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AMINO ACID TRANSPORT VIA THE RED CELL ANION TRANSPORT SYSTEM

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Evidence is presented that the red cell anion-exchange transporter (Band 3) can selectively transport small neutral amino acids, including glycine, serine and cysteine, but not alanine, proline, valine and threonine. This transport is inhibited by micromolar concentrations of SITS (4-acetamido-4′-isothiocyanostilbene-2,2′-disulphonate), and increased by raising the pH from 6.5 to 8.5.

The most abundant transport protein of the human red cell membrane is the Band 3 anion-exchange transporter with approx. 10^6 copies per cell [1], giving a transport capacity of 30 mol/l cells per min for Cl^--HCO_3^- exchange at 37°C [2]. Apart from transporting many inorganic anions [3], and certain cations as anion-pairs (e.g. $LiCO_3^-$) [4,5], the system has a significant affinity for a number of organic acids, including lactate and pyruvate [6,7]. The organic substrate NBD-taurine offers a convenient fluorescent assay of transport function [8]. We report here that this system has the ability to transport selectively some neutral amino acids and we demonstrate that a significant fraction of glycine entry into red cells occurs by this mechanism.

In the course of a series of experiments on glycine transport in human and sheep red cells, we noticed that glycine uptake was always highest in SO_4^{2-} medium, lower in Cl^- and lowest in NO_3^- [9]. This effect was specific for glycine and was not shown by alanine or valine. Glycine fluxes in the three media were inversely related to the known affinities of SO_4^{2-}, Cl^- and NO_3^- for the Band 3 anion-exchange transporter [10], raising the possibility that glycine was competing with these anions for transport by the Band 3 system. In the present series of experiments we therefore investigated the effects of SITS, a specific anion transport inhibitor [11], on glycine entry into human red cells equilibrated in either isotonic K_2SO_4 or KCl media (Fig. 1). Potassium salts were chosen for these experiments to eliminate Na^+-dependent glycine uptake by the Gly and the ASC system [9]. In agreement with earlier results, uptake was approx. 2-fold higher in SO_4^{2-} medium than in the presence of Cl^-. Addition of increasing amounts of SITS inhibited both fluxes to the same final level i.e. the SITS-sensitive component of glycine uptake was greater in SO_4^{2-} than in Cl^-, while the residual SITS-insensitive flux was identical in the two media. In both cases half-maximum inhibition was given at a SITS concentration of 3.5 μM, a value compatible with the affinity of SITS for the Band 3 protein [11]. SITS inhibition of glycine uptake persisted if inhibitor-treated cells were washed with ice-cold medium to remove extracellular SITS before addition of glycine. There was

Abbreviations: SITS, 4-acetamido-4′-isothiocyanostilbene-2,2′-disulphonate; NBD-taurine, 4-(2-sulphoethyl amino)-7-nitrobenz-2-oxa-1,3-diazole.

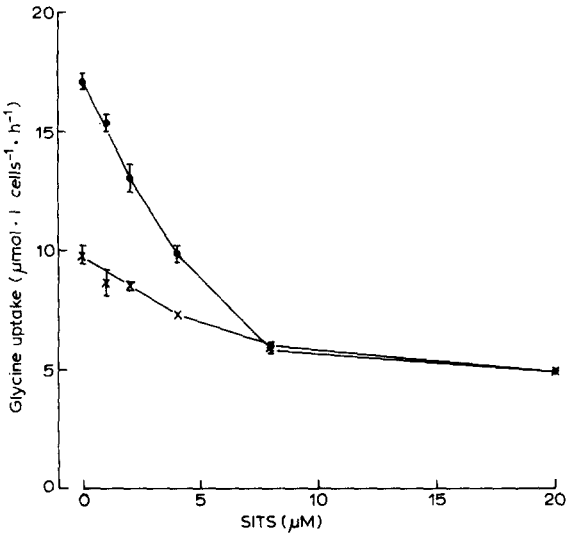

Fig. 1. SITS inhibition of glycine uptake by human red cells equilibrated in either KCl or K_2SO_4 medium. Initial [1-^{14}C]glycine influx rates at pH 7.4 (50 μM amino acid, 15 min incubation) were measured at 37°C in KCl (X) and K_2SO_4 (•) media by the ice-cold start-stop technique described by Ellory et al. [9]. Briefly, uptake was started and stopped by transferring incubation tubes (containing both cells and radioactive permeant) from an ice-bath to a 37°C bath and vice versa. Cells were washed free of extracellular radioactivity using ice-cold incubation medium, lysed in dilute Triton X-100 and deproteinized with trichloroacetic acid. Samples of the protein-free supernatants were counted for radioactivity by β-scintillation spectroscopy with quench correction. Cells were pretreated with SITS at room temperature for 15 min before cooling and addition of amino acid. Values are means ± S.E. of triplicate estimates.

TABLE I

pH-DEPENDENCE OF SITS-SENSITIVE GLYCINE UPTAKE BY HUMAN RED CELLS IN K_2SO_4 MEDIUM

Initial glycine influx rates were measured at 37°C as described in the legend to Fig. 1. Values are means of triplicate estimates.

pH	Uptake (μmol/l cells per h)		
	Control	+10 μM SITS	Δ
6.5	2.11 ± 0.21	1.15 ± 0.03	0.96 ± 0.04
7.5	5.24 ± 0.06	1.76 ± 0.09	3.48 ± 0.01
8.5	24.65 ± 0.18	2.70 ± 0.08	21.95 ± 0.03

therefore no direct interaction between SITS and amino acid.

These results strongly suggest that a significant fraction of glycine uptake into human red cells occurs via the anion-exchange system. Although glycine is a neutral amino acid, its isoelectric point (pI at 25°C) is 6.0 (pK_{a1} 2.3, pK_{a2} 9.7) [12,13], so that at pH 7.4 a small fraction of molecules will be negatively charged. It is this form of glycine which is presumably transported by the Band 3 protein. To test this, we investigated the pH dependence of SITS-sensitive glycine influx. Table I illustrates glycine uptake (50 μM) in K_2SO_4 medium in the presence and absence of 10 μM SITS at pH 6.5, 7.5 and 8.5, and shows that SITS-sensitive glycine uptake was stimulated 22-fold over this pH range while the residual SITS-insensitive flux only increased by 2-fold. Since the pH-dependence of the anion-exchange transporter itself is complex, and differs for the transport of monovalent and divalent substrates [14,15], the same experiment was repeated in KCl medium. There was a 6-fold stimulation of SITS-sensitive glycine uptake between pH 6.5 and 8.5. Over this pH range the ratio $[Gly^-]/[Gly^{total}]$ increases from approx. $6 \cdot 10^{-4}$ to $6 \cdot 10^{-2}$. The results are therefore consistent with glycine transport via the anionic form of the amino acid.

Previous experiments failed to find a significant effect of anion-substitution on alanine and valine fluxes in human and sheep red cells (see above), suggesting that not all neutral amino acids are substrates for the anion-exchange transporter. However, those cells possess additional mediated transport systems for amino acids [18] and several of these systems have relatively high transport capacities which might mask influx by the Band 3 system. We therefore chose Tr^- sheep red cells to investigate the substrate specificity of amino acid uptake by this route. These cells lack the amino acid transport systems found in human and normal sheep (Tr^+) red cells [18,19]. Table II presents data from an experiment where we have measured the influx of 50 μM glycine, proline, alanine and serine into control and SITS-treated Tr^- cells in both K_2SO_4 and KCl media. Only glycine and serine showed increased fluxes in SO_4^{2-} medium compared with

TABLE II

EFFECTS OF SITS ON AMINO ACID UPTAKE BY Tr^- SHEEP RED CELLS EQUILIBRATED IN EITHER KCl OR K_2SO_4 MEDIUM

Initial L-[U-^{14}C]proline, alanine and serine and [1-^{14}C]glycine influx rates (45 min incubation) were measured at 37°C as described in the legend to Fig. 1. The SITS concentration was 20 μM. Cells were washed free of excess inhibitor (two times 20 vol. ice-cold medium) before addition of amino acid. Δ is the difference in uptake rate between control and SITS-treated cells. Values are means ±S.E. of triplicate estimates.

Amino acid	Uptake (μmol/l cells per h)					
	KCl			K_2SO_4		
	Control	+SITS	Δ	Control	+SITS	Δ
Glycine (0.05 mM)	1.01 ± 0.01	0.59 ± 0.03	0.42 ± 0.03	2.60 ± 0.02	0.58 ± 0.01	2.02 ± 0.02
Proline (0.05 mM)	0.51 ± 0.04	0.56 ± 0.07	−0.05 ± 0.08	0.45 ± 0.02	0.44 ± 0.02	0.01 ± 0.03
Alanine (0.05 mM)	1.31 ± 0.02	1.34 ± 0.04	−0.03 ± 0.04	1.50 ± 0.02	1.33 ± 0.03	0.17 ± 0.04
Serine (0.05 mM)	0.85 ± 0.02	0.64 ± 0.02	0.21 ± 0.03	2.61 ± 0.02	0.80 ± 0.02	1.81 ± 0.03
(0.5 mM)	8.06 ± 0.04	5.40 ± 0.10	2.66 ± 0.11	25.0 ± 0.5	7.0 ± 0.2	18.0 ± 0.5
(5.0 mM)	80.6 ± 0.8	57.8 ± 0.4	22.8 ± 0.9	238 ± 4	62 ± 1	176 ± 4

uptake in the presence of Cl^- (2.4- and 3.1-fold, respectively). For both amino acids this effect was abolished in the presence of SITS which also significantly inhibited glycine and serine uptake in Cl^- medium (47 and 25% inhibition, respectively). In contrast, SITS had no significant effect on proline uptake in either medium, while there was a small inhibition of alanine influx in the presence of SO_4^{2-}. Other experiments found no significant inhibition of threonine or valine uptake in SO_4^{2-} medium. Under the same conditions, 50 μM cysteine showed a SITS-sensitive uptake of 1.05 ± 0.15 (mean ± S.E. (n = 3)) μmol/l cells per h (total flux in SO_4^{2-} medium 5.23 ± 0.07 μmol/l cells per h).

The relative abilities of these different amino acids to be transported by the anion-exchange transporter (glycine, serine > cysteine > alanine > proline, threonine, valine) does not correlate with their respective pK_{a1} and pK_{a2} values (pI 6.0, 5.7, 5.1, 6.1, 6.3, 5.6 and 6.0, respectively), suggesting that the anion-carrier has the ability to discriminate between different amino acid structures. Further evidence of selectivity comes from the observation that human and sheep red cells are virtually impermeable to glutamate and aspartate [18–20], amino acids which exist largely as anions at neutral pH. The results in Table II further show that SITS-sensitive serine uptake by Tr^- sheep cells is linear with amino acid concentration up to at least 5 mM in both Cl^- and SO_4^{2-} medium. We have also been able to demonstrate SITS-sensitive serine uptake in human red cells. This flux was also linear with concentration up to 5 mM (0.81 ± 0.12, 9.26 ± 0.96 and 92.4 ± 4.3 (n = 3) μmol/l cells per h at 0.05, 0.5 and 5 mM extracellular serine in Cl^- medium, respectively, and 2.72 ± 0.11, 25.8 ± 1.4 and 305 ± 10 μmol/l cells per h in SO_4^{2-} medium, respectively).

In conclusion, the present results demonstrate that some neutral amino acids can enter red blood cells via the Band 3 anion-exchange transporter. At physiological amino acid concentrations, uptake by this route normally represents a relatively small fraction of the cell's amino acid transport capacity. However, in the case of Tr^- sheep red cells, transport by this mechanism accounts for a major fraction of the total glycine uptake. This is one of the amino acids required for intracellular glutathione biosynthesis.

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