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EFFECT OF ALKALI IONS ON THE ACTIVE TRANSPORT OF NEUTRAL AMINO ACIDS INTO *STREPTOMYCES HYDROGENANS*

K. RING, H. EHLE and B. FOIT

Gustav-Embden-Zentrum der Biologischen Chemie, Abteilung für Mikrobiologische Chemie, Johann-Wolfgang-Goethe Universität, Frankfurt am Main (G.F.R.)

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SUMMARY

The active transport of neutral amino acids into *Streptomyces hydrogenans* is inhibited by external Na^+. There is no indication that in these cells amino acid accumulation is driven by an inward gradient of Na^+. The extent of transport inhibition by Na^+ depends on the nature of the amino acid. It decreases with increasing chain length of the amino acid molecules, i.e. with increasing non-polar properties of the side chain. Kinetic studies show that Na^+ competes with the amino acid for a binding site at the amino acid carrier. There is a close relation between the K_i values for Na^+ and the number of C atoms of the amino acids. Other cations also inhibit neutral amino acid uptake competitively; the effectiveness decreases in the order $Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$. Anions do not have a significant effect on the uptake of neutral amino acids. After prolonged incubation of the cells with 150 mM Na^+, in addition to the competitive inhibition of transport Na^+ induces an increase in membrane permeability for amino acids.

INTRODUCTION

It is well established that the accumulation of distinct amino acids in mammalian cells requires the presence of Na^+ in the extracellular fluid [1–3]. According to the currently most favoured hypothesis, the uphill transport of amino acids into the cells is stoicheiometrically coupled to the downhill movement of Na^+. Although there is some evidence that the Na^+ gradient is not the only driving force for amino acid transport [4–8], the requirement for Na^+ is not questioned.

A fundamentally different situation appears to exist for amino acid transport in microorganisms. Although several microorganisms require Na^+ for growth, no conclusive evidence has been presented that an inward gradient of Na^+ is required to drive transport in these cells. Nevertheless, alkali ions influence specifically amino acid transport in a variety of microorganisms. In a marine *Pseudomonas* species, 2-aminoisobutyric acid uptake strongly depends on a high extracellular Na^+ con-

Abbreviations: Cycloleucine, 1-aminocyclopentane-1-carboxylic acid; POPOP, 2,2'-*p*-phenylene-bis(5-phenyloxazol); PPO, diphenyloxazol.

centration, although no Na^+ gradient is established; intracellular K^+ is required for maximal 2-aminoisobutyric acid accumulation [9–11]. In another marine species, *Halobacterium salinarium*, L-glutamate uptake requires sodium chloride [12]. Specific stimulation of amino acid transport by low extracellular Na^+ has been reported to exist in a variety of bacteria [13–17]. In some of these cases Na^+ appears to lower the K_m values of the transport systems for their substrates [15, 16]. Hence it was inferred that Na^+ is cotransported with the solute, as has been demonstrated for the Na^+-dependent melibiose permease system of *Salmonella typhimurium* [18].

Also, extracellular K^+ has been reported to stimulate active amino acid uptake into a variety of microorganisms, different mechanisms being involved [10, 15, 19–26].

In the present paper, the effects of alkali ions on the initial rates of uptake of neutral amino acids into *Streptomyces hydrogenans* are described. As shown earlier in this laboratory [27–29], amino acids are transported into these cells against large concentration gradients. The transport is unidirectional; no net transport out of the cells was observed. The loss of once accumulated amino acids from the cells is small. Only in a few cases, e.g. when the 2-aminoisobutyric acid or cycloleucine transport was studied, could the rate of efflux be increased by the addition of neutral amino acids to the incubation medium [27, 29]. Extending previous investigations [30] we found that, although this microorganism is able to grow in a natural environment which contains Na^+ and K^+ in high concentrations, the amino acid uptake does neither require, nor is stimulated by Na^+; rather it is inhibited by alkali ions. The inhibition is competitive. Its extent depends on the polar properties of the amino acids. In some aspects our results are similar to those reported by Christensen [3] who observed competitive interference of Na^+ plus a neutral amino acid with the transport of lysine in Ehrlich mouse carcinoma cells.

METHODS AND MATERIALS

1. *Preparation of cell suspensions*

Stm. hydrogenans, strain RT, was used throughout these studies. This strain has been derived from the wild type, designated as HR, by continuous subculturing over three years. As compared to the wild type, RT is characterised by a reduced tendency to form spores in submers cultures, a diminished sensitivity of amino acid transport towards alkali ions, and an increased capacity to enrich amino acids from the medium. No attempt has been made to define the differences between the two strains on a genetic basis. Only the experiment described in Fig. 2 was carried out some time ago with strain HR. All stock cultures were maintained on oat-meal agar slants, as described before [27].

Cells were grown at 30 °C in aerated batch cultures. The medium contained peptone, yeast extract, beef extract, glucose, and salts as described [27]. Midlog phase cells were harvested by centrifugation and washed three times with distilled water. Subsequently the cells were suspended in Tris buffer and chilled rapidly to 0 °C ('cold shock') to remove the amino acid pool as far as possible [31]. After 20 min, the cells were spun down, washed once with cold Tris buffer, and resuspended in buffer to the desired concentration (usually 1 mg dry cell mass per ml suspension).

2. *Transport studies*

Amino acid transport was studied as has been described in detail before [27–29]. Usually 5 ml cell suspension in a 50 ml Erlenmeyer flask equipped with a side arm was warmed to 30 °C in a shaking water bath. The uptake reaction was started by adding the radioactive amino acid from the side arm. If not otherwise indicated the amino acid concentration was between 10 and 60 times the K_m value, corresponding to 0.2–1.2 mM. In most experiments the specific activities were adjusted to 80 μCi/mM.

Aliquots (2 ml) were removed after the desired intervals, and the cells were separated from the incubation fluid by membrane filtration (Sartorius Membranfilter GmbH, Göttingen, G.F.R.; SM 11308, 0.15 μm pore size). The filtrates were collected, and the cells were washed with 2 ml buffer. Then the filters were transferred directly into scintillation vials containing 3 ml of absolute methanol. By this process the cells were extracted instantaneously, and the filter material was partially dissolved. After 30 min, 12 ml of scintillation fluid of the following composition was added: 1000 ml toluene, 600 ml ethanol, 50 mg POPOP, 3 g PPO. Vigorous shaking dissolved the filters completely. The samples were counted in a Packard "Tricarb" liquid scintillation counter. 200-μl aliquots of the filtrates were handled in the same manner. Corrections for adsorption of radioactivity by the filter material for each amino acid were made.

3. *Calculation of the results*

The results are expressed as: (a) relative uptake of radioactivity: $Ru' = u'_c/a'_f$ (ml/g), (u'_c = cpm per g dry cell mass; a'_f = cpm per ml extracellular fluid); or as (b) absolute uptake rates: $Ru' \cdot a_f$ per time = μmol per g per time unit (a_f = μmol amino acid per ml suspension).

4. *Buffers*

For all transport studies described below, Tris · HCl (0.05 M, pH 7.1, containing 1 % glucose) was used as basal incubation medium. Most of the experiments were carried out in the presence of different amounts of salts. Since the rate of amino acid uptake into *Stm. hydrogenans* is influenced by the osmolarity of the medium (Ring, K., unpublished), the solutions were adjusted to the same osmolarities by addition of equivalent amounts of sucrose.

5. *Special chemicals*

Radioactive amino acids were purchased from NEN Chemicals, Dreieichenhain, G.F.R. 2-amino[1-^{14}C]isobutyric acid, 9.79 Ci/mol; L-[U-^{14}C]alanine, 120 Ci/mol; [U-^{14}C]glycine, 102 Ci/mol; L-[U-^{14}C]valine, 252 Ci/mol; L-[U-^{14}C]leucine, 305 Ci/mol; L-[U-^{14}C]isoleucine, 313 Ci/mol; L-[U-^{14}C]phenylalanine, 464 Ci/mol; L-[*Me*-^{14}C]methionine, 11.5 Ci/mol; L-[U-^{14}C]serine, 140 Ci/mol, and from the Radiochemical Centre, Amersham, U.K., L-[U-^{14}C]arginine, 324 Ci/mol; L-[U-^{14}C] aspartic acid 8.7 Ci/mol; L-[U-^{14}C]glutamic acid, 10 Ci/mol; L-[U-^{14}C]lysine, 10 Ci/mol; and 1-amino[1-^{14}C]cyclopentane-1-carboxylic acid, 53 Ci/mol ("cycloleucine"). The unlabelled amino acids were chromatographically pure products of Fluka, Buchs/Switzerland, and of the highest chemical purity obtainable.

Tris, choline chloride, NH_4Cl, $MnCl_2$, and $MgCl_2$ were analytical grade re-

agents, and LiCl, NaCl, KCl, RbCl, and CsCl were "ultrapure" products of E. Merck, Darmstadt/Germany.

RESULTS

1. *The effect of NaCl on the time course of 2-aminoisobutyric acid uptake*

As is shown in Fig. 1, the uptake of 2-amino[^{14}C]isobutyric acid into *Stm. hydrogenans*, strain RT, is markedly inhibited by addition of NaCl to the incubation medium. Both the initial rate of uptake and the accumulation in the steady state are diminished. The degree of inhibition depends on the NaCl concentration in the medium. At 200 mM, the influx is inhibited by about 40 %.

At the beginning of the experiment the cells contained approx. 13 mM Na^+ (Ring, K., unpublished). Thus, in all batches, with the exception of the control, the extracellular Na^+ concentration exceeded the intracellular one. However, in no case was the rate of uptake of 2-aminoisobutyric acid found to be stimulated by external Na^+. Therefore, 2-aminoisobutyric acid uptake into these cells does neither require Na^+ in the incubation medium nor is driven by an inwardly directed Na^+ gradient.

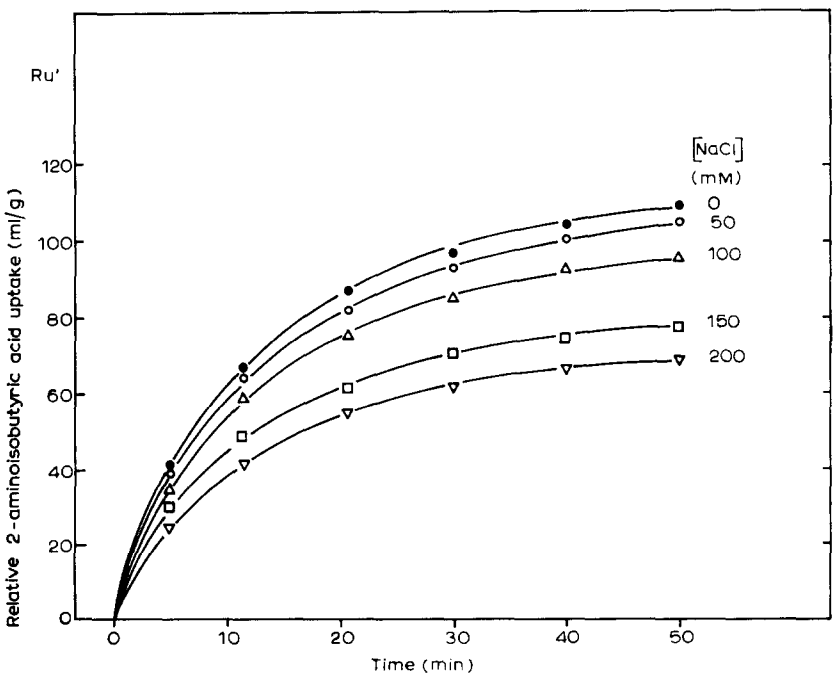

Fig. 1. Time course of 2-aminoisobutyric acid uptake into strain RT in the presence of different amounts of NaCl or KCl. The basal incubation medium was 50 mM Tris buffer, pH 7.1, containing 10 mM of each NaCl and KCl ('Tris/Na^+/K^+-buffer'), to which NaCl was added to the designated final concentrations. Isosmolarity was maintained by additions of sucrose. The 2-amino[1-^{14}C]isobutyric acid concentration in the reaction mixture was 0.3 mM (spec. act. 80 μCi/mmol). Before the uptake reaction was started, the cells were incubated for 30 min at 30 °C in Tris/Na^+/K^+ buffer, containing 400 mosmol sucrose. 2-Aminoisobutyric acid was added at time zero together with the alkali ions.

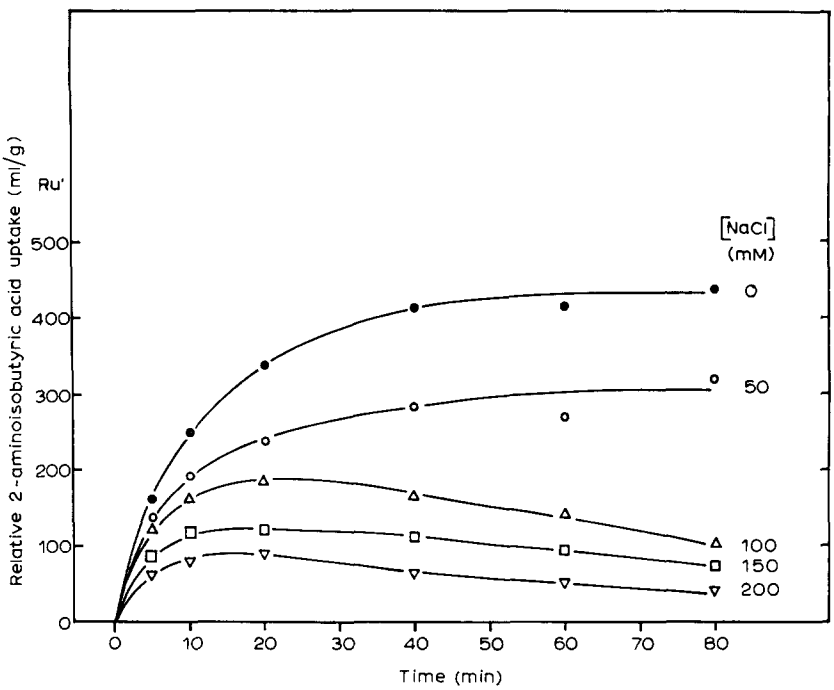

Fig. 2. Time course of 2-aminoisobutyric acid uptake into strain HR in the presence of different amounts of NaCl. The basal incubation medium was 50 mM Tris buffer, pH 7.1, to which NaCl was added to the concentrations as indicated. Isosmolarity was adjusted with sucrose. The 2-amino-[1-^{14}C]isobutyric acid concentration in the reaction mixture was 1 mM. Before the uptake reaction was started, the cells were incubated for 15 min at 30 °C in Na^+-free buffer containing 400 mosmol sucrose. 2-Aminoisobutyric acid was added at time zero together with NaCl.

confirming earlier observations made in this laboratory [30].

Qualitatively similar results were obtained with strain HR under the same experimental conditions (Fig. 2). NaCl at low concentrations diminishes the initial rate of transport and the steady state accumulation of 2-aminoisobutyric acid in a similar way to strain RT. However, an additional effect of NaCl is apparent. During prolonged incubation at NaCl concentrations of 100 mM and above, these cells loose their ability to retain the radioactive amino acid once accumulated. Thus, the conclusion may be drawn that NaCl affects in two different ways the 2-aminoisobutyric acid transport: (a) A first, rapid interference of Na^+ or Cl^- at the transport site for 2-aminoisobutyric acid directly, by which the initial uptake rate is reduced, and (b) a second, slower effect by which the rate of efflux of accumulated 2-aminoisobutyric acid is accelerated, possibly due to changes in the membrane permeability. The differences between strain RT and HR appear to be of quantitative nature, RT being less sensitive towards NaCl.

2. *Influence of NaCl on the efflux of 2-aminoisobutyric acid*

If the conclusions drawn from the previous experiments are correct, direct measurement of the efflux of 2-amino[^{14}C]isobutyric acid from preloaded cells

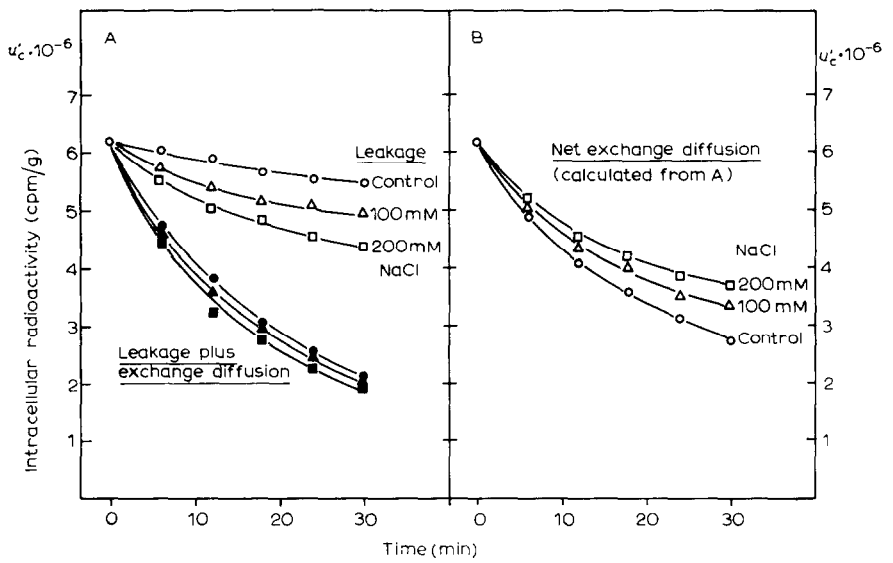

Fig. 3. Effect of Na^+ on the rate of efflux of 2-aminoisobutyric acid. Cells were preincubated at 30 °C in Tris buffer containing 10 mM of each NaCl and KCl, 400 mosmol sucrose, and 1 mM 2-amino[1-^{14}C]-isobutyric acid (spec. act. 80 μCi/mmol). After 60 min, the cells were collected on a membrane filter and resuspended in the same buffer as before but without 2-aminoisobutyric acid. The efflux reactions were started by mixing aliquots of the cell suspension with equal volumes of buffer containing different additions. The final sodium concentrations in the reaction mixtures were 0, 100, or 200 mM, respectively. Isosmolarity was maintained with sucrose. To measure exchange diffusion, unlabelled 2-aminoisobutyric acid (5 mM) was added to the reaction mixture at time zero.

should reveal that the rate of carrier-free efflux will be enhanced by NaCl, whereas the rate of carrier-mediated efflux will be diminished. As Fig. 3a demonstrates, the efflux of 2-amino[^{14}C]isobutyric acid into amino acid-free medium, which can be attributed to free diffusion, is raised markedly in the presence of NaCl. The efflux into amino acid-free medium, which represents the sum of both free and exchange diffusion, is slightly increased by NaCl. By subtracting the efflux due to leakage from the total efflux, the carrier-mediated part of efflux is obtained. As is shown in Fig. 3b, this flux is clearly diminished by NaCl. This finding supports the assumption that the reduction in the steady state levels of 2-aminoisobutyric acid accumulation shown in Fig. 1 can be related to reduced uptake rates and an increase in membrane permeability.

3. *The effects of different ions on the initial rate of 2-aminoisobutyric acid transport*

In the following experiments the effects of various sodium salts and alkali chlorides on the initial rate of uptake of 2-aminoisobutyric acid were studied. The results listed in Table I show that the inhibition of 2-aminoisobutyric acid influx by different sodium salts is rather independent of the nature of the anion. On the other hand, however, a statistically significant dependence on the cation can be observed. Among the alkali ions, Li^+ and Na^+ are the most effective inhibitors. The inhibitory properties decrease according to the position of the cation in the lyotropic series.

TABLE I

INHIBITION OF 2-AMINOISOBUTYRIC ACID INFLUX BY DIFFERENT SALTS

50 mM Tris · HCl, pH 7.1, was used as basal incubation medium, to which the various alkaline chlorides were added. In the control batches, isosmolarity was maintained by addition of sucrose. Before the uptake experiment was started, the cells were incubated for 20 min at 30 °C in buffer containing 200 mosmol sucrose. 2-Amino[1-^{14}C]isobutyric acid (1.2 mM; spec. act. 80 μCi/mmol) was then added together with the various salts. After 2 min, the reaction was stopped by membrane filtration. The results are expressed as relative transport activities ± S.E.M.

Addition	Concentration (mM)	Relative transport rate	±S.E.M. (*n*)
Sucrose (control)	300	1.000	—
NaCl	150	0.741	0.0187 (4)
NaBr	150	0.732	0.0184 (4)
NaI	150	0.731	0.0187 (4)
$NaNO_3$	150	0.759	0.0207 (4)
LiCl	150	0.711	0.022 (14)
NaCl	150	0.741	0.024 (14)
KCl	150	0.842	0.027 (14)
RbCl	150	0.908	0.026 (14)
CsCl	150	0.936	0.022 (14)
$MgCl_2$	100	0.220	— (3)
$MnCl_2$	100	0.760	— (3)
Choline chloride	150	0.930	— (2)
NH_4Cl	150	0.910	— (2)

Other cations, as NH_4^+, choline ion, Mn^{2+}, and Mg^{2+} also inhibit the transport of 2-aminoisobutyric acid, Mg^{2+} exhibiting the strongest effect among all ions tested. Therefore, the inhibition of amino acid uptake cannot be attributed to the specific action of a distinct ion, but is apparently a nonspecific effect due to the cationic properties of the inhibitory substance.

4. *Kinetics of inhibition of 2-aminoisobutyric acid influx by alkali ions*

Since both the transport substrate and the inhibitor are electrically charged, the inhibition of transport might be due to competitive interference at the binding site of the amino acid on the carrier molecule. To test this assumption, initial rates of 2-aminoisobutyric acid uptake at different 2-aminoisobutyric acid concentrations in the medium containing either NaCl or isosmolar sucrose were measured. The results were plotted according to Lineweaver-Burk. From Fig. 4 it is evident that 200 mM Na^+ inhibits the influx of 2-aminoisobutyric acid in a strictly competitive manner provided the cells were not preincubated in the presence of Na^+. After a preincubation period of 15 min, however, not only is the extent of inhibition markedly increased, but the kinetic analysis reveals a mixed type of inhibition. This indicates that in addition to a first, rapid action of Na^+, which is competitive, a second, slower reaction affects 2-aminoisobutyric acid uptake or accumulation. It is possible that the increase of membrane permeability for 2-aminoisobutyric acid described above is responsible for this effect.

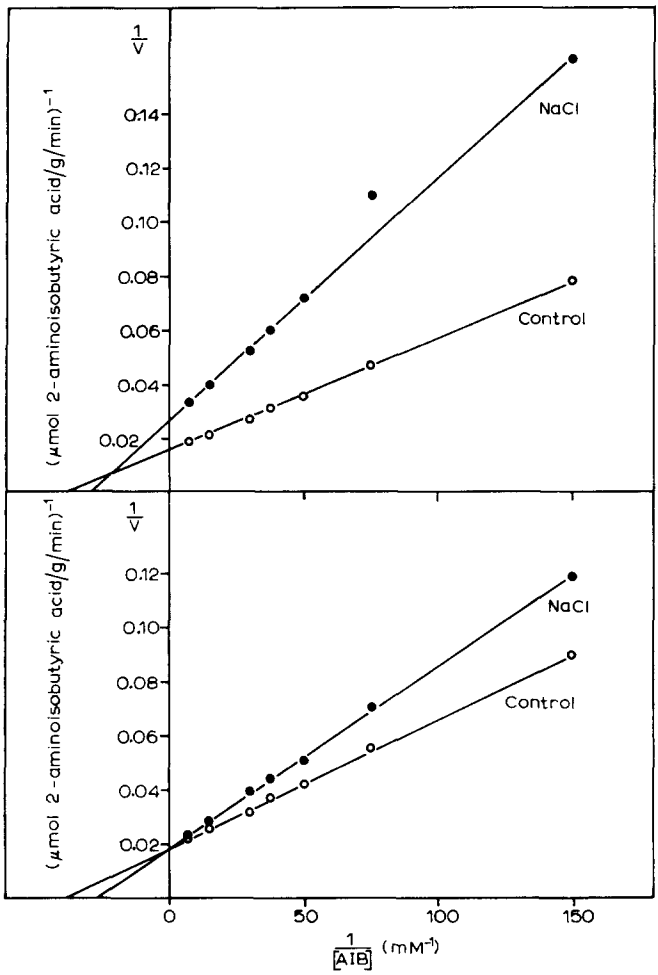

Fig. 4. Kinetics of 2-aminoisobutyric acid transport in absence and presence of NaCl. The incubation fluid was 50 mM Tris buffer, pH 7.1, containing 160 mM NaCl, or isosmolar sucrose. Influx of 2-amino[1-^{14}C]isobutyric acid [AIB] (2 min) into the cells was measured after 0 (lower graph) or 15 (upper graph) min preincubation of the cells in the absence or presence of NaCl. According to Lineweaver-Burk, the reciprocal transport rates were plotted versus the reciprocal 2-aminoisobutyric acid concentrations in the medium. From the lower graph the following kinetic constants were calculated: K_m = 25.0 μM; $K_i(Na^+)$ = 195.1 mM.

5. *Influence of the chain length of the amino acid residue on the effectiveness of transport inhibition by Na^+*

Comparative studies on the uptake of different amino acids, which are summarized in Table II show that the uptake of almost all amino acids tested is inhibited by Na^+. The effectiveness of Na^+, however, is different and depends apparently on the nature of the amino acid. As studies with the homologues series of neutral amino acids, i.e. glycine, alanine, valine, leucine, 2-aminoisobutyric acid, and cycloleucine show, the extent of inhibition by Na^+ decreases with increasing chain length of the

TABLE II

EFFECT OF NaCl ON INITIAL TRANSPORT RATES OF VARIOUS AMINO ACIDS

The incubation procedure was essentially the same as described in Table I. The reaction mixtures contained 120 mM NaCl, or 240 mM sucrose, respectively, and 1.0 mM amino acid, with exception of glutamate and lysine, the concentrations of which were adjusted to 0.05 mM. Uptake was measured over 4 min intervals after the addition of the [^{14}C]-labelled amino acids. The results are expressed as relative transport activities of the Na^+-containing batches.

Amino acid	Relative transport activity	S.E.M. (*n*)
Glycine	0.702	0.017 (11)
Alanine	0.782	0.023 (10)
Valine	0.814	0.024 (7)
Leucine	0.932	0.022 (11)
2-aminoisobutyric acid	0.835	0.022 (11)
Cycloleucine	0.961	0.047 (11)
Serine	0.755	(4)
Methionine	1.010	(4)
Proline	0.862	(5)
Glutamate	0.851	(4)
Lysine	0.790	(4)

amino acid molecule, i.e. with increasing non-polar properties of the side chain.

These results were substantiated by a more detailed kinetic analysis, using different concentrations of amino acids and of Na^+ in the medium. The results were plotted according to Dixon (Fig. 5).

The results confirm and extend the previous observations that Na^+ inhibits the uptake of all neutral amino acids in a strictly competitive manner. The inhibitor constants for Na^+, K_i, increase in the order: Gly > Ala > Ser > 2-aminoisobutyric acid > Val > Ile > Leu > cycloleucine > Phe. As can be derived from Fig. 6, there is a close relation between the K_i values and the number of carbon atoms of the amino cells.

Therefore, we suggest that Na^+ occupies the anionic binding site which is presumably common to each amino acid carrier, or might interact with the carboxylic group of the amino acid itself (see Fig. 7). Such a mechanism would explain the competitive nature of transport inhibition by cations and the observation that transport becomes less sensitive towards cations as non-polar interactions play an increasing role in binding the amino acid to its carrier.

DISCUSSION

The results presented in this paper clearly demonstrate that the uptake of neutral amino acids in *Stm. hydrogenans* does not require an inwardly directed gradient of Na^+, or the presence of Na^+ in the external medium. Thus, a coupled transport of Na^+ and amino acids, which is driven by the electrochemical potential gradient of Na^+ as is postulated for several transport systems in animal cells [1–8], cannot be involved in active amino acid transport in *Stm. hydrogenans*. For energetic reasons this result is not unexpected. By these bacteria, as by others, amino acids such as 2-aminoisobutyric acid or alanine can be concentrated several hundred fold or

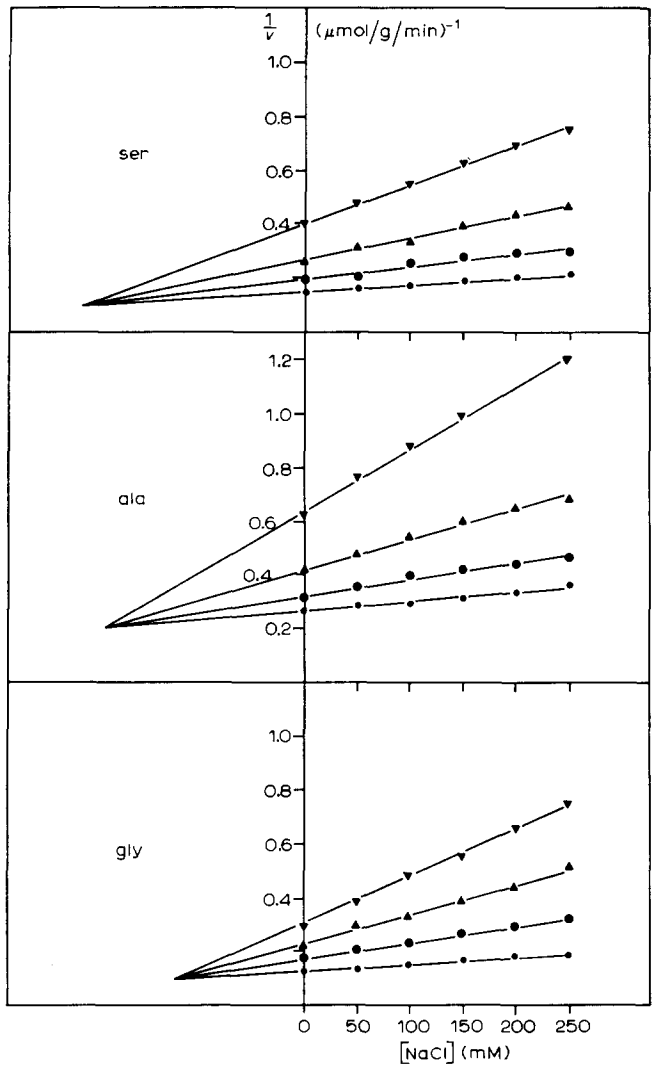

Fig. 5a. See page 626 for legend.

even more. It is obvious that the small Na^+ gradient, which is generally observed in growing cells under physiological conditions can hardly account for the high accumulation ratios. Nevertheless, Na^+-substrate transport mechanisms have occasionally been claimed to be operative also in bacterial transport [13]. Although extracellular Na^+ has been found to enhance the initial uptake rates in certain bacteria [9–17], in no case has conclusive evidence been presented that amino acid accumulation is driven by the Na^+ gradient. On the other hand, there is increasing evidence for several microorganisms that the inward gradient of protons is utilized as the major energy source for substrate accumulation (see ref. 32). Recent observations in our own laboratory indicate that this is also true for amino acid uptake in *Stm. hydrogenans* (Ring, K., unpublished).

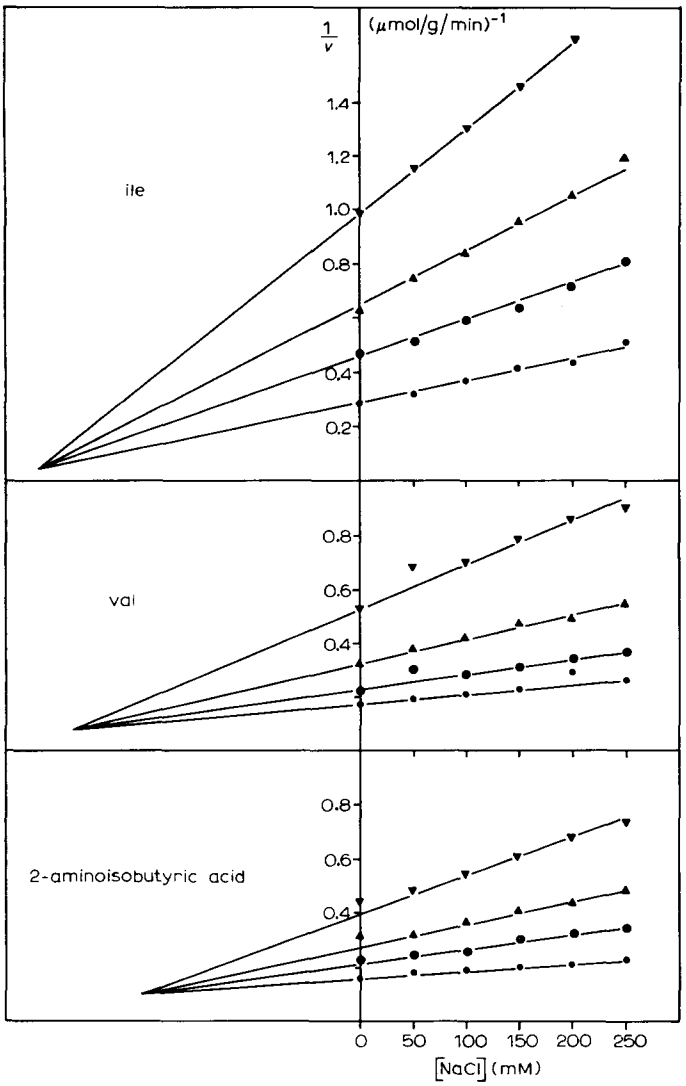

Fig. 5b. See page 626 for legend.

An unexpected finding in our studies was that extracellular alkali ions strongly inhibit amino acid transport in these cells. The inhibitory potency of the different alkali ions is different and depends on the position of the ion in the lyotropic series: Li^+ is the strongest inhibitor, Cs^+ the weakest one. Anions do not have a significant effect.

From the kinetic data it is obvious that the alkali ions compete with the amino acid for a binding site at the amino acid carrier. There is strong evidence that in response to the increasing hydrophobic nature of the side chain of the amino acid molecule the effectiveness of Na^+ decreases. This observation supports the assumption that the inhibitor interferes with one of the two binding sites which are presumably

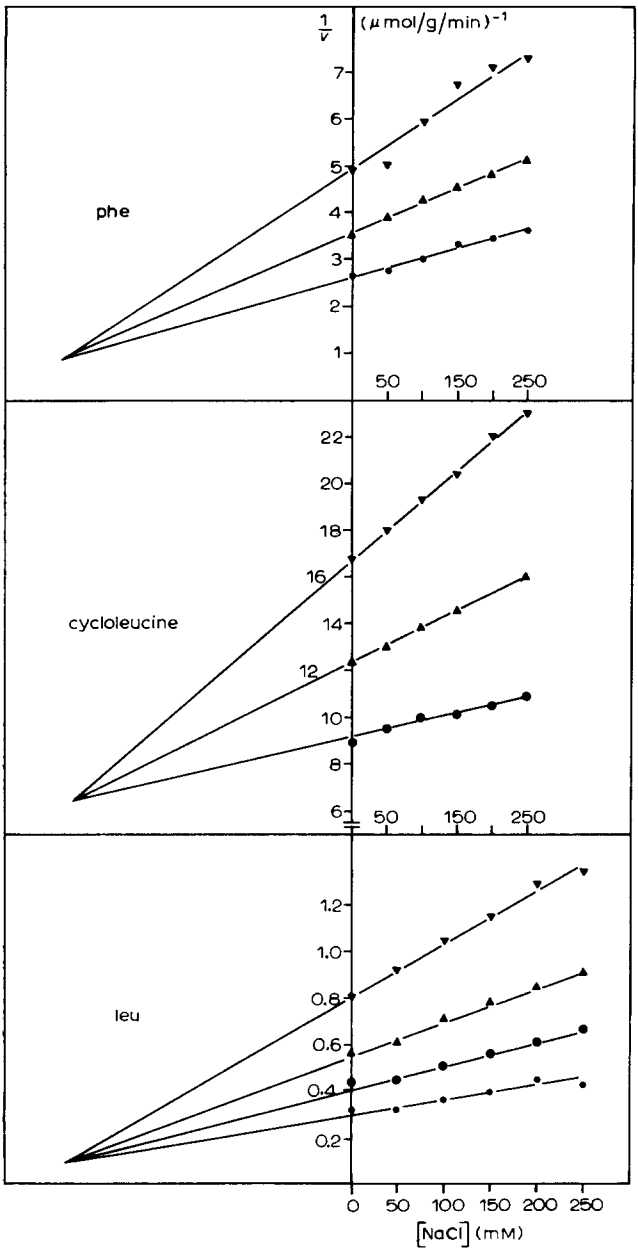

Fig. 5a, b, c. Kinetics of transport of various amino acids in the presence of different amounts of NaCl. Before the uptake experiment was started, the cells were preincubated in 50 mM Tris buffer, pH 7.1, for 15 min at 30 °C. The uptake reaction was started by addition of the ^{14}C-labelled amino acids together with NaCl and sucrose to give the same osmolarities. According to Dixon, the reciprocal uptake rates were plotted versus the Na^+ concentrations in the medium. Amino acid concentrations: 0.3 mM (▼); 0.6 mM (▲); 1.2 mM (●); 3.0 mM (●).

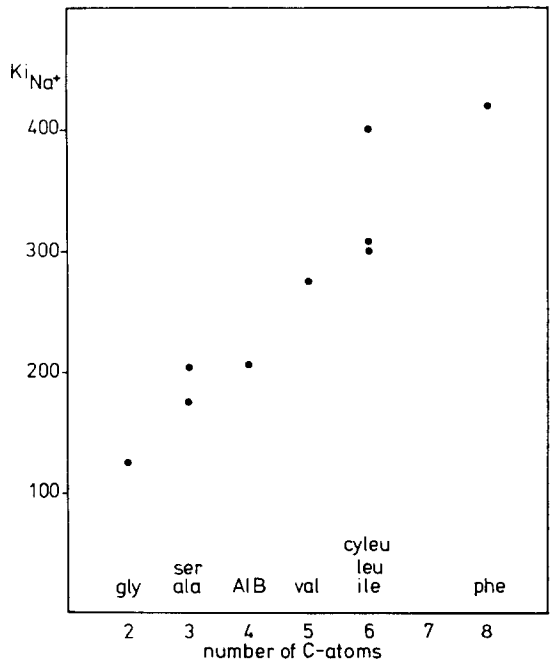

Fig. 6. Dependence of K_i values (mM) for Na^+ on the number of carbon atoms of various amino acids.

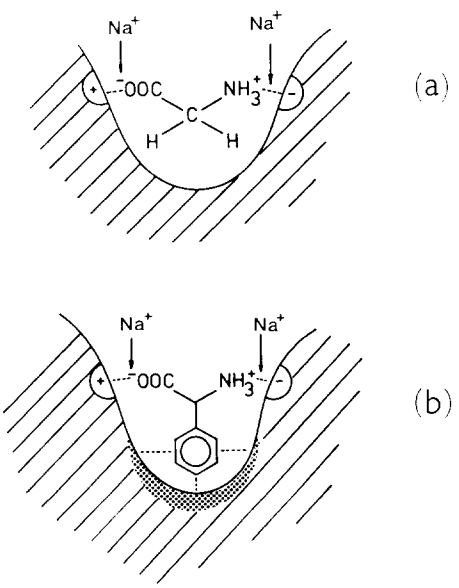

Fig. 7. Schematic representation of a simple model illustrating the competitive inhibition of neutral amino acid transport by alkali ions. Binding sites of the carrier (a) for glycine, (b) for phenylalanine; the shadowed area represents a hydrophobic zone postulated to be involved in the binding of the non-polar amino acid residues.

common to each amino acid carrier (see Fig. 7). As a consequence, the ability of the inhibitor to lower the binding force between the carrier and its "substrate" would be expected to decrease in response to the increasing importance of a third, nonpolar binding site, which presumably is involved in recognition and binding of hydrophobic amino acids. This assumption is consistent with the experimental data.

In some aspects, our results appear to be similar to those reported by Christensen and colleagues [3]. In the rabbit reticulocyte, the uptake of diamino acids is inhibited by Na^+. In contrast to our system, however, inhibition requires the presence of certain neutral amino acids, e.g. homoserine. This need is explained by the assumption that the neutral amino acid serves as analogue for the diamino acid, provided that Na^+ occupies the position on the binding site normally taken by the cationic ω-amino group of the diamino acid.

In addition to the fast action of the alkali ions, a second effect has been observed, which is manifested more slowly. Its nature is less well defined. After prolonged treatment of the cells with Na^+, e.g. 15 min, the transport rate is considerably lowered; the kinetic type of inhibition is formally noncompetitive. As has been previously observed with *Stm. hydrogenans* HR, Na^+ at concentrations above 150 mM irreversibly impairs the barrier properties of the membranes.

The reason for the increased sensitivity towards Na^+, which is observed after prolonged treatment, is still unclear. Monovalent cations at high concentrations are known to modify the permeability properties of artificial and biological membranes. Irreversible disorientation of membrane structures by polycations at much lower concentrations are well documented [33, 34]. It might be possible that the severe impairment of the barrier properties of the membrane in the more sensitive HR cells is based upon such interactions and results in the noncompetitive mechanism of transport inhibition.

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REFERENCES

1 Kromphardt, H., Grobecker, H., Ring, K. and Heinz, E. (1963) Biochim. Biophys. Acta 74, 549
2 Heinz, E. (1974) in Current Topics in Membranes and Transport, (Bronner, F. and Kleinzeller, A., eds.), Vol. 5, pp. 137, Academic Press, New York
3 Christensen, H. N. (1970) in Membranes and Ion Transport, (Bittar, E. E., ed.), Vol. 1, pp. 388, Wiley Interscience, London
4 Heinz, E. (1972) Na^+-linked Transport of Organic Solutes, Springer, Berlin
5 Eddy, A. A. (1968) Biochem. J. 108, 489
6 Gibb, L. E. and Eddy, A. A. (1972) Biochem. J. 129, 979
7 Schafer, J. and Heinz, E. (1971) Biochim. Biophys. Acta 249, 15
8 Heinz, E., Geck, P. and Pietrzyk, C. (1976) Proc. N.Y. Acad. Sci., in the press
9 Drapeau, G. and MacLeod, R. A. (1963) Biochem. Biophys. Res. Commun. 12, 111
10 Thompson, J. and MacLeod, R. (1971) J. Biol. Chem. 246, 4066
11 Sprott, G. D., Drozdowski, J. P., Martin, E. L. and MacLeod, R. (1975) Can. J. Microbiol. 21, 43
12 Stevenson, J. (1966) Biochem. J. 99, 257

13 Shiio, J., Miyajima, R. and Kashima, N. (1973) J. Biochem. Tokyo 73, 1185
14 Frank, L. and Hopkins, J. (1969) J. Bacteriol. 100, 329
15 Halpern, Y. S., Barash, H., Dover, S. and Druck, K. (1973) J. Bacteriol. 114, 53
16 MacLeod, R. A., Thurman, P. and Rogers, H. J. (1973) J. Bacteriol. 113, 329
17 Ariel, M. and Grossowicz, N. (1974) Biochim. Biophys. Acta 352, 122
18 Stock, J. and Roseman, S. (1971) Biochem. Biophys. Res. Commun. 44, 132
19 Gale, E. F. (1953) Advances in Protein Chemistry, Vol. 8, p. 285, Academic Press, New York
20 Davies, R., Folkes, J. P., Gale, E. F. and Bigger, L. C. (1953) Biochem. J. 54, 430
21 Eddy, A. A. and Indge, K. J. (1962) Biochem. J. 85, 35
22 Eddy, A. A. and Nowacki, J. A. (1971) Biochem. J. 122, 701
23 Grimm, E. (1974) Diplomarbeit, Univ. Frankfurt
24 Ring, K., Grimm, E. and Schwarz, M. (1976) Drug. Res. in the press
25 Gale, E. F. and Llewellin, J. M. (1972) Biochim. Biophys. Acta 266, 182
26 Gross, W. (1969) Habilitationsschrift, Univ. Frankfurt
27 Ring, K. and Heinz, E. (1966) Biochem. Z. 344, 446
28 Gross, W. and Ring, K. (1971) Biochim. Biophys. Acta 233, 652
29 Ring, K., Gross, W. and Heinz, E. (1970) Arch. Biochem. Biophys. 137, 243
30 Ring, K. (1965) Z. Klin. Chem. 3, 13
31 Ring, K. (1965) Biochem. Biophys. Res. Commun. 19, 516
32 Harold, F. M. and Altendorf, K. H. (1974) in Current Topics in Membranes and Transport (Bronner, F. and Kleinzeller, A., eds.), Vol. 1, p. 1, Academic Press, New York
33 Mayhew, E., Harlos, J. P. and Juliano, R. L. (1973) J. Membrane Biol. 14, 213
34 Ring, K. (1974) Zbl. Bakt. Hyg. I. Abt. Orig. A. 228, 248