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THE MOVEMENT OF MONOCARBOXYLIC ACIDS ACROSS PHOSPHOLIPID MEMBRANES: EVIDENCE FOR AN EXCHANGE DIFFUSION BETWEEN PYRUVATE AND OTHER MONOCARBOXYLATE IONS

EVERT P. BAKKER and KAREL VAN DAM

Laboratory of Biochemistry, B.C.P. Jansen Institute, University of Amsterdam, Plantage Muidergracht 12, Amsterdam (The Netherlands)

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Summary

In the presence of monensin, the sodium salts of pyruvate, lactate, acetoacetate and β-hydroxybutyrate permeate in increasing order of effectiveness through liposomal membranes. This permeation is strongly pH dependent.

Sodium pyruvate included in liposomes can be liberated into the medium by adding either monensin or other monocarboxylates. The latter process is a demonstration of an exchange diffusion of two compounds across a membrane without the help of a transport protein.

The mechanism by which mitochondria take up pyruvate, an important substrate for the organelles, is still a matter of controversy. Klingenberg [1] has stated that small monocarboxylic acids are freely permeant through the mitochondrial inner membrane. Papa et al. [2, 3], however, have proposed that a specific pyruvate translocator exists, different from those described earlier for adenine nucleotides [4], the phosphate-hydroxyl exchange [5—7], dicarboxylates [8], tricarboxylates [8] and α-oxoglutarate [9]. The argumentation of Papa et al. [2, 3] is based on the following points: (1) [^{14}C]pyruvate included in rat-liver mitochondria, at 0 °C, shows exchange diffusion with added pyruvate and phosphoenolpyruvate but not with added phosphate, Krebs-cycle intermediates or acetate; (2) the exchange diffusion is sensitive to the —SH reagents *N*-ethylmaleimide and mersalyl; and (3) both the rate of uptake of pyruvate and the total amount of pyruvate taken up by the mito-

Abbreviation: MES, 2-[*N*-morpholino]ethanesulphonic acid.

chondria show saturation with respect to the amount of pyruvate added [2, 3].

In this paper we wish to report on the movement of pyruvate across artificial membranes by an exchange-diffusion process that does not involve a specific translocator.

Liposomes containing 3% dicetylphosphate were prepared as described before [10]. Egg phosphatidylcholine was isolated according to the method of Pangborn [11]. Essential phospholipid, a lecithin with highly unsaturated fatty acids (70% linoleic acid [12]) was a generous gift from Dr H. Eikermann, A. Natterman and Cie, GmbH, Köln, Germany.

Sodium pyruvate, sodium D,L-β-hydroxybutyrate, lactate dehydrogenase and NADH were obtained from Boehringer, Mannheim. Lactic acid and dicetylphosphate were obtained from Sigma. Lithium acetoacetate was synthesized by Mr George van Woerkom and transformed to the free acid on Dowex-50 W ion-exchange resin (Sigma). Monensin was a gift from Dr W.C. Pettinga, Eli Lilly and Co., Indianapolis (U.S.A.).

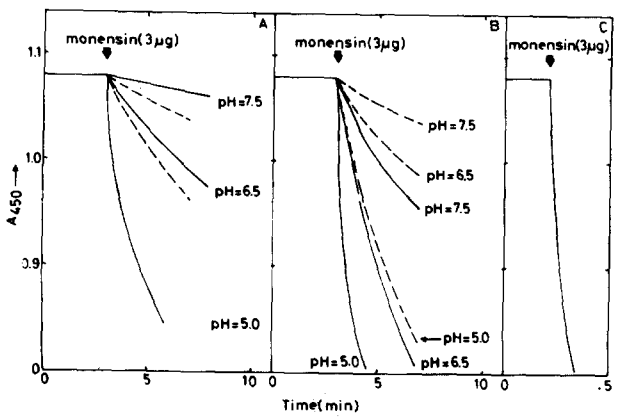

Fig. 1. The swelling, at different pH values, of liposomes suspended in sodium monocarboxylates, mediated by monensin. 0.2 ml non-sonicated liposomes consisting of 97% egg lecithin plus 3% dicetylphosphate, prepared in 1 mM EDTA and 50 mM MES—Tris buffer of the pH indicated was suspended in 1.8 ml 50 mM sodium carboxylates of the same pH. The absorbance at 450 nm was measured as a function of time. At t = 3 min 3 μg monensin was added. A: ———, pyruvate; — — —, lactate. B: ——— β-hydroxybutyrate; — — —, acetoacetate. C: ———, acetate at pH values of 5.0, 6.5 and 7.5.

In Fig. 1, swelling at different pH values of egg lecithin liposomes, caused by the permeation of sodium monocarboxylates, is shown. This swelling is initiated by monensin, a specific Na^+—H^+ exchange ionophore [13]. From the figure it is evident that except for acetate, the permeation of monocarboxylates is strongly pH dependent between pH 5.0 and 7.5. At pH 7.5 pyruvate is only slightly permeant, lactate and acetoacetate are more so, and β-hydroxybutyrate is relatively strongly permeant (Fig. 1A and B); at pH 5.0 all the carboxylates are highly permeant. The possible mechanism according

to which these carboxylates move is given in Fig. 2A. Essentially, it consists of movement of the free acid coupled to a Na^+—H^+ exchange, mediated by monensin. In this process either the movement of the free acid or the monensin-mediated exchange can be rate limiting. In the experiment of Fig. 1, probably the latter is the case for acetate (no pH dependence, Fig. 1C) and the former for the other acids (Fig. 1A and B).

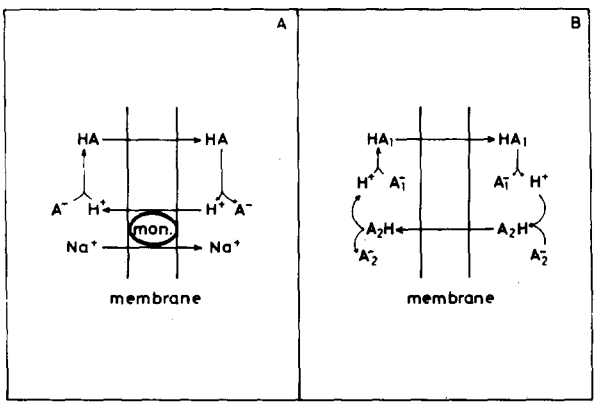

Fig. 2. Proposed scheme for the permeation of monocarboxylates across liposome membranes. A: mediated by monensin (mon.). B: exchange diffusion between monocarboxylates. HA_1 and HA_2 represent different monocarboxylic acids.

TABLE I

THE DISSOCIATION CONSTANTS OF THE WEAK ACIDS USED IN THIS STUDY

Compound	$pK_{a(1)}$	$pK_{a(2)}$
Acetic acid	4.75	—
Pyruvic acid	2.50	—
Lactic acid	3.86	—
Acetoacetic acid	3.58	—
β-Hydroxybutyric acid	4.60	—
Succinic acid	4.19	5.57

In Table I, the pK_a values of the different carboxylic acids tested are given. Comparison of the data from Fig. 1 with these of Table I reveals that the pK_a is only one of the factors determining the rate of transport of the carboxylic acids across the liposomal membrane. Other factors probably are the size and the substituent groups of the molecule.

The permeability of liposomes for Krebs-cycle intermediates and glutamate was also tested with the same technique as that used in the experiments described in Fig. 1. No swelling was observed, not even with succinate at pH 5.0, at which pH most of the molecules are in the monocarboxylate form.

To examine the permeation of pyruvate more closely we included this compound in liposomes and determined its permeation rate by coupling the

appearance of pyruvate to the reaction of NADH in the medium in the presence of lactate dehydrogenase. Pyruvate is liberated from liposomes consisting of the strongly unsaturated lecithin plus 3% dicetylphosphate, either by addition of monensin or by adding acetate or β-hydroxybutyrate to the medium (Fig. 3). The suggested mechanism for the latter type of induced efflux is given in Fig. 2B. From Fig. 3 it can be seen that in the presence of monensin at pH 6.0 the reaction rate is limiting, but that in all the other cases the permeation of pyruvic acid is rate limiting, since (1) both the monensin- and the acetate-induced pyruvate permeation are strongly pH dependent (cf.

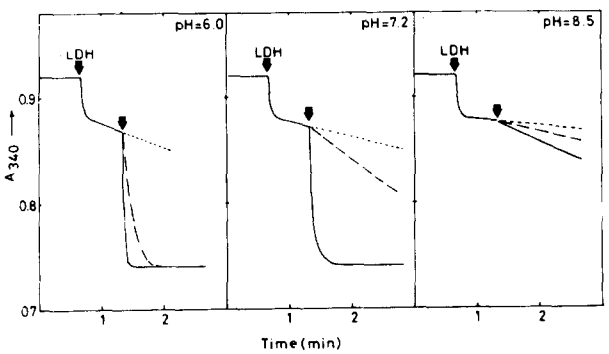

Fig. 3. The efflux of pyruvate from liposomes as measured by coupling pyruvate appearance to the reaction in the medium with NADH mediated by lactate dehydrogenase (LDH). Pyruvate-containing liposomes were prepared by shaking 4 ml 100 mM sodium pyruvate, 1 mM EDTA and 5 mM Tris—HCl (pH 7.2) with 97 mg essential phospholipid lecithin plus 3 mg dicetylphosphate. The liposomes were sonicated 10 × 30 s under Argon atmosphere. External pyruvate was exchanged for 100 mM Tris—HCl on a Sephadex G-75 column (no dilution). The cuvette was filled with 1.8 ml medium consisting of 100 mM Tris—HCl or MES—NaOH of the pH indicated, 100 μM NADH and 0.2 ml liposomes (5 mg). The absorbance at 340 nm was measured as a function of time. At the time indicated, 20 μg previously dialyzed lactate dehydrogenase (at pH 6.0 or pH 8.5) or 5 μg dialyzed lactate dehydrogenase (at pH 7.2) was added.———, addition of 3 μg monensin; – – –, 25 mM acetate or 25 mM β-hydroxybutyrate; . . . . . , 25 mM NaCl.

Fig. 1) and (2) acetate and β-hydroxybutyrate give rise to equal rates of pyruvate permeation.

The same results were obtained in experiments measuring pyruvate efflux from liposomes prepared from egg lecithin plus 3% dicetylphosphate, except that at all pH values the permeation rate was much slower than that with liposomes prepared from the strongly unsaturated lecithin. This result is relevant for mitochondria because it is known that the fatty acids of the phospholipid cardiolipin in this organelle are highly unsaturated [14]. This dependence of the rate of pyruvic acid permeation on the degree of saturation of the fatty acids of the lecithins used is not unexpected since the same has been found for the permeation of glycerol [15] and of water [16].

From the results shown above the following conclusions can be drawn: (a) pyruvate can move across phospholipid membranes by an exchange-

diffusion mechanism not involving a translocator; (b) this mechanism could be operative in mitochondria; (c) although experiments of Papa et al. [2, 3] show that, at 0 °C, acetate does not exchange with pyruvate across the mitochondrial inner membrane, indicating that a special pyruvate translocator may exist, the possibility remains that at least part of the movement of pyruvate is not mediated by a translocator.

Zahlten et al. [17] stated that pyruvate uptake by rat-liver mitochondria could be accounted for completely by the binding of this compound to the mitochondria. Reinvestigation of their experiments both by Papa's group [3] and people from our laboratory (Smits, G.G. and Meijer, A.J., unpublished) showed that their conclusions probably are incorrect because these authors did not correct for the sucrose-permeable space of the mitochondria. Furthermore, with the experiments analogous to the ones shown in Fig. 1, it is easy to demonstrate pyruvate permeation into mitochondria [18].

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