Studies on the Transport of Anions and Zwitterions of Acidic Amino Acids in *Streptomyces hydrogenans*

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In *Streptomyces hydrogenans*, acidic amino acids are taken up either as anions by a specific transport system or as zwitterions *via* a nonspecific one. Variations in the zwitterion concentration caused by changes in pH influence the uptake and exchange diffusion by the nonspecific system. Differences in pH-optima for L-glutamate and L-aspartate transport are due to the different pK_2-values of these amino acids. The anion transport by the specific system is accompanied by a short hyperpolarization of the membrane potential followed by a secondary influx of potassium ions into the cells.

Introduction

In the microorganism *Streptomyces hydrogenans*, there exist multiple transport systems for the active uptake of acidic amino acids. One group of these transport systems that exclusively transport L-glutamate and L-aspartate is termed specific system; another group can be termed as nonspecific system because of accepting both, acidic and neutral amino acids as substrates. Kinetic characteristics and the regulation of transport have been described earlier [1–4] and our present state of knowledge on the uptake of acidic amino acids in *Streptomyces hydrogenans* has been recently reviewed [5]. In this communication we will discuss the question, which ionic species is accepted by each system. The presented evidence leads to the assumption, that acidic amino acids are transported as anions by the specific system and as zwitterions by the nonspecific system.

Materials and Methods

The ^{14}C-labelled amino acids (AIB, cycloleu, glu, asp and aminoadipic acid) were obtained from Amersham-Buchler (Braunschweig, FRG); ^{3}H-la-belled tetraphenylphosphonium chloride was a gift from Dr. P. Geck (Frankfurt, FRG). All other reagents (analytical grade) were obtained from E. Merck (Darmstadt, FRG), Fa. Zinsser (Frankfurt, FRG), Sigma Chemie GmbH (München, FRG) and Biomol (Ilvesheim, FRG).

Streptomyces hydrogenans cells and DGA-cells, which have a much higher transport capacity for acidic amino acids than wild strain cells, were cultured and harvested as previously described [3, 5]. The cells were incubated with the ^{14}C-labelled amino acids in sodium potassium phosphate buffer or McIlvaine buffer. At various time intervals 2-ml-aliquots of the suspensions were withdrawn, and the cells were separated from their medium by filtration through membrane filters (type SM 11378, Sartorius-Membranfilter GmbH, Göttingen, FRG). In experiments with ^{3}H-TPP^{+} we used polycarbonate membranes (Bio-Rad-Laboratories, München, FRG) to which this cation is not bound. Measurements of radioactivity were performed in aliquots of cells and medium in a liquid scintillation counter (Packard Instrument Comp., Inc., Downers Grove, USA)

Abbreviations: AIB, 2-aminoisobutyrate; cycloleu, cycloleucine (= 1-aminocyclopentane-1-carboxylic acid); DGA-cells, D-glutamic acid utilizing cells, a *Streptomyces hydrogenans* species grown in a sugar salt medium with D-glutamate as the sole source of nitrogen; the cells have an induced specific system as compared to wild strain cells; TPP^{+}, tetraphenylphosphonium.

Results and Discussion

1. Transport activity and concentration of zwitterions

If, within a distinct pH-range, the affinity of a transport system to one of its substrates is approximately the same, the transport activity at each pH-value will depend mainly on the substrate concentration available; and if this concentration does not
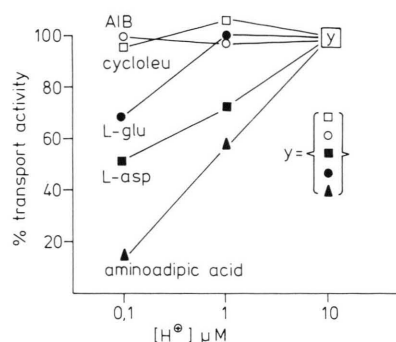
Fig. 1. Dependence of transport activity on the proton concentration of neutral (open symbols) and acidic (dark symbols) amino acids. Wild strain cells were incubated in 50 mM phosphate buffer at 30 °C with the corresponding ^{14}C-labelled amino acids. For better comparison, the 6-minute-influx at 10 μM [H+] was set 100% for all five amino acids (= y).

change by changing the pH, the uptake should be constant, too. This is the case for the uptake of neutral amino acids such as 2-aminoisobutyrate and cycloleucine (Fig. 1), the zwitterion concentration of which is only slightly changed if the proton concentration is enhanced from 0.1 μmol/l to 10 μmol/l. The uptake velocity of these amino acids by the specific system remains fairly constant over that range of proton concentration. Quite different is the situation for the uptake of aminoadipic acid, an acidic amino acid that is, nevertheless, taken up exclusively by the nonspecific system. Enhancing the proton concentration from 0.1 μmol/l to 10 μmol/l, the concentration of the aminoadipic acid zwitterions is strongly increased followed by an increase of the transport activity (Fig. 1). Transport activities of glutamate and aspartate lie in between those of AIB and cycloleu on one hand, and aminoadipic acid on the other. The lower the pH, the higher are the zwitterion concentration and the transport activity of the two amino acids (Fig. 1). On the contrary, increasing pH-values enhance the anion concentration and the transport by the specific system. This may explain why at a proton concentration of 0.1 μmol/l, the transport activity for glutamate is higher than that for aminoadipic acid though the zwitterion concentrations of both are nearly the same. Since the pK-value of the ω-carboxyl group of glutamate is higher than that of aspartate, the zwitterion concentration of glutamate is obviously higher, and at pH 6 and 7 the uptake of glutamate is comparatively faster than that of aspartate.

2. pH-optima and pK-values of the ω-carboxyl groups

Uptake of glutamate by the nonspecific system is optimal at about pH 5.5, that of aspartate at about 4.5. This difference is based on the different pK-values of the ω-carboxyl group of glutamate (pK = 4.32) and aspartate (pK = 3.65), so that, at the given pH- values of the medium, the zwitterion concentration of glutamate preponderates, compared to that of aspartate. However, at the same zwitterion concentration, the transport activity for both amino acids should be the same. In Fig. 2 the uptake activity for glutamate and asparatate (in percent of the maximum uptake activity) is plotted *versus* the difference between the pH-values of the medium and the pK- value of the corresponding ω-carboxyl group. In this way, the transport activities for both amino acids can be compared at equal zwitterion concentrations. The uptake profiles for the two amino acids are fairly the same with a common transport maximum at a pH-value lying one pH-unit above the corresponding pK_2-value.

3. Exchange diffusion and zwitterion concentration

Since only the nonspecific system shows exchange diffusion [5], differences in the zwitterion concentration of glutamate and aspartate at the same proton concentration should influence the extent of this exchange. To prove this, cells of *Streptomyces hydrogenans* were preloaded with ^{14}C-labelled AIB, separated from the ^{14}C-containing incubation medium and resuspended in fresh buffer. After adding
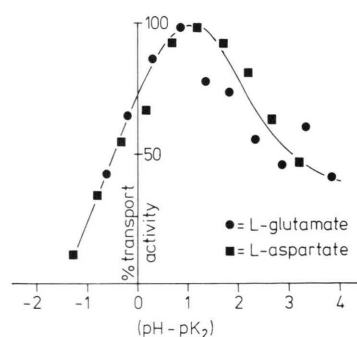

Fig. 2. pH-profiles of L-glutamate and L-aspartate uptake. Cells were incubated in McIlvaine buffer at 30 °C with the ^{14}C-labelled amino acids (extracellular concentration: 0.4 μmol/ml). For direct comparison of the profiles, the 5-minute-influx, at the pH-optimum of each amino acid, was set 100%. The percent transport activity is plotted in dependence on the difference between the pH and the pK-value of the ω-carboxyl group.
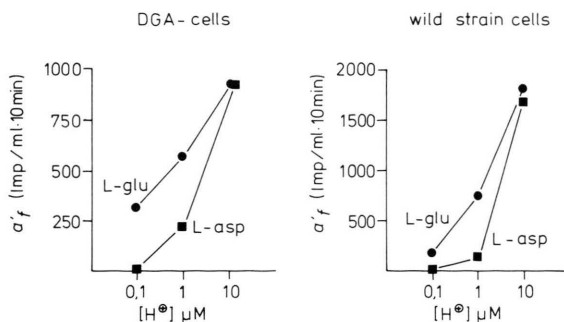
Fig. 3. Exchange diffusion between intracellular AIB and extracellular glutamate or aspartate, at three different proton concentrations. DGA- and wild strain cells were preloaded with ^{14}C-labelled AIB (0.375 μmol/ml medium) at 30 °C. The preloaded cells were resuspended in fresh McIlvaine buffer and incubated with either glutamate or aspartate (extracellular concentration: 3 μmol/ml). The exit of ^{14}C-labelled AIB was followed by determination of radioactivity in the medium ($= a_f^*$).

unlabelled glutamate or aspartate to the resuspended cells, the increase of extracellular radioactivity ($= a_f^*$), as a measure for the extent of exchange between intracellular labelled AIB and extracellular unlabelled amino acid, was determined. Both, for DGA-cells (Fig. 3a) and wild strain cells (Fig. 3b) the exchange diffusion increases as the proton concentration in the medium increases, *i.e.* by increasing the zwitterion concentration of the extracellular acidic amino acids. At each proton concentration, more glutamate than aspartate is taken up into the cells in exchange for intracellular AIB. This is explainable by the higher concentration of zwitterions of glutamate than of aspartate at the same total amino acid concentration and pH-value.

4. Behaviour of membrane potential amino acid transport

Liberman, Skulachev and collaborators [6, 7] were the first to show that synthetic lipophilic ions of the type $[(R_4)P]^+$ are distributed passively across the cell membrane according to an electrical potential, and that these ions can, therefore, be used as indicators for the magnitude of the membrane potential. In some microorganisms, this potential has been measured utilizing dibenzyldimethylammonium (DDA$^+$) [8, 9] and tetraphenylphosphonium (TPP$^+$) [10, 11]. The membrane potential of *Streptomyces hydrogenans* was measured, too [12]. In the present study we only present changes in membranes potential ($\Delta\psi_1 - \Delta\psi_2$) and not absolute values ($\Delta\psi$), thus avoiding uncertainties associated with the TPP$^+$- method. Since we were interested to register rapid changes of the potential, we used TPP$^+$, known to need the shortest time for reaching a steady stade distribution in Ehrlich ascites tumour cells [13]. We investigated the distribution of ^{3}H-labelled TPP$^+$ during the uptake of two acidic and two neutral amino acids. The experimental conditions were chosen such that, on the one hand, acidic amino acids were taken up nonspecifically (at pH 5.1) and on the other hand specifically (at pH 7.1). Fig. 4 (a and b) shows the results of the uptake of glutamate. At pH 5.1, the membrane potential remains constant in DGA- (4a) and wild strain cells (4b). On the contrary, at pH 7.1 a short hyperpolarization up to −10.3 mV is observed. If glutamate is replaced by aspartate (4c and 4d), a similar behaviour of the membrane potential is seen, though in the case of wild strain cells (4d) the hyperpolarization at pH 7.1 is less pronounced and at pH 5.1 a slight depolarization is found.

During the uptake of neutral amino acids like AIB (4e and 4f) or cycloleu (4g and 4h), the membrane potential doesn't change significantly at pH 5.1 or pH 7.1. We interpret these findings as follows: the hyperpolarization during uptake of acidic amino acids at pH 7.1, where transport is mediated by the specific system, is in favour of the hypothesis of anion transport by that system. The enhancement of membrane potential is quite obvious in DGA-cells, in which the specific system is induced. The transport of acidic amino acids at pH 5.1 and of neutral amino acids is mediated by the nonspecific system. In this case, the membrane potential remains fairly constant, an evidence for zwitterion transport by that system. The depolarization observed during uptake of aspartate by wild strain cells at pH 5.1 can be explained by the mode of energization of the nonspecific system: transport is driven by a proton symport [5, 12]. The influx of both, protons and substrate, depolarizes the membrane potential. The depolarization is limited by the action of a proton pump which extrudes the protons out of the cells.

5. Transport of anions and zwitterions and electroneutrality

Based on the electroneutrality, the intracellular accumulation of negatively charged substrates (*e.g.* glutamate anions) needs the accumulation of an
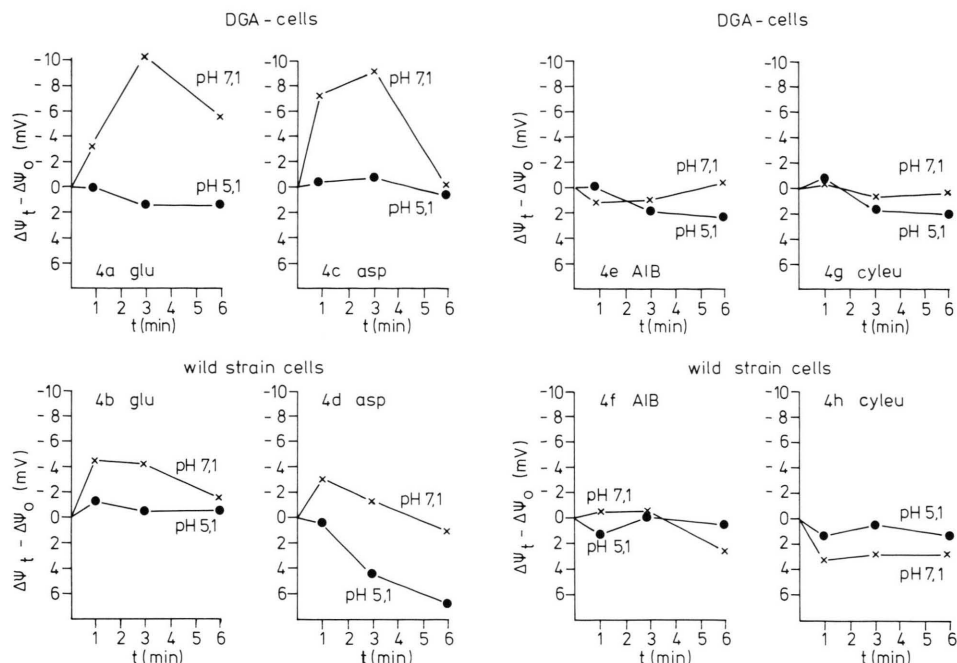
Fig. 4. Behaviour of the membrane potential of *Streptomyces hydrogenans* during uptake of two acidic and two neutral amino acids. The membrane potential of DGA-cells (4a, 4c, 4e, 4g) and wild strain cells (4b, 4d, 4f, 4h) was measured in 50 mM phosphate buffer of pH 5.1 and 7.1 at 30 °C, using the ^{3}H-TPP$^+$-distribution technique. After reaching a steady state distribution of TPP$^+$, unlabelled amino acids (L-glu, L-asp, AIB or cycloleu) were added (extracellular concentration: 20 nmol/ml). At distinct time intervals, the distribution of TPP$^+$ was measured and the membrane potential calculated before and after (1, 3 and 6 minutes) the addition of substrates. The results are given as changes of the potential in mV in relation to the original potential before starting transport.

equivalent amount of positively charged ions. Gross [14] has shown, that in *Streptomyces hydrogenans* the accumulation of 128 µmol glutamate per gram cell dry weight at pH 7.1, where transport is mediated mainly by the specific system, is followed by an increase of the amount of cellular potassium (150 µmol/g dry weight). At pH 5.1, where glutamate is chiefly transported nonspecifically in the zwitterion form, such an increase of the cellular potassium level could not be demonstrated. We assume that the K$^+$ influx into the cells follows that of glutamate anions. Evidence for this is that, during transport of acidic amino acids at pH 7.1, the initial hyperpolarization is followed by a rapid restoration of the starting membrane potential. A symport of potassium ions and glutamate doesn't seem likely to us.

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