PERMEABILITY OF MITOCHONDRIA TO NEUTRAL AMINO ACIDS

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1. Introduction

Although a great deal of work has been carried out on the permeability of the mitochondrial membrane [1, 2], little is known of its permeability to neutral amino acids. What evidence there is suggests that mitochondria are permeable to some amino acids [3–7]. We have investigated the permeability of mitochondria to a selection of neutral amino acids and present a discussion of the mechanism of entry.

2. Materials and methods

Rat liver mitochondria were prepared according to [8], rat brain mitochondria according to [9]. Protein was assayed by a modified biuret method incorporating deoxycholate to solubilise the mitochondria. Swelling was measured at 640 nm as described in [1].

Liposomes were prepared by gentle shaking as described in [10] using crude soy-bean phospholipids or lecithin + 10% dicetyl phosphoric acid; the medium was $\frac{250}{3}$ mM K_2SO_4, 5 mM Tris–Cl, 1 mM EGTA, pH 7.4 or 25 mM KCl, 5 mM Tris–Cl, 1 mM EGTA, pH 7.4 [11]; swelling of liposomes was followed at 400 nm.

All substrates were obtained from B.D.H. Biochemicals, Poole, UK, except for the 3-amino butyric acids which were from Koch-Light Laboratories Ltd., Colnbrook, UK; and 5-amino valeric acid and 6-amino caproic acid which were from Ralph N. Emanuel Ltd., Wembley, Middx., UK.

3. Results and discussion

The rates of swelling in iso-osmotic solutions shown in table 1 are taken as an indication of the relative rates of permeation of the amino acids [1]. Since swelling is not dependent on the presence of

Table 1
Rates of swelling of mitochondria.

Compound	% Of rate in β-alanine	
	Liver mitochondria	Brain mitochondria
Glycine	59	74
L-Alanine	50	67
DL-Alanine	51	60
β-Alanine	(100)	(100)
L-Valine	63	–
DL-Valine	61	–
DL-Nor-valine	53	50
L-Isoleucine	18	–
DL-2-Amino-n-butyric acid	28	29
2 Amino-iso-butyric acid	24	25
DL-3 Amino-n-butyric acid	23	25
3-Amino-iso-butyric acid	43	32
4-Amino-n-butyric acid (GABA)	23	26
5-Amino valeric acid	6	–
6-Amino caproic acid	3	–
DL-Serine	21	–
DL-Threonine	2	–
L-Cysteine	7	–
L-Methionine	4	–
L-Proline	33	–

Mitochondria (2–4 mg protein) were suspended in 2.5 ml of a 250 mM solution of the amino acid containing 5 mM Tris–Cl, 1 mM EGTA, 1 μg rotenone, and 1 μg antimycin A at pH 7.4. The initial value of ΔE_{640} was measured and is expressed in arbitrary units relative to the fastest change observed (with β-alanine). This rate is about one seventh of that with NH_4 acetate, an extremely rapid penetrant.

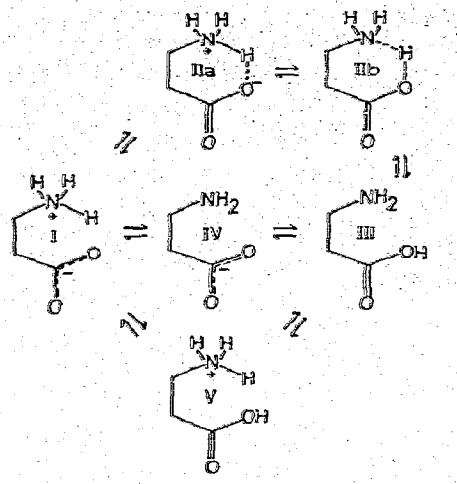
agents allowing dissipation of a membrane potential, the permeant species must have no net charge. There is no evidence for a requirement for linked movement of any other species. The permeant form must therefore be either the zwitterion or a form related to it by internal proton movements.

Carriers have been postulated for glycine [3] and for neutral amino acids [5]. We feel that there is no conclusive evidence for these carriers. Garfinkel [3] suggested a glycine carrier on the basis of experiments in which the 'equilibrium' optical density of mitochondria suspended in glycine solutions was measured. These results are not meaningful since mitochondria will swell in solutions of permeant solutes until membrane damage occurs and allows efflux of internal osmotically active species, for if there is no additional impermeant species outside, the permeant species can never provide the permanent osmotic support needed for true equilibrium.

Gamble and Lehninger [5] propose a general carrier for neutral amino acids from their finding that rat liver mitochondria swell in glycine, alanine, proline, valine, and citrulline, but rat heart mitochondria do not swell in citrulline. The mechanism of entry of citrulline is open to debate [12], and our results show that other amino acids do not show tissue differences. We therefore feel that this generalisation is not valid.

Liposomes have been shown to be permeable to amino acids [11, 13]; the rate of penetration is dependent on the hydrophobicity of the amino acid. This rate is however very much less than in mitochondria, as is confirmed by our results. We found there was no significant swelling of liposomes prepared in either medium (see Methods) over a 10-min period under the same conditions as for mitochondria, although liposome swelling was apparent in iso-osmotic ammonium acetate and in hypo-osmotic K_2SO_4. This might be taken as circumstantial evidence for carrier-mediated uptake of amino acids in mitochondria, but it must be borne in mind that the mitochondrial membrane is very different from the liposomal system, particularly since the mitochondria contain much protein.

There are several lines of evidence which imply that there are no specific transport mechanisms for neutral amino acids. Brand and Chappell [14] found that rates of swelling of both brain and liver mitochondria were proportional to the concentration of 4-amino butyric acid (GABA) in the suspending medium; no evidence was obtained for saturation kinetics. We feel that apparent values of K_m for transport of more than about 100 mM are meaningless: models of diffusion across a membrane can easily produce saturation at high substrate concentrations.

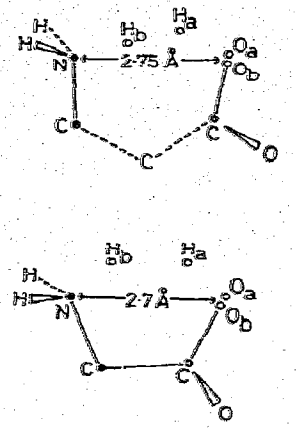


Scheme 1. Possible forms of β-alanine. IIa and IIb could be either tautomers or canonical forms of a resonance hybrid. Ring form(s) are referred to as II in the text.

Table 1 shows that: a) L- and DL-alanine enter at similar rates, the same is true of L- and DL-valine; b) the relative rates show no correlation with the physiological importance of entry of the amino acids into the mitochondria, and c), the relative permeabilities of liver and brain mitochondria are very similar although metabolism of for example GABA is very dissimilar in the two tissues. This lack of evidence for saturation and specificity argues against carrier-mediated uptake of amino acids.

At first sight, however, it is difficult to see how the amino acids are able to cross the mitochondrial membrane by diffusion at the observed rates. On the basis of macroscopic dissociation constants the calculated fraction unionised is similar to that for dicarboxylic acids, which are generally accepted to be impermeant in the absence of a specific carrier [15]. We suggest that the ability of the amino acids to form rings with intra-molecular hydrogen bonds may be important in their permeation through membranes.

Scheme 1 shows the possible rings formed by β-alanine. Note that since a) the angle at the carboxyl carbon is 120°, not 109°, and b) the energy of a hydrogen bond increases as it approaches linearity, we



Scheme 2. Possible ring structures for glycine and β-alanine. Scale drawings were made from Dreiding molecular models, marking positions (•, ○) of atoms in the plane of the possible O·····H·····N hydrogen bond. Positions marked (O) are possible alternatives *calculated* from normal bond lengths; O_a and H_a would form a normal C-OH group; H_b would be the position of an amino hydrogen; O_b would be in a normal C = O.

would expect the optimal ring sizes to be greater than in alicyclic rings. Scheme 2 shows ring structures for glycine and β-alanine. The hydrogen available for hydrogen bonding is much closer to the nitrogen—oxygen line in β-alanine than it is in glycine. The hydrogens on C-2 and C-3 of β-alanine are staggered.

The results in the table 1 show that β-alanine and 3-amino-iso-butyric acid (6-membered rings) penetrate twice as fast as their isomers α-alanine and 2-amino-iso-butyric acid respectively (5-membered rings). However 2-amino-*n*-butyric acid penetrates slightly faster than 3-amino-*n*-butyric acid. 4-amino-*n*-butyric acid (7-membered ring) penetrates at the same rate as 3-amino-*n*-butyric acid. 5-amino valeric acid (8-membered ring) and 6-amino caproic acid (9-membered ring) penetrate at very much slower rates, although the similar-sized nor-valine penetrates fairly rapidly. The trend of these results seems to be 6-membered rings $> 5,7 > 8 > 9$.

Ring formation could be involved in permeation in several ways. Models can be proposed where the rate of penetration is dependent on the rate of formation or on the stability of the ring formed. The species actually existing in the hydrophobic interior of the membrane is probably II or III (scheme 1). If the latter, II could be on a pathway for the formation of III near the surface of the membrane which is faster than pathways via IV or V. Possibly II could exist in significant concentrations in aqueous solutions: this would be reflected in microscopic pK_a values. Clearly the nature of the side chain is also important in determining permeability, but it is difficult to explain the effect of different side chains purely on the basis of size and hydrophobicity.

We therefore feel that ring formation may be important in diffusion of amino acids through mitochondrial membranes. We have been unable to obtain suitable stereochemically fixed model compounds in order to test this idea further.

Acknowledgements

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