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The transport of glycolic acid by *Chlamydomonas reinhardtii*

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Evidence for a transport system for glycolate in *Chlamydomonas* was obtained. [^{14}C]Glycolate was taken up rapidly, reaching an equilibrium in less than 2 s at 4°C. Glycolate uptake was stimulated by valinomycin and high KCl or high KCl alone and inhibited by *N*-ethylmaleimide. This uptake was not dependent on temperature or pH in contrast to uptake of benzoate by diffusion which decreased by orders of magnitude with increasing external pH. Based on these data, a transporter for glycolate is proposed.

Glycolate; Organic acid transport; *Chlamydomonas*

1. INTRODUCTION

Glycolate, pK_a 3.8, exists primarily as an anion at physiological pH and it is postulated that there exists a protein transporter for glycolate which may be essential to cell survival and must be present constitutively. Howitz and McCarty [1,2] have described a glycolate transporter in chloroplasts and Renstrom et al. [3] have described a glycolate transporter in *Anabeana*.

When *Chlamydonomas reinhardtii* is switched from high-CO_2 growth conditions to low-CO_2 growth conditions, there is an adaptation to low-CO_2 that takes about 3–5 h. During this time there is a large efflux of glycolate from the cells as a result of photorespiration. While the magnitude and timing of this excretion are well documented, little is known about the actual transport process of glycolate across the cell membrane for excretion. Saturation of glycolate transport has been claimed previously [4,5] but it has often been difficult to separate transport from subsequent metabolism [6]. Published data on glycolate uptake by algae have described the process over long periods of time (hours) and as has been shown in this laboratory [7], these experiments measure glycolate metabolism rather than the kinetics of uptake.

In this report experiments to test the existence of a transport system in *C. reinhardtii* are described. These experiments include concentration dependence, time and temperature dependencies different from simple diffusion and inhibition of transport. Results of these studies are consistent with the hypothesis that glycolate is transported across the plasma membrane by a protein transporter.

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2. MATERIALS AND METHODS

2.1. *Algae*

Chlamydomonas reinhardtii strain 90 was grown in 1 liter of the minimal medium of Sueoka [8] in 3 liter Fernbach flasks at 23–25°C with shaking, constant illumination at 125 $\mu E \cdot m^{-2} \cdot s^{-1}$ and aeration with air enriched with 5% CO_2. Cells were harvested after 3–4 days by centrifugation at 900 × *g* for 5 min at 4°C and washed once with water before resuspension in buffer as noted in figure legends. Chlorophyll was determined by the method of Arnon [9].

2.2. *Erythrocytes*

Erythrocytes were obtained by venous puncture from a human volunteer into a vacutainer and blood was allowed to clot on glass beads. After centrifugation, cells were resuspended in 6.5 mM sodium phosphate buffer, pH 7.4, containing 4.5 mM glucose and 140 mM NaCl to a hematocrit of 50% and kept at 4°C [10]. Protein was determined by a modified Folin-Lowry procedure [11].

2.3. *Uptake of monocarboxylic acids*

Uptake was measured using the silicone oil centrifugation method as modified by Howitz and McCarty [1,2]. Short time measurements of about 2 s were accomplished by pelleting the cells suspended in an upper layer of 200 µl through a 50 µl layer containing ^{14}C-labeled acid in buffer containing 10% Percoll into 20 µl of 4 N NaOH. The cells, ^{14}C-labeled acid and NaOH layers were separated from each other by layers of AR 20 and AR 200 silicone oils mixed 2:1 and 1:1, respectively. For longer time points, ^{14}C-labeled acid was added to the 200 µl of cells before centrifugation through a single layer of the 1:1 silicone oil mixture. Label in the cell pellet was determined by liquid scintillation counting. Internal volume of the cells was determined by incubation with ^3H_2O and [^{14}C]sorbitol as described by Moroney and Tolbert [7].

2.4. *Materials*

[^{14}C]Glycolate was obtained from ICN Biomedicals (Costa Mesa, CA), and ^3H_2O, [^{14}C]sorbitol and [^{14}C]benzoate were from New England Nuclear (Boston, MA). Percoll was from Pharmacia and Wacker silicone oils AR 20 and AR 200 were a gift from SWS Silicones Corp., Adrian, MI. Common laboratory chemicals were reagent grade and all solutions were prepared in deionized glass distilled water.

3. RESULTS AND DISCUSSION

3.1. *Time course and temperature and pH dependencies*

The time courses for glycolate and benzoate uptake are shown in Fig. 1. The uptake of glycolate was very rapid, reaching equilibrium within the time of the shortest technically possible time point in this procedure (about 2 s) at 4°C. Since glycolate uptake did not increase with time, it is unlikely that metabolism was involved in the measurement of the transport process. Glycolate metabolism at 4°C has not been measured but glycolate uptake and subsequent metabolism at 22°C have been shown to increase with time and to be light dependent as well [12]. In contrast, glycolate uptake for 2 s was not different in the light or dark (data not shown).

No difference in glycolate uptake at 4°C and 22°C was detectable (data not shown). In contrast, uptake of benzoic acid was more rapid at 22°C than at 4°C as expected for a diffusion process (Fig. 1). Since equilibrium for glycolate is already reached within 2 s at 4°C, it is expected that equilibrium would be achieved within that time at a higher temperature and no difference in uptake data would be observed. However, as shown in Fig. 1, higher temperature substantially increased benzoate uptake. The glycolate transporter described in chloroplasts is also very rapid reaching equilibrium within 10 s [1].

The time course for glycolate uptake was the same at pH 3 and pH 4 but the time courses for benzoate uptake were different at pH 3 and pH 4. Because of its lipophilic nature, benzoate would be expected to be more readily taken up than glycolate but the uptake of glycolate was more rapid than the uptake by diffusion of benzoic acid which has a similar pK_a. Benzoate uptake required 30 s to reach equilibrium at pH 4 unlike glycolate which had already reached equilibrium. At pH 3 where the concentration of the protonated species for diffusion is greater, benzoate had not reached equilibrium in the time shown in Fig. 1. This makes the difference in equilibration times between transport of glycolate and diffusion of benzoic acid more pronounced.

The external pHs chosen for the time course data shown in Fig. 1 are pHs at which high concentrations of the protonated species would favor diffusion. The data are consistent for the diffusion of benzoate. The uptake of glycolate and benzoic acid at different external pH was compared (Fig. 2). Benzoic acid uptake was strongly dependent on the pH. At pH 4 the internal concentration of benzoate is much less than at pH 3; at pH 7 the internal concentration is barely detectable. Glycolate uptake is measurable at pH 7 and is essentially constant across the pH range despite similar pK_a values between the two molecules. If glycolate crossed the membrane only as the undissociated acid, the uptake pattern should be similar to benzoic acid. Even at

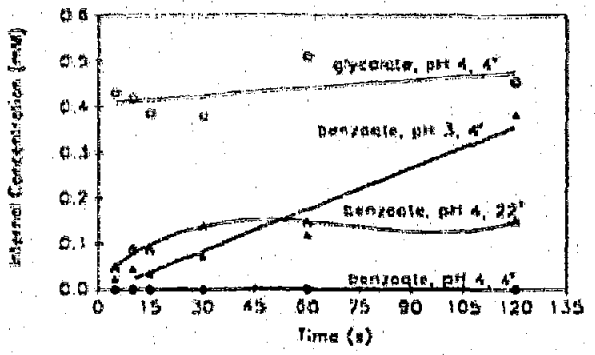

Fig. 1. Time course of glycolate and benzoate uptake. CO_2-grown *Chlamydomonas* (50 μg chl/ml) cells were incubated in the dark at 4°C with 1 mM [^{14}C]glycolate in 20 mM Na-citrate, pH 4, or with 0.82 μM [^{14}C]benzoate in 20 mM Na-citrate, pH 3 or 4, for the times indicated. Glycolate (○); benzoate, pH (▲); benzoate, pH 4 (●); benzoate, pH 4, 22°C (△).

low pH (3 and 4) where some diffusion would be expected due to higher concentration of undissociated acid, internal glycolate came to the same equilibrium value as at higher pH. Since the uptake of glycolate is much more rapid than the temperature- and pH-dependent diffusion of benzoic acid, a transport system for glycolate is indicated.

3.2. *Saturation*

Because lactate transport in erythrocytes has been reported to saturate [13] and because lactate and glycolate are transported by the same carrier in erythrocytes, glycolate uptake in *Chlamydomonas* was compared to erythrocytes. Using the silicone oil centrifugation procedure, glycolate uptake by algae or erythrocytes did not saturate but reached equilibrium with respect to external concentration, even up to 50

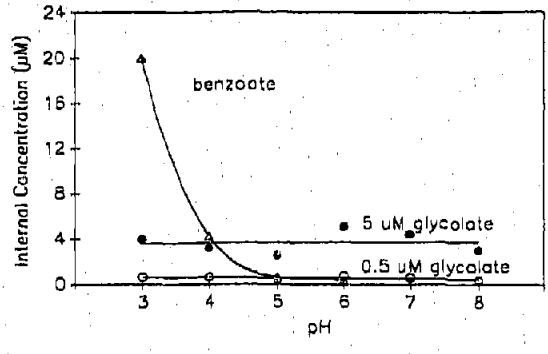

Fig. 2. Uptake of glycolate and benzoate as a function of external pH. CO_2-grown *Chlamydomonas* (50 μg chl/ml) were centrifuged (for less than 2 s) through 0.5 (○) and 5 μM (●) [^{14}C]glycolate or 0.82 μM [^{14}C]benzoate (△) at 4°C in the dark in 20 mM Na-citrate, pH 3, 4 and 5, 20 mM Mes, pH 6, 20 mM Hepes, pH 7 and 20 mM Epps, pH 8.

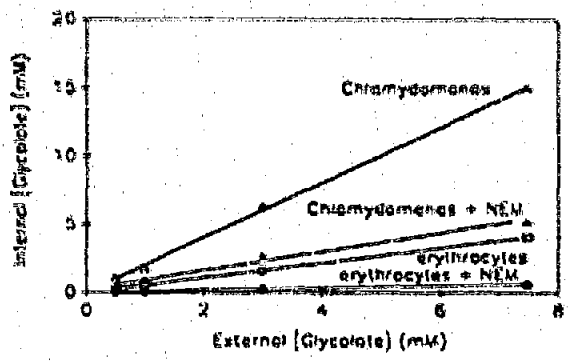

Fig. 3. Glycolate uptake by *Chlamydomonas reinhardtii* or erythrocytes. Control (○) or pretreated with 10 mM NEM for 15 min (●) human erythrocytes (40% hematocrit) and control (△) or pretreated with 10 mM NEM for 15 min (▲) CO_2-grown algae (300 μg chl/ml) centrifuged through a buffer layer of 6.5 mM sodium phosphate, pH 7.4, containing 4.5 mM glucose and 140 mM NaCl at 4°C in the dark and 0.5, 1, 3 and 7.5 mM [^{14}C]glycolate.

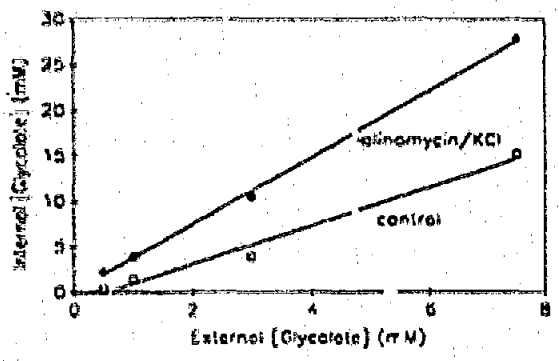

Fig. 4. Stimulation of uptake by valinomycin and KCl. Addition of 0.2 ng valinomycin/ml and 100 mM KCl to the [^{14}C]glycolate-20 mM Hepes, pH 7, layer increased uptake at 4°C by CO_2-grown *Chlamydomonas* (300 μg chl/ml) in the dark. Glycolate concentrations were 0.5, 1, 3 and 7.5 mM.

mM, in contrast to literature reports [13]. Halestrap [13] measured uptake by incubating the erythrocytes with glycolate followed by centrifuging and washing of the cells during which exchange of glycolate would have occurred, giving rise to misleading results. Saturation of a transport system cannot be demonstrated if the system is at equilibrium. The inability to demonstrate saturation of glycolate uptake is due to the rapid equilibration shown in the time course.

Glycolate did not accumulate in *Chlamydomonas* nor in human erythrocyte cells but reached an equilibrium between internal and external concentrations (Fig. 3). Since the system had already reached equilibrium within these short incubations, it is not possible from these data to determine if the system saturates. A different technique allowing less than 2 s incubations would be required to determine saturation.

3.3. *Inhibition*

Incubation of erythrocytes with *N*-ethylmaleimide (NEM) severely inhibited the uptake of glycolate (Fig. 3). As with *Anabeana* [3] 10 mM NEM inhibited the uptake of glycolate in *Chlamydomonas* (Fig. 3) but inhibition was not as great as in erythrocytes.

Decreased glycolate uptake by competition with structural analogs such as acetate, lactate and glycerate was not observed. The difference between *Anabeana* [3] and *Chlamydomonas* in regard to inhibition by substrate analogs may be partly due to differences in experimental design and the rapid equilibrium in *Chlamydomonas*. Studies of uptake of glycolic acid by *Chlamydomonas* in contrast to the chloroplasts and *Anabeana* may be complicated by transport across organelle membranes as well as across the plasma membrane. No attempt has been made to distinguish between these processes.

3.4. *Stimulation*

Addition of 0.2 ng valinomycin/ml and 100 mM KCl to the glycolate layer increased uptake (Fig. 4) with optimal stimulation at 60 mM KCl with 0.2 ng valinomycin/ml. Similar to the stimulation by valinomycin and K^+ of glycolate uptake in *Chlamydomonas*, Buckhout [14] observed a two-fold stimulation by valinomycin and K^+ in sucrose transport. These data were used to support a sucrose-H^+ symport mechanism for transport by the sucrose transporter. Although the rapid equilibration of glycolate uptake precludes attempts at further characterization of the transport system, the ionophore and K^+ stimulation indicate involvement of a transport system for glycolate other than diffusion.

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