Biochimica et Biophysica Acta, 942 (1988) 33–37
Elsevier

BBA 74036

Uptake of fructose by the cyanobacterium *Nostoc* sp. ATCC 29150

Georg Schmetterer * and Enrique Flores

MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI (U.S.A.)

(Received 16 November 1987)

Key words: Fructose uptake; Permease induction; Cyanobacterium; (*Nostoc*)

Fructose uptake was studied in the facultatively heterotrophic, dinitrogen-fixing cyanobacterium *Nostoc* sp. ATCC 29150. A fructose uptake system showing a K_m of 1.4 mM was evident in fructose-grown cells. It was induced by fructose even in the light and was more active when assayed in the light than in the dark. In addition, a low-affinity process of fructose uptake showing a K_m of about 100 mM was observed in both photoautotrophically and chemoheterotrophically grown cells.

Introduction

Photoautotrophy is the most common mode of growth of cyanobacteria [1,2]. Facultative photoheterotrophy (i.e., growth with organic electron donors in the light) or chemoheterotrophy has been demonstrated, however, for a number of cyanobacteria, mainly filamentous strains [1–3]. About half of the filamentous strains described by Rippka et al. [3] were able to grow photoheterotrophically on a sugar. Fructose, glucose and sucrose were, in that order, the most commonly used substrates [3]. A significant fraction of the filamentous, sugar-assimilating strains were dinitrogen-fixers, most of them belonging to three genera, namely, *Calothrix, Fischerella* and *Nostoc*.

Studies on sugar uptake by cyanobacteria have mainly concentrated on the assimilation of glucose [4–8]. These studies have indicated the presence, in glucose-assimilating strains, of permeases showing K_m values for glucose of about 0.1–1.0 mM [6–10]. Fructose is a good substrate for growth for many cyanobacteria (see e.g. refs. 11–13), but fructose uptake has been studied with only one facultatively heterotrophic strain, *Anabaena variabilis* ATCC 29413, for which K_m values for fructose of 0.05 and 0.15 mM have been reported [9,14].

Our interest in transport systems of cyanobacteria arose from the fact that very little information is available on the functions of the cytoplasmic membrane of the organisms. Although physical separation of the cytoplasmic membrane from the intracellular photosynthetic membranes is now possible with several strains [15,16], only two activities, a Ca^{2+}-ATPase [17,18] and a cytochrome-*c* oxidase (see e.g. Ref. 19), have been tentatively localized on the cytoplasmic membrane. Apart from porins in the outer membrane, systems for the uptake of substrates into the cell must be located in the cytoplasmic membrane. An investigation of the uptake system for fructose in *Nostoc* sp. ATCC 29150 is reported here. This dinitrogen-fixing strain can grow photoautotrophically, photoheterotrophically [3] or chemoheterotrophically [20], and is capable of gene transfer

* Present address: Institute of Physical Chemistry, University of Vienna, Währingerstrasse 42, A-1090 Vienna, Austria.

Correspondence (present address): E. Flores, Instituto de Bioquímica Vegetal y Fotosíntesis, Universidad de Sevilla-C.S.I.C., Facultad de Biología, Apartado 1095, 41080-Sevilla, Spain.

from *Escherichia coli* [20] by the method of Wolk et al. [21].

Materials and Methods

Nostoc sp. strain ATCC 29150 (PCC 7107) was grown axenically in the light or in darkness, at 30 °C, in the liquid medium of Allen and Arnon [22] diluted 8-fold and supplemented with 2.5 mM KNO_3 and 2.5 mM $NaNO_3$ (AA/8 NO_3^-). For growth in the dark the cultures were supplemented with D-fructose, sterilized by filtration, at 30 mM unless otherwise indicated.

To study fructose transport, cells were harvested by low-speed centrifugation at room temperature (24 °C), washed (when the cells that were used had been grown with fructose, they were washed several times), and finally resuspended in fresh AA/8 NO_3^- medium at a concentration of 2.5 to 5.0 μg of chlorophyll [23] per ml. Suspensions of cells (final volume, 5 ml) were incubated with shaking at 30 °C under white light (10 cm from a 15-W Daylight fluorescent lamp, General Electric) or in the dark, and 0.1–333 mM (0.4–0.8 μCi per ml) D-[U-^{14}C]fructose (specific activity 219 mCi/mmol, from ICN Radiochemicals, Irvine, CA, U.S.A.) was added. All of the assays were carried out for 20 min and, at different times (5, 10, 15, and 20 min), 1 ml samples were removed and filtered (0.45-μm pore size Millipore HA filters), and the cells on the filters were washed with 5 ml of AA/8 NO_3^- medium. The filters were dissolved in a dioxane-based scintillation liquid and the radioactivity determined in a scintillation counter. The amount of fructose taken up by the cells was always less than 3% of the total fructose added.

Growth rates were determined from the increase in the concentration of chlorophyll [23] in the cultures. The specific growth rate constant (μ') as used here corresponds to $\log_{10}2/t_d$, where t_d is the doubling time.

Results

A kinetic study of fructose uptake by dark grown cells, for a wide range of sugar concentrations (from 0.1 to 333 mM), showed the presence of two different components in the graph of fructose uptake by *Nostoc* sp. ATCC 29150 (Fig. 1). The high-affinity component showed a K_m of 1.4 mM, and the low-affinity one showed a K_m of about 100 mM.

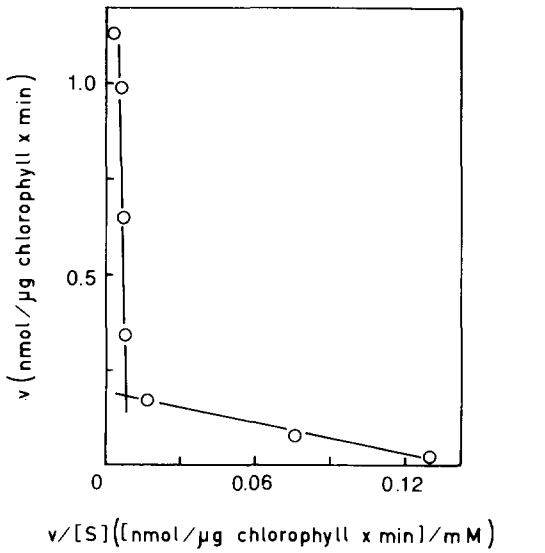

Fig. 1. Eadie-Hofstee plot of the effect of the concentration of fructose on the rate of fructose uptake by *Nostoc* sp. ATCC 29150. Cells grown on fructose in the dark were harvested and washed, and their fructose uptake activity determined in the light for different concentrations of [^{14}C]fructose. v, fructose uptake rate; [S], concentration of fructose.

Dark-grown cells of *Nostoc* sp. took up fructose when assayed in the light or in darkness (Fig. 2). Rates of uptake of fructose at low concentrations (0.1–10 mM) of the sugar were higher in the light than in darkness, whereas at high concentrations of fructose (33–150 mM) the rates were similar under both conditions (Fig. 2).

The level of activity of each uptake system was dependent upon conditions of cell growth (Fig. 2). The high-affinity system was present in cells grown in the dark with fructose (V_{max}, 0.15–0.3 nmol · μg^{-1} chlorophyll · min^{-1}), but its activity was extremely low in photoautotrophically grown cells (V_{max}, 0.005–0.01 nmol · μg^{-1} chlorophyll · min^{-1}). The difference between light- and dark-grown cells with regard to the low-affinity system was however not nearly as great. When the uptake of fructose was tested at high concentrations of sugar (66–150 mM), where the low-affinity system was operative, fructose uptake rates were 0.25–0.5 nmol · μg^{-1} chlorophyll · min^{-1} for dark-grown

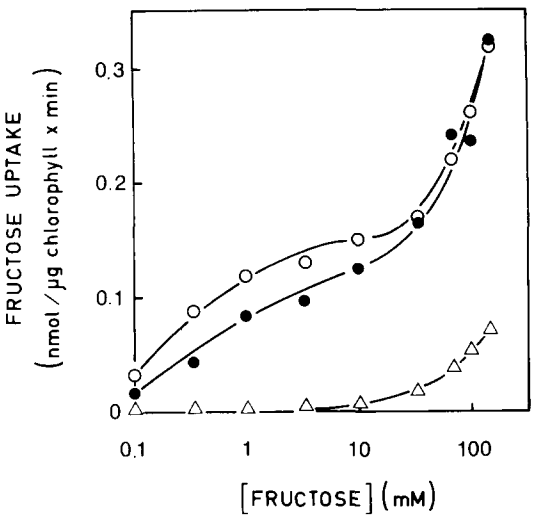

Fig. 2. Effect of the concentration of fructose on the rate of uptake of fructose in photoautotrophically or chemoheterotrophically grown *Nostoc* sp. cells. Note that the data are presented in a semi-logarithmic format. Uptake of [^{14}C]fructose was measured in the light (○) or in the dark (●) with chemoheterotrophically (fructose)-grown cells, and in the light with photoautotrophically-grown cells (△).

cells and 0.05–0.1 nmol · μg^{-1} chlorophyll · min^{-1} for light-grown cells (Fig. 2). If, for dark-grown cells, the activity of the high-affinity system is substracted from the uptake activity observed at high concentrations of fructose, the resulting values (0.1–0.2 nmol · μg^{-1} chlorophyll · min^{-1}) are similar to those observed with light-grown cells (0.05–0.1 nmol · μg^{-1} chlorophyll · min^{-1}). It thus appears that, in strain ATCC 29150, the high-affinity system for fructose uptake is inducible, its cellular level changing up to 40-fold, whereas there was only about a 2-fold difference in the activity of the low-affinity system between light- and dark-grown cells.

When photoautotrophically-grown cells were incubated in the dark in the presence of fructose, development of the high-affinity fructose uptake took place in less than 48 h; incubation in the light in the presence of fructose also resulted in induction (Fig. 3). However, photoautotrophically-grown cells incubated for 48 h in the dark without fructose exhibited an extremely low activity for uptake of fructose at a low sugar concentration (Fig. 3). It thus appears that fructose, rather than incubation under dark conditions, is the factor that decisively determines the induction of the high-affinity system for uptake of fructose by strain ATCC 29150. As a matter of fact, the growth rate of this strain in the light was enhanced by the presence of fructose in the culture medium. Under our experimental conditions, the specific growth rate constant (μ') of photoautotrophic cultures was 0.32 d^{-1}, whereas that of cultures supplemented with 15 mM fructose and incubated under the same conditions was 0.45 d^{-1}.

To test the possible contribution to fructose-dependent growth of the two affinity components of fructose uptake, the growth rates of cultures incubated in the dark with a wide range of concentrations (1–333 mM) of fructose were determined. As shown in Fig. 4, the specific growth rate constant (μ') increased slightly as the concentration of fructose increased from 1 to 33 mM. Higher concentrations of sugar did not result in increased growth rates but, rather, hampered growth to some extent (Fig. 4); for instance, the growth rate at 333 mM fructose was 60% of the value at 33 mM. It thus appears that growth on fructose in the dark involves fructose uptake by the high-affinity system, whereas growth was negatively affected by concentrations of fructose

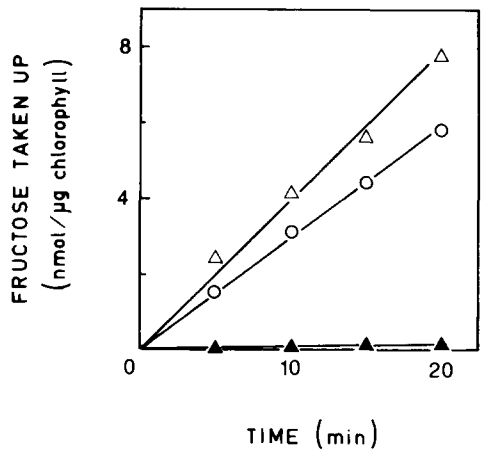

Fig. 3. Adaptability of the high-affinity fructose uptake system of *Nostoc* sp. Photoautotrophically-grown cells were incubated in AA/8 NO_3^- medium, at 30 °C with shaking, for 48 h, in the light with 15 mM fructose (○), or in the dark with (△) or without (▲) 15 mM fructose. The cells were then harvested and washed, and their fructose-uptake activity determined in the light with 1 mM [^{14}C]fructose.

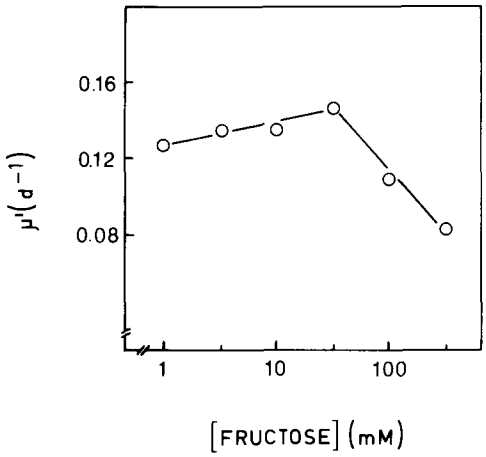

Fig. 4. Effect of the concentration of fructose on the growth rate of *Nostoc* sp. in the dark. Washed dark-grown cells were inoculated at 75 ng chlorophyll·ml^{-1} in AA/8 NO_3^- medium containing different concentrations of fructose, and incubated with shaking in the dark at 30 °C. μ', specific growth rate constant (see Materials and Methods).

that allowed uptake of fructose by the low-affinity component as well.

Discussion

A biphasic pattern for uptake of fructose was evident in *Nostoc* sp. ATCC 29150 (Figs. 1 and 2). The component showing higher affinity for fructose (K_m 1.4 mM) appears to be the one involved in heterotrophic growth on fructose. It may well correspond to a permease taking up fructose. The affinity of this uptake system for fructose is similar to the affinities for glucose or fructose reported for other cyanobacteria [6,7,9,10,14]. Although these affinities are relatively low [9], it has been reported (see review in Ref. 24) that cyanobacteria frequently occur in soils and in freshwater habitats rich in organic matter. The possible significance of the uptake of sugar by cyanobacteria in relation to the sugar concentrations usually found in nature has been discussed [8,9].

The high-affinity activity of fructose uptake was subject to regulation, being induced by fructose (Fig. 3). Since metabolism of fructose is probably taking place in the course of our experiments, either the transport or the metabolism (or both) of fructose could be the regulated step in fructose uptake. However, because increasing the concentration of fructose allowed fructose uptake in photoautotrophically grown cells (Fig. 2), we suggest the transport step is the regulated one. Adaptation of a sugar transport system has also been shown for glucose uptake in a filamentous cyanobacterium, *Plectonema boryanum*, by Raboy et al. [5,6]; those authors did not distinguish between induction by the sugar or by incubation in the dark. In the filamentous, dinitrogen-fixing strain *Nostoc* sp. Mac and in the unicellular strain *Synechocystis* sp. PCC 6714, on the other hand, glucose transport activity was constitutive [4,7]. Whereas *Nostoc* sp. ATCC 29150 was isolated as a free-living organism [3], *Nostoc* sp. Mac was an endophytic symbiont [25]. In addition to short-term induction of sugar transport systems, long-term adaptation to growth under heterotrophic conditions has also been observed in cyanobacteria [5,20,26], but it is not currently understood.

Uptake of fructose by the high-affinity component is higher in the light than in the dark (Fig. 2). Since, for cyanobacteria, light is a much more efficient energy source than is heterotrophic metabolism [1,2], this result could be interpreted as an energy requirement for uptake of fructose by the high-affinity system. In contrast, the low-affinity process of fructose uptake was not affected by light; moreover, it was not subject to regulation (Fig. 2) and did not appear to contribute to growth on fructose (Fig. 4). The physiological significance of fructose uptake at high concentrations of fructose is thus uncertain. Whether the low-affinity process is mediated by a permease or proceeds by passive diffusion through the cell membrane is not clear. In the latter case, the K_m of the process might correspond to an intracellular reaction involved in the metabolism of fructose, since the incubation times used (20 min) were relatively long and most probably allowed metabolism of the substrate.

Not every cyanobacterium able to grow photoheterotrophically is able to grow chemoheterotrophically in the dark [27]. Thus, the presence of a sugar permease, which appears to be required to grow on a sugar [9], does not guarantee growth of the corresponding strain in the dark on the sugar. What role has such a sugar permease in the physiology of cyanobacteria? Organic substrate-depen-

dent growth in the light, in the absence of CO_2, has been reported in cyanobacteria [28,29], as has sugar-dependent growth in dim light which by itself failed to support autotrophic growth [12,30,31]. In addition, in strain ATCC 29150, the growth rate was higher in the light in the presence of fructose than under photoautotrophic conditions, as it is also the case for some other filamentous cyanobacteria [14,32]. It is thus possible that photoheterotrophy and mixotrophy (a nutritional regime in which CO_2 and organic carbon are assimilated simultaneously [9]) are important modes of growth for sugar-assimilating cyanobacteria. In this context it is most interesting that the fructose uptake system of strain ATCC 29150 was induced by fructose even in the light, since the evolution of a given regulatory function is most probably physiologically meaningful, suggesting that fructose may contribute to cell growth in nature under phototrophic conditions.

Acknowledgments

This work was supported by the U.S. Department of Energy under contract DE-ACO2-76ERO-1338. E.F. was the recipient of a Fulbright/M.E.C. (Spain) fellowship. We thank C.P. Wolk for facilities and encouragement, and J. Martín-Nieto for help in the preparation of the manuscript.

References

1 Wolk, C.P. (1973) Bacteriol. Rev. 37, 32–101.
2 Stanier, R.Y. and Cohen-Bazire, G. (1977) Annu. Rev. Microbiol. 31, 225–274.
3 Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M. and Stanier, R.Y. (1979) J. Gen. Microbiol. 111, 1–61.
4 Pelroy, R.A., Rippka, R. and Stanier, R.Y. (1972) Arch. Mikrobiol. 87, 303–322.
5 Raboy, B., Padan, E. and Shilo, M. (1976) Arch. Microbiol. 110, 77–85.
6 Raboy, B. and Padan, E. (1978) J. Biol. Chem. 253, 3287–3291.
7 Beauclerk, A.A.D. and Smith, A.J. (1978) Eur. J. Biochem. 82, 187–197.
8 Smith, A.J. (1983) Ann. Microbiol. (Inst. Pasteur) 134 B, 93–113.
9 Smith, A.J. (1982) in The Biology of Cyanobacteria (Carr, N.G. and Whitton, B.A., eds.), pp. 47–85, Blackwell Scientific Publications, Oxford.
10 Flores, E. and Schmetterer, G. (1986) J. Bacteriol. 166, 693–696.
11 Watanabe, A. and Yamamoto, Y. (1967) Nature 214, 738.
12 Hoare, D.S., Ingram, L.O., Thurston, E.L. and Walkup, R. (1971) Arch. Mikrobiol. 78, 310–321.
13 Wolk, C.P. and Shaffer, P.W. (1976) Arch. Microbiol. 110, 145–147.
14 Haury, J.F. and Spiller, H. (1981) J. Bacteriol. 147, 227–235.
15 Omata, T. and Murata, N. (1983) Plant Cell Physiol. 24, 1101–1112.
16 Omata, T. and Murata, N. (1984) Arch. Microbiol. 139, 113–116.
17 Lockau, W. and Pfeffer, S. (1982) Z. Naturforsch. 37C, 658–664.
18 Lockau, W. and Pfeffer, S. (1983) Biochim. Biophys. Acta 733, 124–132.
19 Peschek, G.A. (1984) Subcell. Biochem. 10, 85–91.
20 Flores, E. and Wolk, C.P. (1985) J. Bacteriol. 162, 1339–1341.
21 Wolk, C.P., Vonshak, A., Kehoe, P. and Elhai, J. (1984) Proc. Natl. Acad. Sci. USA 81, 1561–1565.
22 Allen, M.B. and Arnon, D.I. (1955) Plant Physiol. 30, 366–372.
23 Mackinney, G. (1941) J. Biol. Chem. 140, 315–322.
24 Fogg, G.E., Stewart, W.D.P., Fay, P. and Walsby, A.E. (1973) The Blue-Green Algae, Academic Press, New York.
25 Bowyer, J.W. and Skerman, V.B.D. (1968) J. Gen. Microbiol. 54, 299–306.
26 Fay, P. (1965) J. Gen. Microbiol. 39, 11–20.
27 Rippka, R. (1972) Arch. Mikrobiol. 87, 93–98.
28 Ingram, L.O., Calder, J.A., Van Baalen, C., Plucker, F.E. and Parker, P.L. (1973) J. Bacteriol. 114, 695–700.
29 Ingram, L.O., Van Baalen, C. and Calder, J.A. (1973) J. Bacteriol. 114, 701–705.
30 Van Baalen, C., Hoare, D.S. and Brandt, E. (1971) J. Bacteriol. 105, 685–689.
31 White, A.W. and Shilo, M. (1975) Arch. Microbiol. 102, 123–127.
32 Rozen, A., Arad, H., Schönfeld, M. and Tel-Or, E. (1986) Arch. Microbiol. 145, 187–190.