Competitive Inhibition for Amino Acid Uptake by the Indigenous Microflora of Upper Klamath Lake

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The uptake of a specific $^{14}$C-amino acid by the heterotrophic microorganisms in the epilimnion of an eutrophic lake was influenced by the presence of other amino acids. The effect of unlabeled serine on $^{14}$C-glycine uptake was shown to be caused by competitive inhibition, which changed the interpretation of the kinetic parameters, the turnover time, $T_t$, and the sum of a transport constant, $(K_t + S_a)$, and the natural substrate concentration. The maximum velocity of uptake, $V_{max}$, is unaffected by the competitive inhibition.

The kinetic approach to study the uptake (transport) of organic solutes in natural waters was recently used to investigate heterotrophic activity in freshwater lakes (1, 2, 13, 16, 20, 28-31), the oceans (9-11, 21, 25, 26, 28), and sediments (12). This kinetic approach was derived from the work of Parsons and Strickland (21) and modified by Wright and Hobbie (29). The technique has been applied to pure bacterial cultures (10, 16, 20) to determine the kinetics for glucose uptake.

Various controllable factors, which influence the kinetic analyses of organic solute uptake in natural waters, have been determined, such as: (i) temperature, (ii) time, (iii) sample homogeneity (since $V_{max}$ is proportional to the number of bacteria), and (iv) low substrate concentration. A second uptake mechanism, simple diffusion into algal cells, became evident when high substrate concentrations (>0.5 mg/liter) were used (29-31).

In most investigations of natural waters, the uptake of organic substrates by heterotrophic plankton followed a Michaelis-Menten type of kinetics. As a result, a modified Lineweaver-Burk plot has been used in determining the kinetic parameters, $V_{max}$, $T_t$, and $(K_t + S_a)$. $V_{max}$ is the maximum velocity attained when all uptake sites are saturated with substrate. $T_t$ is the turnover time (time in hours) required for the natural population to completely remove the natural substrate ($S_a$), and $K_t$ is a transport constant (30, 31, 32).

Competitive inhibition effects in natural habitats have been mentioned briefly in the literature. Mannose was found to competitively inhibit glucose uptake, but the transport system was 20 times more sensitive to glucose (31). Wright (28) demonstrated that lactate competitively inhibited glycolate uptake. In their estuary studies, Hobbie, Crawford, and Webb (15) found aspartate uptake was only affected by the addition of glutamate and not by any other common amino acids.

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MATERIALS AND METHODS

Study area. Water samples were obtained at the south end of Upper Klamath Lake, a naturally eutrophic lake. The lake is located at the eastern foot of the Cascade Mountains in south central Oregon. It is approximately 37-km long and 8-km wide, has a mean depth of 2.4 m, and a storage capacity of 7.75 by $10^9$ m$^3$ (584,000 acre-feet) (19).

Competitive inhibition effects on uptake. Stock solutions (10 $\mu$g/ml) of unlabeled amino acids (inhibitors) were made by using sterile distilled and deionized water. Concentrations (1 to 50 $\mu$liters) of the inhibitors were added, by using a Hamilton syringe, to 50-ml serum bottles containing 0.05 $\mu$Ci of uniformly labeled $^{14}$C-amino acids. Ten milliliters of Upper Klamath Lake water was added, and the flasks were incubated at 6 °C (in situ temperature) in the dark, unless otherwise stated. The incubation bottles and respiration assembly used have been described by Harrison, Wright, and Morita (12).
Uptake was terminated by adding H$_2$SO$_4$ (0.2 ml, 5 n), resulting in a final pH of approximately 2.2. The respirated $^{14}$CO$_2$ was determined by the techniques of Hobbie and Crawford (14). Plankton was removed from the acidified samples by filtration through a 0.45-$\mu$m membrane filter (Millipore) and dried at 37 C. The combined radioactivity (filtered plankton plus respirated $^{14}$CO$_2$) was compared to uninhibited controls, arbitrarily set as 100% uptake, and consisting of lake water containing only a labeled amino acid, incubated, and processed similarly.

The kinetics of the $^{14}$C-glycine uptake mechanism was measured by the techniques of Wright and Hobbie (30). The series consisted of adding 50, 100, 200, and 300 $\mu$liters of labeled glycine (0.5 $\mu$Ci/ml) to the incubation bottles. This uptake experiment was measured in the presence and absence of 28 $\mu$g of unlabeled serine per liter.

Amino acids. The concentrations of unlabeled amino acids (Sigma Chemical Co., St. Louis, Mo.) used were determined by using the methods of Spackman, Stein, and Moore (22) with a Spinco model 120B automatic amino acid analyzer. The uniformly labeled $^{14}$C-amino acids were obtained from Amersham-Searle Corp., Arlington Heights, Ill. The specific activities of aspartate, glutamate, glycine, and leucine were 100, 125, 109, and 150 $\mu$Ci/\(\mu\)mole, respectively.

Assay for radioactivity. Radioactivity of the filtered plankton and respirated $^{14}$CO$_2$ was measured with a Nuclear Chicago Mark I liquid scintillation counter. The scintillation fluid was composed of 0.01% 1,4-bis-2-(4-methyl-5-phenyloxazolyl)benzene and 0.4% 2,5-diphenyloxazole in toluene. All counts were corrected for counting efficiency by the channels ratio method.

RESULTS AND DISCUSSION

Small volumes of amino acids (labeled and unlabeled) were added to lake water to avoid diluting the balance of in situ dissolved, free amino acids. The presence of glycine, serine, and alanine affected the uptake of $^{14}$C-leucine more than $^{14}$C-aspartate uptake (Table 1). Isoleucine and valine also influenced $^{14}$C-leucine uptake.

Increasing concentrations of unlabeled glutamate, aspartate, and serine decreased the uptake of labeled glutamate (Fig. 1). Serine and glycine usually represent a large fraction of the dissolved free amino acids found in freshwater lakes (3, 8). In Upper Klamath Lake 31.9 and 15.3 $\mu$g of serine and glycine per liter, respectively, were found in the surface water on 23 Feb. 1971 (B. K. Burnison, Ph.D. thesis, Oregon State Univ., Corvallis). Serine, alanine, and, to a lesser extent, leucine decreased uptake of $^{14}$C-glycine (Fig. 2). Aspartate did not significantly affect glycine uptake at the low concentrations tested. The data show that the uptake of a specific amino acid by the heterotrophic

| TABLE 1. Influence of various amino acids on the uptake of aspartic acid and leucine |
|---------------------------------|------------------|-----------------|------------------|
| Labeled substrate | Inhibitor conc ($\mu$g/ml) | Committed radioactivity ($\mu$Ci/ml) | Noninhibited uptake (%) |
|-------------------|------------------|-----------------|------------------|
| Aspartic acid | None | 0.7 | 5,712 | 100.0 |
| (0.046 $\mu$m) | Aspartic acid | 6.2 | 1,663 | 29.1 |
| | Alanine | 18.4 | 4,558 | 79.8 |
| | Glycine | 34.9 | 5,015 | 87.8 |
| | Serine | 22.5 | 3,992 | 69.9 |
| | Leucine | 16.9 | 5,467 | 95.7 |
| Leucine | None | 0.0 | 11,230 | 100.0 |
| (0.032 $\mu$m) | Leucine | 16.9 | 1,363 | 12.1 |
| | Isoleucine | 18.1 | 4,942 | 44.0 |
| | Valine | 20.8 | 2,901 | 25.8 |
| | Aspartic acid | 6.2 | 11,761 | 104.7 |
| | Alanine | 18.4 | 4,563 | 40.6 |
| | Glycine | 14.0 | 7,403 | 65.6 |
| | Serine | 9.0 | 6,539 | 58.0 |
| | 22.5 | 4,659 | 41.5 |

* Incubated for 2 hr at 5 C.

* Radioactivity of filtered plankton plus $^{14}$CO$_2$. All duplicate values are well within an experimental error of 5%, most being closer to 2%. DPM, Disintegrations per minute.

* Noninhibited uptake was determined by incubating lake water containing only labeled $^{14}$C-aspartic acid or $^{14}$C-leucine for 1 or 2 hr, respectively, at 5 C.

* Incubated for 1 hr at 5 C.

![Fig. 1. Effect of unlabeled serine, aspartic acid, and glutamic acid on the uptake of $^{14}$C-glutamic acid. The 100% uptake control was obtained by incubating lake water containing $^{14}$C-glutamic acid (1.9 $\times$ 10$^{-3}$ $\mu$m) at 6 C for 2.75 hr and represents a combined radioactivity of 20,040 disintegrations per minute.](image-url)
plankton in Upper Klamath Lake was affected by the presence of other amino acids.

The mode of action of the inhibiting amino acids is undoubtedly complex. Stumm-Zollinger (24) has shown enzyme inhibition and repression by cell metabolites to be a significant phenomenon in natural microbial communities. However, relatively high substrate concentrations (2.5 to 200 mg/liter) were used and, therefore, the occurrence of these metabolic regulations in natural heterotrophic populations remains undetermined. Harrison, Wright, and Morita (12) demonstrated an induction phenomenon for glucose transport in lake sediments by using high concentrations of substrate.

The presence of 28 μg of serine per liter greatly affected the uptake of labeled glycine (Fig. 3). The turnover time, $T_t$, increased from 11.7 to 97.5 hr. The value representing the natural substrate concentration plus a transport constant, $(K_t + S_a)$, increased from 4 to 27.5 μg/liter, whereas the maximum uptake velocity, $V_{\text{max}}$, remained constant. Serine is, therefore, by definition (7) a competitive inhibitor of glycine uptake.

In pure culture studies, amino acids which have similar structures share the same transport systems (4, 17, 18, 21), as do some structurally unrelated amino acids (5, 6). The concentrations of all the amino acids, and of other compounds, which may conceivably inhibit a specific substrate uptake, must be taken into account to attain meaningful interpretation of the kinetic data.

Although defining the natural uptake velocity, $v_t$, is the goal of the kinetic approach, this value is elusive when considering competitive inhibition. Therefore, $V_{\text{max}}$, although overestimating $v_t$, is the most significant parameter and should still be used to compare the "heterotrophic potential" of natural waters. The other kinetic parameters, $T_t$ and $(K_t + S_a)$, were both increased due to competitive inhibition, and thus were not useful. The $T_t$ increase would indicate the time required for the natural population to remove compounds which share a common transport system. The $(K_t + S_a)$ would reflect the natural concentrations of compounds sharing the common transport system and a very complex "comprehensive transport constant" for the substrates.

There may be noncompetitive inhibition of substrate transport by other naturally occurring compounds. This type of inhibition, if analogous to enzyme kinetics, would affect the $V_{\text{max}}$ and $T_t$. These compounds should be considered as part of the natural conditions influencing the rate at which organic compounds are transported.

The results presented indicate that the kinetic approach to determine amino acid uptake in natural waters must be interpreted with caution. The validity of the flux data obtained by Hobbie, Crawford, and Webb (15) is thus subjected to question. Although their study was in an estuarine environment, competitive inhibition data should be necessary to correctly interpret their results.

Factors which influence the kinetic analysis must be known to properly interpret the results. Nevertheless, as R. T. Wright (personal communication) states, "the basic approach is gradually giving us insight into what the heterotrophic bacteria are doing in nature, and there may be no other way to get that information."
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