ANNAL. BIOL. TIHANY	32	247–253	HUNGARIA 1965

THE GLYCINE UPTAKE OF *SCENEDESMUS OBTUSIUSCULUS*

EDITH CSEH and ERNŐ SZABÓ

Received: March 15th 1961

While the metabolism of carbohydrates, the most important compounds of the Krebs cycle and lipids is fairly well-known in the case of algae (DANFORTH 1962), few investigations of the amino acids have been made in this direction. Previous data in literature were presented by SAUDERS (1957), while recent data can be found in the works of SYRETT (1962). Questions relating to the uptake, utilization and metabolism of amino acids deserve particular attention not only from the point of view of algal-physiology (as carbon and nitrogen source), but they also have a distinct role to play in nature, in the extremely important process of the self-purifying ability of waters as well as the mineralization of organic nitrogen compounds.

Several authors (ALGEUS 1948 a, b, c; BELCHER and FOGG 1958) maintain that the algae are capable of utilizing glycine as a nitrogen source. Examinations referring to the absorption of glycine, however, are still to be made. So far only SHRIFT and SPROUT (1963) are known to have carried out investigations to this end by examining the methionine uptake of *Chlorella vulgaris* and its incorporation into proteins. Based on the inhibitive effect of 2—4 DNP they have suggested that the methionine uptake is an active process.

Method

Scenedesmus obtusiusculus (CHODAT strain 5618) from the algal collection of the Biological Research Institute (Tihany) was used in the experiments. The suspension was grown in KNOP—PRINGSHEIM solution (PRINGSHEIM 1946) and aërated by 5 per cent carbon dioxide in air.

The nutrient solution of the culture before use was found to contain nitrate only in traces since it had already been absorbed by the cells. This thick suspension was then diluted 15 to 20 times by another dose of nutrient solution, depending on the number of cells of the culture. The experimental solution contained glycine and $NaHCO_3$ (350 mg/l) which served as nitrogen and carbon source respectively. To promote the gas exchange of the suspension and prevent sedimentation the samples were either incubated by a shaker (New Brunswick Co. thermostatic shaker which provides for giratory shaking) or they were rotated in light thermostate.

Fluorescent tubes of an intensity of 2000 luxes were used in the examinations in the light with thermostatic shaker. In the light thermostate which had been constructed at the Chair of Plant Physiology of Eötvös University

as suggested by STEEMANN NIELSEN (1961) the samples were placed in the compartments of a wheel held in a waterbath of constant temperature, in 100 ml reagent bottles. The waterbath was illuminated by F 29 fluorescent tubes and mercury-vapour lamps of low pressure (maximum intensity 50,000 luxes). Illumination in the compartments can be regulated by the neutral filter series of Chance and Co. A more detailed description of the light thermostate used will be given in a future publication.

After the time of exposition 10—10 ml aliquots of the suspension were filtered by a membrane filter (Co 5 filter plate, Membranfilter Gesellschaft Göttingen), then they were rinsed with nutrient solution containing inactive glycine of the same concentration; after drying up the activity of the 10^7 cells was measured on membrane plates by FRIESECKE—HOEPFNER methane gas flow counter.

Results

The time curve of glycine uptake is presented in *Figure 1.* The nutrient solution contained 5 mM glycine as nitrogen source. The period of the experi-

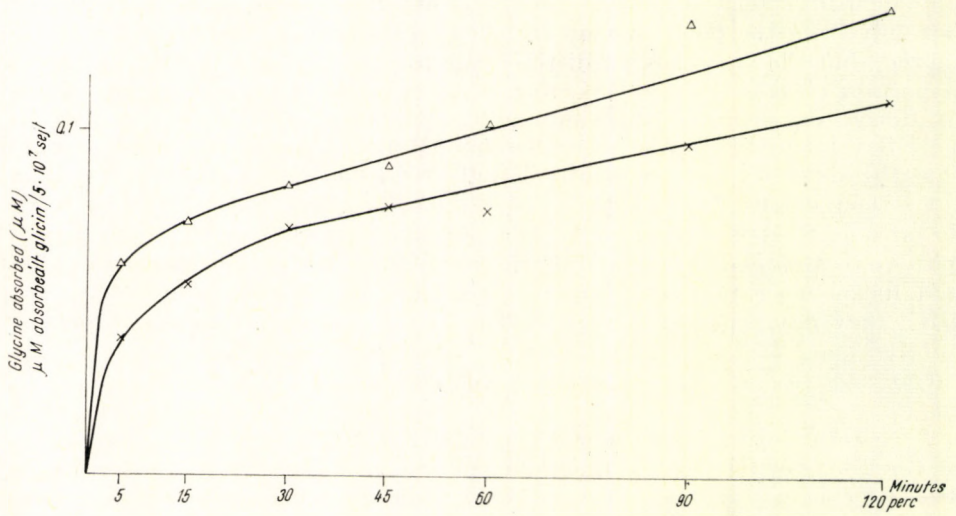

Fig. 1. Changes in the glycine uptake by *Scenedesmus obtusiusculus* (5 × 10^7 cells) in the light △ and dark × in relation to the time of incubation

1. ábra. A *Scenedesmus obtusiusculus* (5 × 10^7 sejt) glicinfelvételének változása fényen △ és sötétben × az inkubálási idő függvényében

ment ranged from 5 to 120 minutes. The absorption was examined in samples incubated in the light and in dark. At the beginning the uptake was found to be very rapid in cells kept either in the light or in dark. During the first five minutes the uptake of the illuminated cells accounted for 45% of the total quantity absorbed during two hours as against 35% by cells kept in the dark. In both cases the rate of uptake was reduced after 30 minutes but it remained steady during the subsequent period of up to 120 minutes. The

glycine absorbed by cells kept in the light was generally 30% higher on the points of measurement of the time curve.

It is noteworthy that the stimulatory effect of light can be detected as early as during the first five minutes and if expressed in percentage it shows no essential change throughout the whole period of examination. Since it is of common knowledge that the uptake of photosynthetic plants is likely to

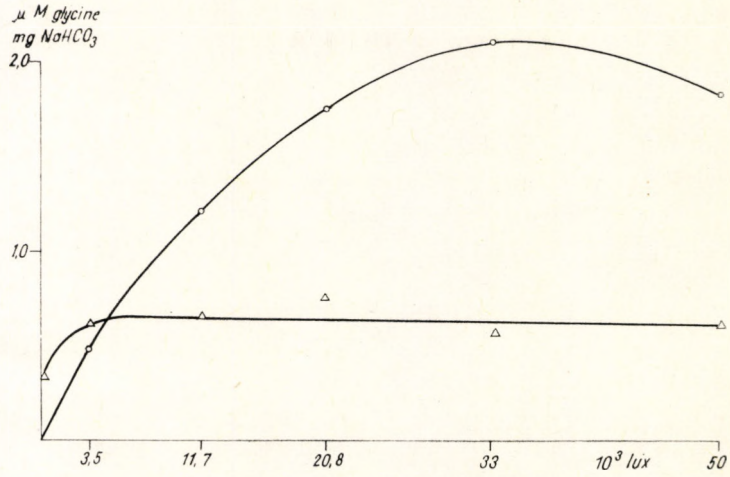

Fig. 2. The intensity of photosynthesis ○ and glycine uptake △ by *Scenedesmus obtusiusculus* (10^8 cells/2 hours) in relation to the changes in the intensity of light

2. ábra. A *Scenedesmus obtusiusculus* (10^8 sejt/2 óra) fotoszintézisintenzitása ○ és glicinfelvétele △ a beeső fényenergia változásának függvényében

be indirectly stimulated by light, we carried out investigations in light thermostate in order to determine optimum intensity. The results of the two hour experiments are presented in *Figure 2*. The effect of even 1200 luxes on glycine uptake can be considered optimum because more light failed to bring about a substantial increase in absorption. The intensity of photosynthesis shows the usual light saturation curve, reaching its maximum at 33,000 luxes (66%) under our experimental conditions. Since the course of the curve of glycine uptake is completely different from that of photosynthesis, the stimulatory influence of light is presumably of an indirect character.

The concentration curves of glycine uptake were also made in light thermostate, with the glycine concentration of the nutrient solution varying between 0.01—10.0 mM. In *Figure 3.* the changes in glycine absorption are given in relation to the glycine concentration in double logarithmic plot so as to present the results of the experiments over the whole concentration range. The same data are also presented in *Figures 4* and *5* on a linear scale. Unfortunately SHRIFT and SPROUT have failed to publish concentration curves referring to L-methionine so we give the concentration curve of glycine uptake by excized wheat roots in *Figures 3* and *4* for the sake of comparison (CSEH and BÖSZÖRMÉNYI 1964). According to the view formed during the studies

on the mechanism of ion uptake the active (carrier-mediate) absorption saturates at a certain external concentration. Here the rate of uptake reaches its maximum and becomes independent of the external concentration. Recently, however, the experiments made by a number of authors (BANGE and OVERSTREET 1960, BÖSZÖRMÉNYI and CSEH 1964, BÖSZÖRMÉNYI 1965, EPSTEIN,

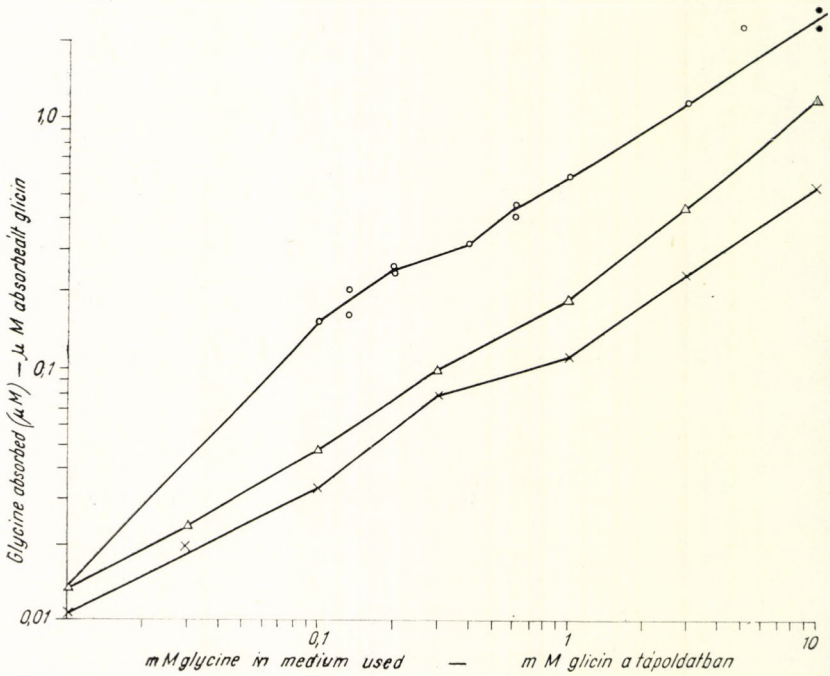

Fig. 3. Changes in glycine uptake by *Scenedesmus obtusiusculus* (10^8 cells/2 hours) in the light △ and dark × and by wheat seedling ○ (20 plants/1 hour) in relation to the glycine concentration of the nutrient solution

3. ábra. A *Scenedesmus obtusiusculus* (10^8 sejt/2 óra) fényen △ és sötétben × és a búzacsíranövény ○ (20 növény/1 óra) glicinfelvételének változása a tápoldat glicinkoncentrációjának függvényében

RAINS and ELZAM 1963, ELZAM, RAINS and EPSTEIN 1964) have pointed to the possibility that two or more uptake systems may play a part in the absorption of the same ion. We may run into difficulties when trying to divide the concentration curve into saturation stages if a sharp distinction cannot be made between the operation of the systems concerned by adopting mathematical and experimental methods.

The glycine uptake curve fails to indicate pronounced saturation either in the case of algae or wheat roots. The largest deviation of the curve from the linear one can be found with 0.2—0.4 mM and 0.3—1.0 mM external concentrations in the case of wheat roots and algae respectively. The slight deviation between the two materials may also stem from the fact that the concentration curve is not sufficiently detailed.

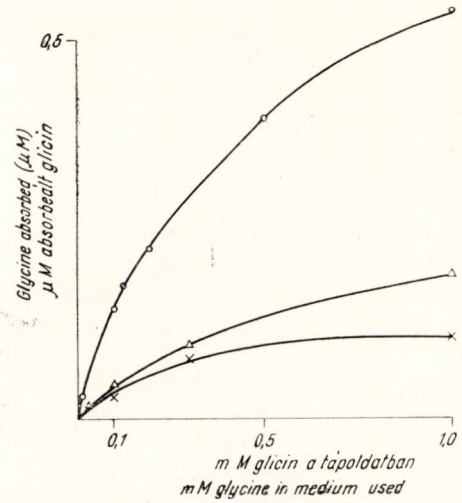

Fig. 4. Changes in glycine uptake by *Scenedesmus obtusiusculus* (10^8 cells/2 hours) in the light △ and dark × and by wheat seedlings (20 plants (1 hour) ○ in relation to the glycine concentration of the nutrient solution

4. ábra. A *Scenedesmus obtusiusculus* (10^8 sejt/2 óra) fényen △ és sötétben × és a búzacsíranövény ○ (20 növény/1 óra) glicinfelvételének változása a tápoldat glicinkoncentrációjának függvényében

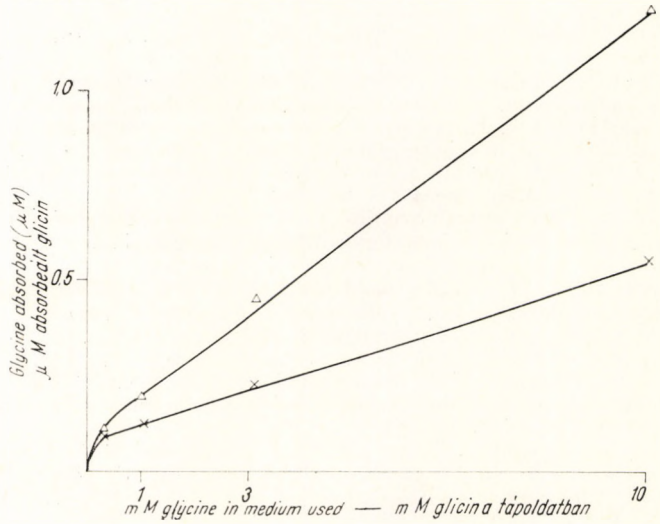

Fig. 5. Glycine uptake by *Scenedesmus obtusiusculus* (10^8 cells/2 hours) in the light △ and dark × in relation to the glycine concentration of the nutrient solution

5. ábra. A *Scenedesmus obtusiusculus* (10^8 sejt/2 óra) glicinfelvétele — fényen △ és sötétben × a tápoldat glicinkoncentrációjának függvényében

Summary

The uptake of glycine-C^{14} by *Scenedesmus obtusiusculus* has been examined in relation to the period of absorption, the intensity of light and the changes in the glycine concentration of the nutrient solution.

It could be concluded on the basis of the time curves made in the light and in darkness that in both cases there was an initial very rapid uptake period followed by a slower linear stage. The stimulatory effect of light began to operate right at the beginning of illumination and lasted until the end of the experiment.

Even a minimum light was found to stimulate absorption. Further increase in the intensity of light had neither a stimulatory nor inhibitive influence on uptake.

The extent of the stimulatory effect of light is dependent on the medium glycine concentration; there was a slight stimulation at 0.01 mM, but absorption was found to have been increased by more than 100% at 10.0 mM external concentration.

Despite the wide concentration interval used during the experiment, pronounced saturation cannot be observed on the concentration curves.

LITERATURE

ALGÉUS, S. (1948a): Glycocoll as a source of nitrogen for Scenedesmus obliquus. — *Physiol. Plantarum* **1**, 66—84.

ALGÉUS, S. (1948b): The utilization of glycocoll by Chlorella vulgaris. — *Physiol. Plantarum* **1**, 236—244.

ALGÉUS, S. (1948c): The deamination of glycocoll by green algae. — *Physiol. Plantarum* **1**, 382—383.

BANGE, G. G. J. and R. OVERSTREET (1960): Some observations on absorption of cesium by excised barley roots. — *Plant Physiol.*, **35**, 605—608.

BELCHER, J. H. and FOGG, G. E. (1958): Studies on the growth of Xanthophyceae in pure culture. III. Tribonema aequale Pascher. — *Arch. Mikrobiol.* **30**, 17—22.

BÖSZÖRMÉNYI Z. (1965): The ion-uptake of excised barley roots with special reference to the low concentration process. — (in press.)

BÖSZÖRMÉNYI, Z. and E. CSEH (1964): Studies of ion-uptake by using halide ions. Changes in the relationships between ions depending on concentration. — *Physiol. Plantarum* **17**, 81—90.

CSEH E.—BÖSZÖRMÉNYI Z. (1964): Magasabbrendű növények aminosavfelvételi mechanizmusának vizsgálata. — *VI. Biokémiai Vándorgyűlés Tihany.*

DANFORTH, W. F. (1962): Substrate assimilation and heterotrophy. In R. A. LEWIN: Physiology and biochemistry of algae. — *Academic Press* 99—123.

ELZAM, O. E., D. W. RAINE and E. EPSTEIN (1964): Ion transport kinetics in plant tissue: Complexity of the chloride absorption isotherm. — *Biochem. Biophys. Res. Comm.* **15**, 273—276.

EPSTEIN, E., D. W. RAINS and O. E. ELZAM (1963): Resolution of dual mechanisms of potassium absorption by barley roots. — Proc. Nat. Acad. Sci. **49**, 684—692.

PRINGSHEIM, E. G. (1946): Pure cultures of algae, their preparation and maintenance. *Univ. Press. Cambridge* 1—119.

SHRIFT, A. and M. SPROUT (1963): Nature of the stable adaptation induced by selenomethionine in Chlorella vulgaris. — *Biochim. Biophys. Acta* **71**, 332—344.

STEEMANN NIELSEN, E. and HANSEN, V. KR. (1961): Influence of surface illumination on plankton photosynthesis in Danish waters (56° N) throughout the year. — *Physiol. Plant.* **14**, 595—613.

A *SCENEDESMUS OBTUSIUSCULUS* GLICIN FELVÉTELE

Összefoglalás

Cseh Edit és *Szabó Ernő*

Scenedesmus obtusiusculus C^{14} jelzett glicin felvételét tanulmányoztuk az inkubálási időtartam, a fényintenzitás és a tápoldat glicinkoncentráció változásának összefüggésében.

A sötétben és fényen felvett időgörbék alapján megállapítható volt, hogy mindkét esetben, egy kezdeti igen gyors felvételi szakaszt egy lineáris lassúbb periódus követ. A fény serkentő hatása a megvilágítás kezdetén jelentkezik és tart az egész kísérlet alatt.

A felvétel szempontjából már minimális fény serkentő hatású. A fényintenzitás további növekedése a felvételre sem gátló, sem serkentő hatással nincsen.

A fény serkentő hatásának nagysága függ a medium glicin koncentrációjától 0,01 mMol esetén alig figyelhető meg, addig 10,0 mMol külső koncentrációnál a felvételt több mint 100%-kal növeli.

A kísérlet során használt széles koncentráció intervallum ellenére a koncentráció görbéken kifejezett telítődés nem figyelhető meg.

УСВОЕНИЕ ГЛИЦИНА У SCENEDESMUS OBTUSIUSCULUS

Эдит Чех, Э. Сабо

Было изучено усвоение глицина с меченной C^{14} Scenedemus obtusiusculus в зависимости от времени инкубации, интенсивности света и изменения концентрации глицина в питательной среде.

На основе кривых времени полученных при регистрации во свете и в темноте, было устоновлено, что в обоих случаях имеется начальная быстрая фаза, вслед за которой следует более медленный линеарный период в усвоении глицина. Стимулирующее влияние света появляется в начале освещения и продолжается в течении всего эксперимента.

С точки зрения усвоения глицина уже минимальное количество света обладает стимулирующим действием. Дальнейшее увеличение интенсивности света не оказывает ни тормозящего ни стимулирующего влияния усвоения глицина.

Стимулирующее действие света зависит от концентрации глицина в среде; при концентрации 0,01 мМ/л стимуляция усвоения глицина почти не наблюдается, а при концентрации 10 мМ/л усвоение глицина увеличивается в 100%.

Несмотря на широкие предели, применяемых концентрации, в ходе экспериментов не отмечалась насыщенность на концентрационных кривых.