LIGHT-DRIVEN ACTIVE UPTAKE OF 3-O-METHYLGLUCOSE VIA AN INDUCIBLE HEXOSE UPTAKE SYSTEM OF CHLORELLA

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Summary:

D-Glucose adapted Chlorella cells take up 3-O-methylglucose-C^{14} linearly for more than 1 hour; cells not adapted show a lag of 6o min. 99 % of the radioactivity taken up is unchanged 3-O-methylglucose. The uptake proceeds against an inside concentration more than loo times higher than the concentration in the medium. Under anaerobic conditions the uptake is strongly stimulated by light (no CO_2 present). The uptake is inhibited by D-glucose and other hexoses, however, no inhibition was exerted by disaccharides.

It has been shown previously that glucose uptake and assimilation by Chlorella vulgaris proceeds with a lag phase of 4o to 6o minutes (1). This adaptation requires energy and it is completely prevented by actidion (1.5×10^{-5}M); the same concentration of actidion did not at all affect the uptake and assimilation of glucose by adapted cells (1). It has also been shown that the postulated protein induced by glucose is turning over: under non-growing conditions the cells lost their ability for linear glucose uptake in 1o to 15 hours (2). Since the hexokinase activity did not change during adaptation it had been suggested that Chlorella cells possess an inducible hexose uptake system (1). Evidence will be presented here that this is indeed the case.

Materials and Methods

The strain of Chlorella vulgaris and the culture conditions

have been the same as in previous work (1). 3-O-Methyl-glucose-^{14}C (3MG) was purchased from New England Nuclear Corp., non-radioactive 3MG from Calbiochem. 6-Deoxy-6-fluoro-D-galactose was a generous gift of Dr. John Barnett. Adaptation of the cells: Approximately 5oo µl packed cells were incubated in lo ml o,o4 M Na-phosphate buffer pH 6,5 in the presence of 8 mM D-glucose for 2-3 hours. Under these conditions the D-glucose was used up in 1 1/2 hrs, but the cells stayed induced for more than lo hrs (2). Other experimental conditions are given in the legends. Radioactivity was determined with the Liquid Scintillation Spektrometer, Beckmann LS loo.

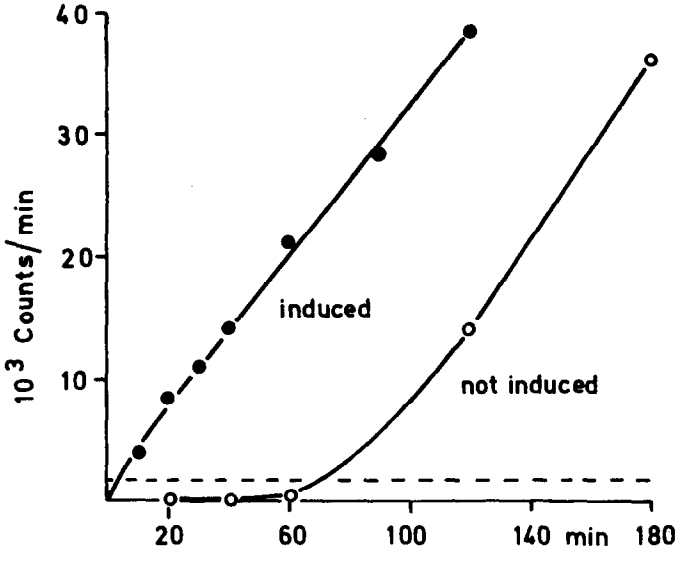

Fig.1. Time course of 3MG-^{14}C uptake by Chlorella vulgaris pretreated and not pretreated with D-glucose. In a total volume of 4,2 ml Na-phosphate buffer o,o3 M pH 6,5 15o µl packed cells were incubated in a small Erlenmayer flask and rapidly shaken in the dark. The medium contained o,45 µC of 3MG (s.A. 1 µC/17,4 µmoles; lo ooo cpm = o,11 µmole). Aliquots of o,3 ml were taken at the times indicated, filtered through membrane filters, washed with 2 times 2 ml cold buffer, and the cells subsequently extracted with 8o % ethanol. Each point represents the radioactivity per o,3 ml aliquot; the dashed line indicates the concentration equilibrium.

Results

Fig. 1 shows the difference in the time course of 3-O-methyl-glucose-^{14}C (3MG) uptake of Chlorella vulgaris adapted and non-adapted for glucose. The fact that non-adapted cells start to take up 3MG after 4o to 6o min can be explained by the observation that 3MG itself acts as an inducer (2). The radioactivity taken up by each sample was chromatographed after extraction in ethylacetate-n-butanol-acetic acid-water = 3:4:2,5:4, and n-butanol-pyridin-acetic acid-water = 6o:4o:3:3o. The amount of radioactivity corresponding to 3MG was in all cases larger than 99 %.

In another experiment the disappearance of radioactivity from the medium was also measured (fig.2). Each pair of experimental points was obtained by analyzing an aliquot of 8oo µl of cell

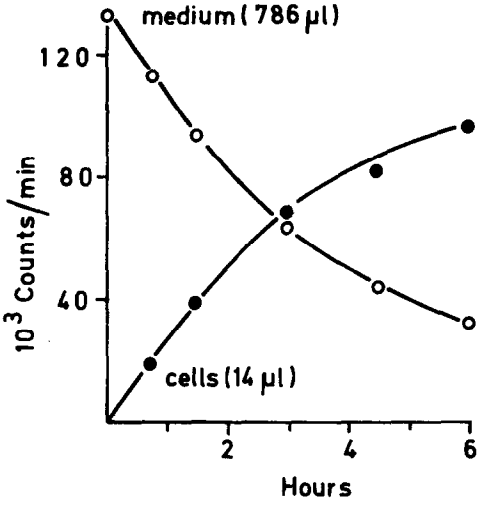

Fig.2. Increase in ethanol-extractable radioactivity of the cells and decrease of radioactivity in the medium. In a total volume of 8,4 ml 147 µl of packed cells were incubated. o,9 µC of 3MG-^{14}C was present; other conditions as in fig.1. o,8 ml aliquots were taken, rapidly centrifuged at 0°. o,1 ml of the clear supernatant was withdrawn to determine the radioactivity in the medium; then the cells were resuspended and treated as in fig.1.

suspension containing 14 µl of packed cells[1] for radioactivity in the cells and in the medium. The sum of the ethanol-extractable radioactivity plus the radioactivity remaining in the medium amounts in all cases to more than 97 % of the total initial activity, except for the values at 4 1/2 hours, where only 93 % have been recovered. The uptake after 4 1/2 hours proceeds against a 1o5 fold concentration gradient and after 6 hours the concentration in the cells is 164 times of that in the medium.

That 3MG is actively taken up is furthermore seen in the energy requirement of the process (Table 1). The uptake is strongly reduced under anaerobic conditions. In the light, however, the uptake proceeds under anaerobic conditions as well as under

Table 1

Energy requirement for 3-O-methylglucose uptake

Conditions	Time (min)	Counts/min taken up	Conc.inside / Conc.outside
Air/Dark	3o	16 39o	3,9
Air/Dark	6o	32 43o	8,7
N_2/Dark	3o	1 26o	o,27
N_2/Dark	6o	2 12o	o,45
N_2/Light	3o	23 45o	5,9
N_2/Light	6o	41 1oo	11,8
Air/Light	3o	24 81o	6,2

In a total volume of 1,3 ml Na-phosphate buffer o,o25 M pH 6,5 4o µl packed cells were incubated in a Warburg vessel. 0,1 µC of 3MG (s.A. 1 µC/63 µmoles; 1o ooo cpm = o,41 µmole) was added from the side arm at time 0. The anaerobic samples were flushed with purified N_2 and contained alkaline pyrogallol in the center well. Where indicated white light of 2 ooo lux was used. At the various times o,8 ml aliquots were filtered through membrane filters and treated as in fig.1. The radioactivity given in column 3 accounts for the uptake of the total sample.

1) Without correction for extracellular space, which would reduce the actual cell colume by one third (6).

aerobic ones, although no CO_2 is present which would allow the production of oxygen. Most likely the light dependent uptake of 3MG is brought about by cyclic photophosphorylation as this has been shown to be the case for glucose uptake and -assimilation (3,4,5). Under aerobic conditions in the dark it was observed that the endogenous respiration increases by more than 5o % due to the addition of 3MG. The uptake of 3MG showed Michaelis-Menten kinetics and a K_m of 5×10^{-4} was determined. The uptake was strongly inhibited by D-glucose and the K_i-value was found to be $1,5 \times 10^{-4}$. The uptake of 3MG at a concentration of 2×10^{-3}M was inhibited by other hexoses (5×10^{-3}M) to the following extent: D-galactose 64 %, D-mannose 29 %, 2-deoxyglucose 73 %, 6-deoxy-6-fluoro-D-galactose 73 %. There was no inhibition, however, with L-glucose, maltose, melibiose, and sucrose.

Discussion

These experiments show that *Chlorella vulgaris* cells are able to accumulate hexose analogues actively against a concentration gradient. This ability is inducible. The same has also been observed with *Chlorella pyrenoidosa*, whereas *Ankistrodesmus* and *Scenedesmus* seem to possess a constitutive hexose uptake system (2). Uptake of sugars against a concentration gradient in higher plants has been reported before (7,8); also the uptake of ions by *Chlorella* is possible against an electrical potential difference (6).

The light dependent anaerobic uptake of 3MG is of special interest. In anaerobic photoassimilation of D-glucose light energy of cyclic photophosphorylation is undoubtedly used for sucrose and starch synthesis (4,9,1o), although it has been

suggested that the actual uptake process, too, is supported by this light driven photophosphorylation (11). The data reported here, together with previous ones clearly show, that light energy is used for both processes. Two ATP have to be supplied, therefore, by light to assimilate one glucose (5,9) and a third energyrich phosphate is possibly required for the uptake. Since a quantum requirement of 4 per glucose taken up and assimilated has been determined at 712 mμ (5), it seems necessary to postulate more than one phosphorylating site in cyclic photophosphorylation.

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References:

1. Tanner, W., and O.Kandler, Z.Pflanzenphysiologie 58, 24 (1967).
2. Tanner, W., R.Grünes, and O.Kandler, Z.Pflanzenphysiologie, in print.
3. Kandler, O., Z.Naturforsch. 9b, 625 (1954).
4. Kandler, O., and W.Tanner, Ber.dtsch.Bot.Ges. 79, 48 (1966).
5. Tanner, W., E.Loos, W.Klob, and O.Kandler, Z.Pflanzenphysiologie 59, 3o1 (1968).
6. Barber, J., Biochem.Biophys.Acta 15o, 618 (1968).
7. Bielesky, R.L., Australien J.Biol.Sci. 13, 2o4 (196o).
8. Grant, B.R., and H.Beevers, Plant Phys. 38, 78 (1964).
9. Tanner, W., E.Loos, and O.Kandler, in Currents in Photosynthesis, ed. J.B.Thomas and J.C.Goedheer, A.D.Donker, Rotterdam 1966, p.243.
lo. MacLachlan, G.A., and H.Porter, Proc.Royal Soc. 15o, 46o (1959).
11. Smith, F.A., J.Exp.Botany 18, 348 (1967).