and appear less healthy.

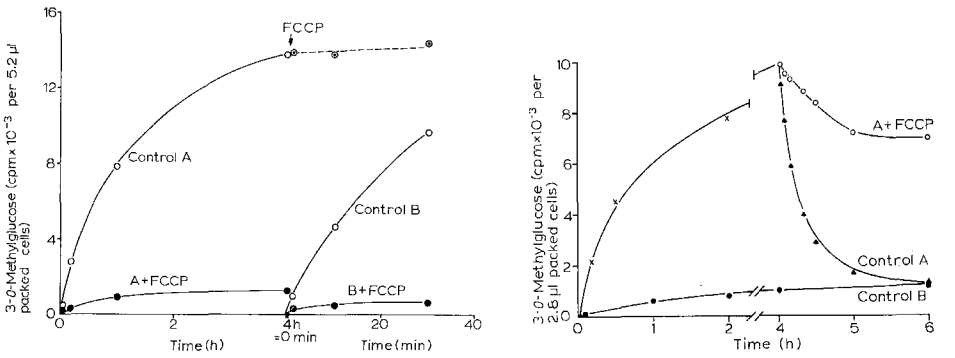


Fig. 1. Inhibition of influx and steady-state influx by FCCP. Sample A: In a total volume of 6.7 ml *Chlorella* suspension (70 µl packed cells) in 0.025 M sodium phosphate buffer (pH 6.5), 0.5 µCi 3-*O*-methylglucose (30 µmoles) were present from time zero. At 4 h FCCP ($5 \cdot 10^{-5}$ M) was added to this control. A second sample contained this amount of FCCP from time zero. Sample B: From time 0 to 4 h the same composition as A except that only 30 µmoles nonradioactive 3-*O*-methylglucose were present. At 4 h 0.5 µCi 3-*O*-methyl[^{14}C]glucose (spec. act. 10 µCi/µmole) was added. To a parallel sample FCCP ($5 \cdot 10^{-5}$ M) was added together with the radioactive sugar. Note the difference in time scale after 4 h.

Fig. 2. Inhibition of exchange of accumulated 3-*O*-methylglucose by $5 \cdot 10^{-5}$ M FCCP. 144 µl packed cells were incubated in 12 ml 0.032 M sodium phosphate buffer (pH 6.5) with 1 µCi 3-*O*-methyl[^{14}C]glucose (0.05 µCi/µmole). Samples of 0.5 ml were taken and the sugar content in the cells determined (Materials and Methods). After 4 h samples of 4 ml were withdrawn and 1 ml 3-*O*-methylglucose (180 µmoles) with or without FCCP (final concn $5 \cdot 10^{-5}$ M) was added (A). In a parallel experiment (B) 48 µl packed cells were shaken in 5 ml 0.026 M sodium phosphate buffer (pH 6.5) with 0.33 µCi 3-*O*-methyl[^{14}C]glucose (1.77 nCi/µmole).

Effect of uncouplers on influx, efflux and exchange transport of 3-O-methylglucose

$5 \cdot 10^{-5}$ M FCCP, a concentration acting optimally as uncoupler in *Chlorella*[2], has been shown to strongly inhibit 3-*O*-methylglucose uptake[2]. Since the accumulation plateau reflects a steady-state situation[2] it was expected that the addition of FCCP to cells during the steady-state plateau would result in a rapid efflux. However, as can be seen in Fig. 1, this is not the case. The same concentration of FCCP which inhibits the initial influx by almost 90 % does not initiate any efflux when added during the steady-state plateau. That FCCP does inhibit the steady-state influx to the same extent as it does the initial influx can be seen in the same figure (realize the different time scale; the steady-state influx is more rapid than the initial influx reflecting a transmembrane effect).

During the steady state an efflux does, of course, exist which can be detected by the addition of an excess of nonradioactive 3-*O*-methylglucose (Fig. 2) or other sugars transported by the same uptake system[2]. Fig. 2 shows that the radioactive

sugar is rapidly lost under these conditions until a new steady-state is reached. The new plateau is identical to that which is reached when cells are incubated from time zero with a low specific activity of 3-*O*-methyl [^{14}C]-glucose (Fig. 2). The efflux which is detected by the means of the exchange transport is also strongly inhibited (85 %) by FCCP (Fig. 2); at a FCCP concentration of $1.2 \cdot 10^{-4}$ M this exchange efflux was even 100 % inhibited. This result is in logical agreement with the results of Fig. 1. However, both are surprising when compared to other sugar transport systems of bacteria or fungi[10–15].

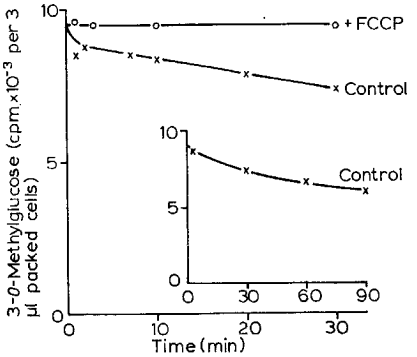


Fig. 3. Effect of dilution during steady-state. Insert: The same experiment observed for 90 min. 144 μl packed cells were shaken in 12 ml 0.032 M sodium phosphate buffer (pH 6.5) together with 1.0 μCi 3-*O*-methyl[^{14}C]glucose (spec. act. 0.033 μCi/μmole). For measurement of net efflux caused by dilution 5 ml were centrifuged, the supernatant discarded and the cells resuspended in 500 ml 0.032 M sodium phosphate buffer (pH 6.5) with or without FCCP (final concn $5 \cdot 10^{-5}$ M). Samples of 50 ml were withdrawn and the radioactivity in the cells determined.

The simplest explanation for the results of Figs 1 and 2 seems the following: the efflux of the sugar is not passive by diffusion but requires the same carrier which is necessary for influx. The carrier-substrate complex "moves" from the outside to the inside (*i.e.* at a high rate) only when metabolic energy is available. In the absence of energy no carrier will move in and thus no carrier is available for efflux. This explanation requires an additional assumption, unloaded carrier should not "move" to a significant extent even when sufficient energy is available, otherwise transport energy would be used up without any useful transport.

If the given explanation were correct certain predictions could be tested, *e.g.* (1) very little sugar should flow out of the cells when no or only a little sugar is present in the outside medium, *i.e.* after high dilution, and a large positive transmembrane effect should therefore be observable, for efflux, (2) carrier-mediated influx should be energy dependent already below concentration equilibration.

As can be seen from Fig. 3, the rate of efflux is indeed very low when cells, after having reached the steady-state, are centrifuged and resuspended in a volume 100 times the initial volume but without sugar present. The efflux rate in this experiment was 28 μmoles/h per ml packed cells, which corresponds to a relative rate of 0.3 ml/h per ml packed cells or 0.04 ml/min per g dry weight. The initial influx, which gives a minimum value for the steady-state influx, was 162 μmoles/h per ml packed cells. As a consequence of this slow efflux the accumulation of 3-*O*-methyl-

TABLE II

TRANSSTIMULATED EFFLUX OF HEXOSES

1.1 ml suspension of *Chlorella* cells (corresponding to 66 μl packed cells) which were in the steady-state of taking up 3-*O*-methylglucose and 6-deoxyglucose, respectively, were centrifuged to remove the medium. The pellet was resuspended in 110 ml either of the same medium as before but without any sugars or with 3-*O*-methylglucose ($1 \cdot 10^{-3}$ M) or 6-deoxyglucose ($1 \cdot 10^{-3}$ M) present. After dilution the radioactivity in the cells was determined by filtering aliquots of 20 ml.

Expt	*Sugar concn in the cells (M)*	*Rate of net efflux (μmoles/ml packed cells per h)*	*Rate of exchange efflux against*		*Ratio of fluxes exchange efflux / net efflux*	
			3-O-Methylglucose	*6-Deoxyglucose*	*3-O-Methylglucose outside*	*6-Deoxyglucose outside*
			(μmoles/ml packed cells per h)			
1	3-*O*-Methylglucose $5 \cdot 10^{-4}$	0.29	10.25	14.0	35	48
2	6-Deoxyglucose $1.3 \cdot 10^{-2}$	4.1	158	328	38	80

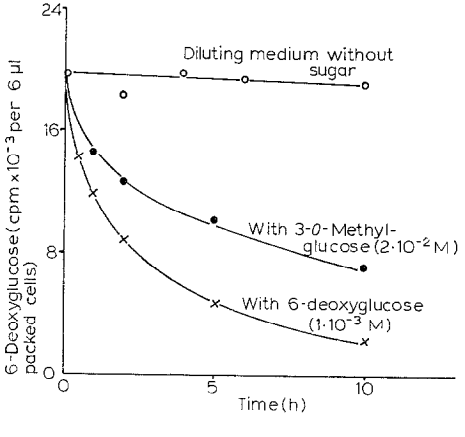


Fig. 4. Transstimulated efflux of 6-deoxyglucose. Experimental conditions see Table II.

glucose within the cells was still 15 000-fold the outside concentration after 90 min.

In a parallel experiment the accumulation ratio was checked when cells were incubated with the low concentration of 3-*O*-methylglucose which was present in the above sample after dilution: in the steady-state the inside concentration was now only about 320 times the outside one.

However, the efflux is greatly enhanced when the same dilution as above is carried out, except that the dilution medium also contains transportable sugars. In Table II data are given: it can be seen that the rate of efflux is increased by a factor of 30–80. The rates of Expt 2 were calculated using the 1-min values of the curves in Fig. 4. From this figure it is obvious that no exact rates, especially of the net efflux, can be determined. However, the large transstimulation can be clearly seen and the ratios given in Table II can be taken as conservative estimates of the fluxes. This kind of transstimulation has been first observed for amino acid transport in Ehrlich ascites cells[14] and has since been found for many uptake systems. However, the effect seems to be especially large in *Chlorella*.

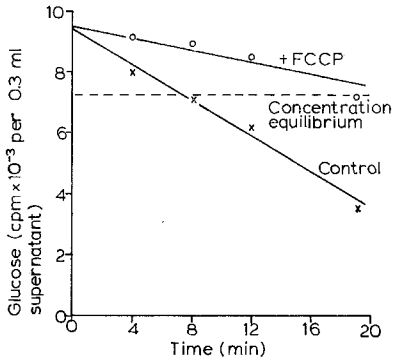


Fig. 5. Inhibition of glucose uptake below concentration equilibrium. 890 μl packed cells were shaken in 4 ml 0.032 M sodium phosphate buffer (pH 6.5) with 0.025 μCi [^{14}C]glucose (0.5 nCi/μmole). Samples of 0.8 ml were withdrawn and rapidly centrifuged in the cold. The radioactivity of 0.3 ml of the supernatant was determined.

The second testable prediction mentioned above, the energy requirement for uptake even below concentration equilibration, has actually already been tested and a positive result has been obtained[2]. However, these data were obtained by the standard membrane filter technique including washing the sample on the filter. Therefore a possible objection could be raised concerning these experiments: the washing could remove a certain fraction of the cell content and it would remain difficult to say whether one really measures uptake below concentration equilibration. To avoid this possible interference the disappearance of [^{14}C]glucose from the medium was measured after centrifugation.

From the known cell volume and total incubation volume the minimal amount of sugar was calculated which had to disappear from the medium for equilibrium concentration to be reached. Fig. 5 shows that FCCP clearly inhibits the disappearance of this amount of sugar. The fact that glucose is rapidly metabolized after entering the cells and a real concentration equilibrium is hardly reached at all does not affect the above considerations.

Efflux

The rate determining reaction for net sugar efflux is different from that of exchange efflux, the latter being considerably faster than the former. Accepting the assumptions made above, each of the following reactions could be rate limiting for net efflux, since they should not be of importance during exchange efflux: (1) passive diffusion, (2) free carrier outside ——→ free carrier inside, *i.e.* some carrier might be moving towards the inside although neither substrate is present in the outside medium nor energy is used. (3) Free carrier outside $\xrightarrow{+\text{ energy}}$ free carrier inside, *i.e.* some carrier can be moved using energy, although the carrier is not loaded.

The observation that FCCP ($5 \cdot 10^{-5}$ M) present in the dilution medium inhibits net efflux completely (Fig. 3) can be taken as an indication that Reaction 3 is required for this process. This reaction would take part to a negligible degree only in the recycling of the carrier during net influx and steady-state influx and efflux (Fig. 7). Therefore it seemed possible that some characteristics of net efflux, *e.g.* the temperature dependence, are different from the temperature dependence of these other processes.

Net efflux was measured by centrifuging steady-state cells and taking them up in 100 times the initial volume without sugar. Table III gives the Q_{10} values for the various fluxes. Although the values fluctuated considerably it is obvious that the energy of activation is very much larger for net efflux than for the three other fluxes measured. The very high value of around 8 for net efflux is reminiscent of the Q_{10} of about 7 for thiomethylgalactoside influx into poisoned *E.coli* cells[15].

TABLE III

Q_{10} VALUES OF THE VARIOUS FLUXES

Influx measurement: 54 µl packed cells were incubated in 6 ml 0.025 M sodium phosphate buffer (pH 6.5) with 5 mM 3-*O*-methylglucose. For net influx measurement the sugar was labelled from the very beginning, whereas for the estimation of steady-state influx traces of labelled sugar were added during steady-state as in Fig. 1. Efflux measurements: Cells were incubated with labelled 3-*O*-methylglucose as above until steady-state was reached. Then aliquots were withdrawn, centrifuged and resuspended in a 100-fold volume of 0.025 M sodium phosphate buffer (pH 6.5) with unlabelled 3-*O*-methylglucose of the steady-state concentration of the medium. Net efflux was measured in the same manner without 3-*O*-methylglucose in the dilution medium. The measurements were normally performed at 17, 22 and 27 °C.

Expt	*Net influx*	*Steady-state influx*	*Net efflux*	*Steady-state efflux*
1	1.8	3.1	—	3.7
2	3.0	2.8	8.9	2.3
3	3.15	2.4	6.9	1.75
4	3.24	2.95	10.0	1.9

Transmembrane effect and the concept of diffusion barrier

It has been pointed out that a large transmembrane effect for efflux is a prerequisite for the postulated model, which will be discussed in greater detail below. There is no "need" to assume an influx transmembrane effect, but there does exist a definite although slight transmembrane effect for 3-*O*-methylglucose influx: the uptake into preloaded cells is 1.3–1.5 times faster than into "empty" cells; for 6-deoxyglucose the factor is even around 2. Although this effect would not be

predicted from the model, it does not contradict it. The transmembrane effect for influx will be discussed in the subsequent paper[5]. However, the large asymmetry of the transmembrane effect should be noted in this context. Robbie an Wilson[16] have also observed an asymmetry with the β-galactoside uptake system of *E.coli*. The transmembrane factor for efflux was observed to be 7, whereas for influx it was less than 2. The authors explained this phenomenon by assuming an additional diffusion barrier outside the plasma membrane.

The crucial experimental data taken as evidence for this concept came from the observation that the transmembrane stimulation for influx increased with increasing outside concentration[16]. It was tested whether this is also the case for sugar uptake in *Chlorella*. Table IV shows that there is no significant change in the ratio of the influx rates with increasing sugar concentration in the outside medium. Therefore in *Chlorella* there seems to be a true asymmetry of the transmembrane effect, not simulated by a diffusion barrier which exists in addition to the plasma membrane.

TABLE IV

TRANSSTIMULATION OF INFLUX WITH A CONSTANT INSIDE CONCENTRATION BUT INCREASING OUTSIDE CONCENTRATION

To 3 ml *Chlorella* cells (60 μl packed cells) either preloaded with the sugars indicated or "empty" radioactive 3-*O*-methylglucose or 6-deoxyglucose was added at the concentrations given. Uptake of radioactive sugars was measured as usual. The concentration of sugar in the preloaded cells was $1 \cdot 10^{-3}$ M in Expt 1, $1.8 \cdot 10^{-3}$ M and $1.5 \cdot 10^{-2}$ M in Expts 2 and 3, respectively.

Expt	*Sugar concn outside (M)*	*Rate of influx (μmoles/ml packed cells per h)*		*Ratio of rates preloaded/nonpreloaded*
		Into non-preloaded cells	*Into preloaded cells*	
1	3-*O*-Methylglucose			
	$1 \cdot 10^{-3}$	31.6	46.3	1.46
	$5 \cdot 10^{-3}$	58.7	86.1	1.46
	$2 \cdot 10^{-2}$	90.6	121.3	1.34
2	3-*O*-Methylglucose			
	$1 \cdot 10^{-3}$	37.6	56.2	1.51
	$5 \cdot 10^{-3}$	68.8	106.3	1.54
	$2 \cdot 10^{-2}$	98.4	158.3	1.61
3	6-Deoxyglucose			
	$1 \cdot 10^{-3}$	134.5	246	1.83
	$5 \cdot 10^{-3}$	161.5	311	1.92
	$2 \cdot 10^{-2}$	206	342	1.66

DISCUSSION

When compared to other active sugar transport systems[7–13, 15–20] the system of the green alga *Chlorella vulgaris* differs quite considerably. No phosphorylation of the sugar is required for transport[2] as is the case in some bacterial and possibly yeast systems[17–20] and no Na^+ seem to be co-transported (Table I) as seen, for example, in intestine transport[7, 8] and so far at least in one bacterial system[9]. When compared to uptake systems[10, 12, 13] sharing these properties with *Chlorella* two results

are especially striking. First, uncoupling agents which strongly inhibit net and steady-state influx do not initiate efflux when added in the steady-state; second, the steady-state efflux, detected by inhibiting ^{14}C-labeled sugar influx by large dilution of the outside hexose with a nonradioactive sugar (exchange transport), can be completely inhibited by uncouplers (with 2,4-dinitrophenol the same results as with FCCP have been obtained). Similar results have so far only been reported by Höfer and Kotyk[3] for *Rhodotorula gracilis*.

These results cannot be explained by any of the common models for active transport. According to the model proposed for amino acid uptake in Ehrlich carcinoma cells by Heinz and Walsh[21], for example, a large part of steady-state influx and efflux proceed independent of metabolic energy and should therefore not be inhibited by uncoupling agents.

As pointed out by Jacquez[22] it is sufficient that the rate constant of one of four reactions (α_1, β_2, k_{+2} and k_{+1}; see Fig. 6) increases due to its linkage to metabolism to change a facilitated diffusion transport system to an actively transporting one. In active amino acid transport in the intestine the reaction in question has been assumed to be k_{+1}[21], whereas Wong *et al.*[23] have come to the conclusion that an increase in β_2, *i.e.* the dissociation constant of the carrier–substrate complex in the cells, causes β-galactoside accumulation in *E.coli*. This agrees with the earlier suggestion of Winkler and Wilson[24]. When metabolism is blocked by poisons all corresponding rate constants become equal again ($k_{+1} = k_{-1}$; $\beta_1 = \beta_2$) and the transport system can only bring about concentration equilibration. The main difference for *Chlorella* is that no observable transport whatsoever is found to take place when the energy supply is blocked completely. By the reasoning given above this means that the rate constant of the reaction linked to metabolism approaches zero when energy is lacking. It also means, according to Jacquez[22], that the rate constant corresponding to the one increased by metabolism (*e.g.* β_1 in case β_2 were increased by metabolic energy) would have to be close to zero all the time. This consideration immediately excludes the possibility that in *Chlorella* α_1 or β_2 are the reactions linked to metabolism, at least not as the only ones, since reactions β_1 and α_2 are fast enough to allow rapid exchange reactions.

If it is assumed that k_{+1} is increased due to energy supply and that in the presence of poisons k_{+1} equals k_{-2} and both are extremely small then no net efflux should occur when metabolism is poisoned during steady-state. This is indeed observed. However, exchange fluxes should not be affected by the poison in this case, but they are, in fact, severely affected. If, on the other hand, the same assumptions are made for k_{+1} and k_{-2} the experimental findings are in agreement with the model. It is suggested, therefore, that k_{+2} is increased due to energy coupling and that the reaction

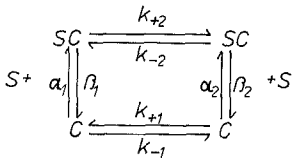
$$CS_{out} \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} CS_{in}$$

is extremely slow in the absence of energy supply.

However, all results reported cannot be explained by this model. Therefore a more complex model depicted in Fig. 7 had to be proposed for hexose uptake of *Chl. vulgaris*.

In the paragraphs to follow this model shall be explained and the evidence for

it summarized. Net uptake of sugar, *i.e.* uptake immediately after the sugar has been added to the cells, is thought to proceed by the sequence shown in A, steady-state fluxes by the sequence shown in B. Evidence for the statement that the reversal of reaction 1, *i.e.* 1_{out}, produces energy will be given in the subsequent paper[5].



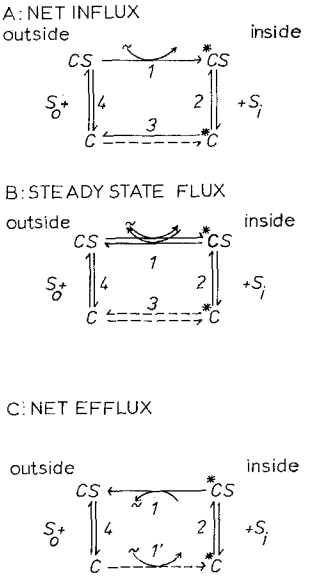


Fig. 6. Model for active uptake taken from Fig. 2 of Jacquez[22].

Fig. 7. Model for active sugar uptake of *Chlorella vulgaris*. The reactions suggested for net efflux (C) are thought to take place all the time, *i.e.* also during conditions A and B. They are omitted there since the velocity of the rate limiting step in C is negligible when compared to the rates of the cycles in A and B. Other explanations see Discussion.

The translocation of the free carrier C from the outside to the inside by reaction 3_{in} must be a very slow reaction since otherwise it should bring about a high rate of net efflux which should also not be inhibited by FCCP and dinitrophenol. The rate constant for efflux of the carrier *via* this reaction (3_{out}) must be high, since this reaction is required for net influx. This means that the two rate constants of reaction 3 have to differ greatly, which can be explained, for example, assuming that C_{inside} (= $*C$) is different from $C_{outside}$.

Thus, essentially it is assumed here, according to Jacquez[22], that the linkage to metabolism results in speeding up two reactions: the influx of CS and the efflux of the free carrier. Chemically this can be easily visualized, *e.g.* by phosphorylation of CS during influx and dephosphorylation of $*C$ (= the phosphorylated carrier) during efflux. Basically this part of the model would correspond to the model proposed by Kennedy[25] for β-galactoside uptake of *E. coli*.

Steady-state efflux does proceed by reaction 1_{out}, whereas reaction 3 should be almost zero during steady-state since no net movement of free carrier does occur. This is brought about by lowering the amount of $*C$ considerably, due to the large amount of internal sugar.

Finally, for net efflux a new reaction has to be assumed: 1′ (Fig. 7C). It should be considerably faster than the reaction $C \rightarrow {}^*C$ (3_{in}), since net efflux can be completely inhibited by uncouplers. The other reactions during net efflux would be the same as for steady-state efflux (Fig. 7).

The transmembrane effect and especially the large asymmetry of the effect can be explained by the model: The limiting reaction for net efflux 1′ is much smaller than reaction 1_{in} giving rise to a very dramatic transmembrane stimulation for efflux. A transmembrane stimulation for influx should arise if efflux of *C (reaction 3_{out}) during net influx is slower than the efflux reaction 1_{out}, which basically corresponds to the explanation given by Heinz and Walsh[21].

Finally it should be pointed out that from the experiments carried out so far it cannot be excluded that the dissociation of *CS inside the cells differs from the dissociation of CS at the outer surface of the cells. Essentially this means that even a third reaction might be speeded up by energy dissipation.

The uptake model presented explains that hexoses are rapidly transported by *Chlorella* as long as sugars are only outside or are outside as well as inside, but are transported very much slower when they are only inside the cells. This system thus has the properties of a valve. In addition *Chlorella* cells containing a large amount of accumulated sugar do not run empty as rapidly as bacterial cells, for example, whenever there is a lack of energy. Therefore from a physiological point of view this transport system seems to have great advantages when compared to bacterial sugar transport systems.

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