Relations in the Transport of \( \beta \)-Alanine and the \( \alpha \)-Amino Acids in the Ehrlich Cell*

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\[ L-\alpha,\beta \text{ Diaminopropionic acid appears to enter the Ehrlich ascites tumor cell by the means serving characteristically for glycine, } \alpha\text{-aminoisobutyric acid, and alanine (1). This means is assumed to be principally the so-called alanine-prefering transport system (2), and to concern the species, } \text{N}^1\text{H}_2\text{CH}_2\text{CH(}\text{N}^2\text{H}_2\text{)}\text{-COO}^- . \text{ The cationic form } \text{N}^+\text{H}_3\text{CH}_2\text{CH(}\text{N}^2\text{H}_2\text{)}\text{-COO}^- , \text{ is not abundant at pH 7.4 (3), and besides appears to have rather low affinity for any recognized transport system (1). A third species, } \text{N}^+\text{H}_3\text{CH}_2\text{CH(NH}_3\text{)}\text{-COO}^- , \text{ might be expected to be the most abundant one, bringing to mind the possibility that it might also encounter an effective transport-mediating receptor. The present results show that a component of the entry of } \alpha,\beta-\text{diaminopropionic acid is sensitive to inhibition by } \beta\text{-alanine at concentrations much lower than is the entry of L-alanine. A population of transport-mediating sites could accordingly be discerned which accept a somewhat wider separation of the amino and the carboxyl groups, than do the ones serving principally for glycine, alanine, phenylalanine, lysine, and similar amino acids.} \]

The uphill transport of \( \beta \)-alanine into the Ehrlich cell (4) and its sensitivity to the presence of glycine and alanine (5) have been recognized for several years, but details of the relationship of its transport to that of other amino acids have not been described heretofore.

EXPERIMENTAL PROCEDURE

\( \beta \)-Alanine-2-\( ^14 \)C, L-phenylalanine-1-\( ^14 \)C, and L-alanine-1-\( ^14 \)C were obtained from Calbiochem. The uniformly labeled L-\( \alpha,\beta \)-diaminobutyric acid-\( ^14 \)C and uniformly labeled DPA-\( ^14 \)C were our own preparations. Chromatography of over 3 \( \mu \)c of the labeled \( \beta \)-alanine on paper showed but a single component, \( R_f \) 0.50 in tert-butyl alcohol-formic acid-water (70:15:15). The methods of collection and study of the Ehrlich cell have already been described (2, 6). Rates of uptake of \( ^14 \)C-labeled amino acids were determined for approximately 4 to 10\% suspensions of the cells in Krebs-Ringer-bicarbonate medium at 37\°. In selected cases tests were made with 25 to 30\% suspensions, so that a larger proportion of the radioactive amino acid would be taken up, to guard against misleading actions on the uptake of possible radioactive impurities. Time intervals were 1 minute for L-alanine, L-lysine, or L-phenylalanine, 2 minutes for DPA, and 1 or 5 minutes for \( \beta \) alanine. In the study of the effect of pH, 1-minute intervals were used. These intervals were short enough so that accumulation within the cells remained a small factor in returning the labeled amino acids to the suspending fluid. Uptake was terminated as usual by dilution with ice-cold 0.9\% NaCl solution, and the cells separated by centrifugation. Radioactive disintegrations were counted in the extracellular phase, and in a hot-water extract of the cells, by the liquid scintillation method (2). Amino acids added as inhibitors replaced part of the NaCl, so that the toxicity would not be disturbed. Significant observations were replicated, and typical results taken for illustration.

RESULTS

Fig. 1 shows the time course of the uptake of 1 mm \( \beta \)-alanine by the Ehrlich cell at 37\°. Because the net uptake proceeds almost unslowed for the first hour, with no suggestion of an approach to a steady state, I assumed that the cells would deteriorate excessively before a steady state could be reached, and therefore used an indirect method to look for its position. Apparent 7 to 8 mm levels of \( \beta \)-alanine in the cellular water were obtained by incubating the cells in a 10 mm \( \beta \)-alanine-\( ^14 \)C solution for 15 minutes. The suspension was then diluted by 20 to 200 times with Krebs-Ringer-bicarbonate medium, and incubated for an additional 30 minutes at 37\°. If the distribution ratio had a value less than 30 to 40 at the beginning of this 30-minute period, it increased during the interval; if it was more than 40 to 50 it decreased during the interval. The distribution ratio remained essentially stable if it lay initially at a value which ranged between 35 and 45 in several experiments. After the total incubation period of 45 minutes, chromatography of the hot-water extract of the cells on paper, with tert-butyl alcohol-formic acid-water in the ratio 70:15:15 as the ascending phase, showed only one radioactive spot, corresponding to unchanged \( \beta \)-alanine. The initial rate of uptake shown in Fig. 1 is about 30 times the efflux (\( K_a \) 0.01 per minute) indicated by the rate of decline of high distribution ratios, in good agreement with the estimate of 40 for the steady state distribution ratio.

Figs. 2 and 3 contrast the inhibitory action of \( \beta \)-alanine on the uptake of 1 mm L-alanine and DPA. The action on alanine uptake is not yet significant at 10 mm \( \beta \)-alanine. Its gradual emergence at higher levels is consistent with a \( K_a \) of about 0.9 mm (Fig. 2) as determined by the Dixon method of plotting (7). Since the \( K_a \) predominating for L-alanine entry is only 0.0005 mm (2), this result indicates that the so-called A system, in reacting

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1 The abbreviations used are: DPA, L-\( \alpha,\beta \)-diaminopropionic acid; AIB, \( \alpha \)-aminoisobutyric acid.
FIG. 1. Time course of p-alanine-1-W uptake by Ehrlich cells at 0° and at 37°. A 4% suspension of cells in 1 mM p-alanine-14C in Krebs-Ringer-bicarbonate solution was used.

FIG. 2. Inhibition by increasing levels of p-alanine of the uptake by the Ehrlich cell of uniformly labeled DPA-W and of n-alanine-l-l% from 1 mM solutions, during 2 and 1 minutes, respectively.

Fig. 3. Plot of the reciprocal velocity of the uptake of l-alanine (left) and of DPA (right) from 1 mM solutions, against the concentration of p-alanine. The slope of the curve at left corresponds to a $K_i$ of about 0.2 m, and that at the right of 0.005 m; in the latter case the $K_i$ for DPA entry was taken to be 0.0003 m and the $V_{max}$ (as for glycine, alanine, and AIB 3) to be about 5 mmoles per kg of cell water per minute.

FIG. 4. Plot of the reciprocal velocity of the mediated uptake of p-alanine (1 mM) against the concentration of l-alanine.

with p-alanine and L-alanine, discriminates against the former by a factor of roughly 400.

Through the inhibitory action of AIR (1) and that of alanine on the uptake of DPA, an apparent $K_i$ of about 0.0003 m was observed for the latter for the transport system shared predominantly by these three (i.e. the A system). Ideally this result should be multiplied by an activity coefficient representing the fraction, still unknown, of DPA in the effective form. From this result we may anticipate that rather similar levels of p-alanine should be required to inhibit DPA uptake and to inhibit l-alanine uptake, were a single system involved for the uptake of these three. In Fig. 2 the lower curve shows, however, that the action of p-alanine on DPA uptake is much the greater, being already significant at a 0.005 m level. Its increase with concentration corresponds (Fig. 3, right hand scale) to an apparent $K_i$ unmistakably far lower (crudely, 0.005 m) than that shown for inhibition of L-alanine entry (about 0.2 m). The unexpected effectiveness of p-alanine in reducing DPA entry shows that these two must react with a common transport system, and one that plays at most a minor role in the total entry of L-alanine. For convenience we will tentatively refer to this mediating system as the p system.

This comparison does not mean, however, that the new route shared by p-alanine and DPA is inaccessible to a-amino acids. In fact, the inhibitory action of l alanine on p alanine uptake is as great as that of p-alanine in saturating its own uptake. The slope described by the points of Fig. 4 corresponds to a $K_i$ of 0.003 to 0.004 m for L-alanine in inhibiting p-alanine entry, whereas we shall see below that p-alanine shows a $K_i$ for entry...
of about 0.004 m. The accessibility of the β system to the inhibitory action of α-amino acids no doubt accounts for the ability of AIB (1) or alanine at fairly low levels to suppress almost totally the entry of DPA, even though a significant part of that entry appears to occur by the β rather than the L system.

β-Alanine at sufficiently high concentrations inhibits the uptake not only of L-alanine and glycine, but also that of L-phenylalanine (Fig. 5) and L-leucine. The latter two, particularly phenylalanine, enter principally by a different system, the so-called L system (2). If the values for phenylalanine, Kα 0.0004 m and Vmax 2 mmoles per kg of cell water per minute apply, the inhibitory action of β-alanine corresponds to a Kι of about 0.00 m. The converse action of phenylalanine in the 10 to 100 mM range on the uptake of β-alanine (Fig. 5) corresponds to a Kι of about 0.025 m, with the values for β-alanine, Kα 0.004 m and Vmax 1.6 mmoles per kg of cell water per minute, as determined below. Fig. 5 reveals, however, an undue effectiveness of inhibition by phenylalanine when its concentration lies in the 1 to 10 mM range, an inhibition which must correspond to a different process of β-alanine entry. That is, β-alanine appears to enter to a minor extent by a process for which phenylalanine has a rather high apparent affinity, which may be the L system; and to a major extent by one for which phenylalanine has less affinity, which we designate here as the β system.

Table I shows that β-alanine at levels up to 100 mM does not inhibit measurably the uptake of 1 mM L-lysine, and only slightly that of 10 mM L-α,ω-diaminobutyric acid. Conversely, 50 mM L-lysine has no action on the uptake of 1 mM β-alanine. Accordingly the β system is distinct from the agencies (1) serving for the uptake of the diamino acids.

Figs. 3 and 5 fail to give any significant indications that L-alanine and L-phenylalanine enter by the β system, an action that should be revealed by a sensitivity to low levels of β-alanine, greater than that predicted by the slope applying at higher concentrations, a relation illustrated in the upper curve of Fig. 5. The question was tested further by examining for sensitivity to the presence of β-alanine, the uptake of alanine at 10 mM, and that of phenylalanine at 5 mM. These levels are 12 to 20 times the Kι values applying to the principal means of entry of these two amino acids, and should therefore minimize entry by those routes, and presumably increase the portions (if any) entering by the system represented by the Kι values of 3 to 4 and 25, respectively, for alanine and phenylalanine. At these elevated concentrations, however, much less, rather than more, sensitivity to the presence of β-alanine could be observed (Table I). Accordingly very little if any entry of α-amino acids appears to occur by the β-system despite their appreciable reactivity with that system. Indeed the greater sensitivity of DPA entry than of L-alanine entry to the presence of β-alanine would again become inexplicable if the β system handled α-amino acids as rapidly as it does β-amino acids.

The pH sensitivity of β-alanine uptake at 1 mM was found to be intermediate between that characteristic of the A and L systems (2), the rate being 50% as fast at pH 5.0 as at pH 7.4. No significant stimulation of the counterflux of β-alanine by the presence of unlabeled β-alanine, either inside or outside the Ehrlich cell, could be shown. These two features bring the β system into a greater similarity to the A than to the L system.

The effect of β-alanine at various concentrations on the rate of its own uptake is illustrated by the Lineweaver-Burk plot in Fig. 6. In this figure, β-alanine entry by an apparently nonsaturable process, perhaps diffusion, with an estimated Kι of 0.07 per minute, has been deduced in calculating Y, the rate of mediated entry (8). The plot indicates the presence of a second uptake process with a high apparent Kα, somewhat above 0.1 m. The justification of the correction for a nonsaturable uptake process may be questioned in this case, and its accuracy certainly is poor. The similarity of the above value of Kα to the values observed for the nonsaturable entry of other amino acids (2) encourages the interpretation that a nonspecific means of migration accounts for the value. On the other hand, the tiny rate of uptake of β-alanine (see Fig. 1) at pH 7 (as for other amino acids) seems to leave little place for diffusion as a factor in the distribution of β-alanine. The same conclusion is supported by the very low rate constant Kι = 0.01 per minute calculated for the exodus.
uptake corrected for an apparently nonsaturable component with the contribution of the first.

These assignments, and those for alanine and phenylalanine, are of a line to describe the second entry process, after correcting for a $K_d$ of 0.07 per minute (8). The triangles represent the position second mediated mode of entry now shows an apparent $K_i$ of migration, we obtain a plot resembling Fig. 6, except that the second mediated mode of entry now shows an apparent $K_d$ of perhaps 0.5 M, i.e. too high to be measured. In either case we reach the same conclusion, that a difficulty saturated but mediated migration of $\beta$-alanine occurs, which may well utilize the $A$ system, for which Fig. 2 supports a value for the $K_i$ of $\beta$-alanine of about 0.2 M. A contribution by the $L$ system, for which $\beta$-alanine shows a $K_i$ of 0.06 M (Fig. 5), is less likely. An observation that the uptake of $\beta$-alanine at a 100 mM level is inhibited to the extent of 50% by 10 mM L-alanine, to the extent of 48% by 10 mM L-methionine, and to the extent of only 16% by 10 mM L-phenylalanine supports the interpretation that the $\beta$-alanine entry process with the high $K_i$, does indeed occur largely by the $A$ system. At this high $\beta$-alanine level, added amino acids would be expected to have no significant inhibitory action on $\beta$-alanine entry by the $\beta$ system, for which it shows a $K_i$ of 0.004 M.

The other uptake process, predominating at lower levels, must then be the one tolerating better the $\beta$ position of the amino group. It shows a $K_i$ of about 0.004 M, similar to the estimate of 0.005 M obtained for the $K_i$ of $\beta$-alanine in Fig. 3 (right). These assignments, and those for alanine and phenylalanine, are summarized in Table II.

The above results show that the reactive site in question transfers certain $\beta$-amino acids into the cell, and that it reacts with either $\alpha$- or $\beta$-amino acids, although its contribution to the transport of the former appears to be negligible. When the amino group was at the $\gamma$ position, however, as in $\gamma$-aminobutyric acid, little inhibition at 5 mM was seen for the uptake of 1 mM $\beta$-alanine; nor did 10 mM DL-$\omega$-aminocaproic or -capryllic acid inhibit the uptake of 1 mM $\beta$-alanine. The transport system in question accordingly does not tolerate as wide a separation of the amino and carboxyl groups as does a transport system reported for brain for which $\beta$-alanine, $\gamma$-aminobutyric acid, and $\omega$-aminocaproic acid compete (9). DL-$\beta$-Aminoisobutyric acid at 10 mM caused an 18% inhibition of the uptake of $\beta$-alanine, although the same level of DL-$\omega$-aminocaproic acid was without significant effect. This loss of affinity on adding an ethyl group to the end of the carbon chain of $\beta$-alanine is perhaps consistent with the lower apparent affinity of phenylalanine than of alanine for the $A$-system (Table II). The finding of an insignificant inhibitory action of 10 mmoles per liter of DL-isoaurine on the uptake of $\beta$-alanine (or on the uptake of various $\alpha$-amino acids) also shows the rather special advantage conferred by the structure of $\beta$-alanine, among $\alpha$-amino acids, on entry by the new system.

$\alpha$-Amino-$\beta$-dimethylaminopropionic acid, $pK_z$ 6.8, is like DPA in being mainly without net charge at pH 7.4. This factor makes it more an inhibitor of the transport of leucine and phenylalanine (Fig. 7) than of DPA or lysine. As the pH is lowered, however, converting the amino acid into a cation, its inhibitory action on phenylalanine uptake largely disappears (Fig 7) as would be predicted from the change in the state of charge. This behavior, along with the high apparent affinity of DPA for the $A$ transport system, and the observation that benzoylation of DPA leads mainly to the $\beta$-N-benzoyl derivative, emphasizes the contribution of the $\beta$ amino group to $pK_z$. The factor of charge on the sidechain, as a function of pH, deserves special consideration in the evaluation of "azaleucine" (10) as a leucine analogue.

Table II

<table>
<thead>
<tr>
<th>Constant for</th>
<th>By inhibition of uptake of</th>
<th>System to which attributed</th>
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<tr>
<td>$K_i$, $\beta$ alanine</td>
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<tr>
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</table>

Fig. 6. Lineweaver-Burk plot of the kinetics of $\beta$-alanine uptake by the Ehrlich cell. The symbol $Y$ represents the velocity of uptake corrected for an apparently nonsaturable component with a $K_P$ of 0.07 per minute (8). The triangles represent the position of a line to describe the second entry process, after correcting for the contribution of the first.

A subsequent determination gave a value, however, of 0.008 M for the $K_i$ of $\gamma$-aminobutyric acid in inhibiting uptake of labeled taurine from a 0.001 M solution during 5 minutes.
The means by which β-alanine is slowly accumulated to high gradients by the Ehrlich cell appears to include one of the known systems for neutral amino acids; but most of the entry at ordinary concentrations occurs by a distinct system with which the α-amino acids are also reactive, although one that ordinarily participates little if at all in their total uptake. Its low affinity even for β-alanine makes its contribution to amino acid transport so small that it would probably not be noticed did not the previously studied transport systems discriminate rather sharply against the larger separation of the amino and the carboxyl groups. An exceptional sensitivity of the uptake of L-α,β-diaminopropionic acid to the presence of β-alanine indicates that an appreciable part of the entry of this diamino acid probably occurs by the same agency. When the β-amino group of diaminopropionic acid bears two methyl groups, however, the resulting amino acid is reactive with the leucine-preferring system, with a pH sensitivity which identifies a dipolar form without net charge as the reactive species.

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