Biochimica et Biophysica Acta, 600 (1980) 577—580

BBA Report

BBA 71474

AMINO ACID TRANSPORT IN PLASMA MEMBRANE VESICLES FROM ISOLATED RAT LIVER PARENCHYMAL CELLS

HERMAN J. SIPS, MAX E.A. APITULE and KAREL VAN DAM

Laboratory of Biochemistry, B.C.P. Jansen Institute, University of Amsterdam, Plantage Muidergracht 12, 1018 TV Amsterdam (The Netherlands)

(Received March 14th, 1980)

Key words: Amino acid transport; Membrane vesicle; (Rat liver parenchymal cell)

Summary

Plasma membrane vesicles were prepared from isolated rat liver parenchymal cells. The transport of several amino acids was studied and found to be identical to that in membrane vesicles from whole liver tissue.

Several studies have recently been devoted to the uptake of amino acids in rat liver parenchymal cells [1—4]. In order to be able to study the membrane-associated component(s) which are responsible for the observed transport phenomena, we developed an in vitro system of isolated plasma membrane vesicles [5]. In this preparation, derived from intact liver tissue, the uptake of the gluconeogenic substrate L-alanine has been characterized [6].

However, the liver consists of various cell types, 35% of which is non-parenchymal [7]. Moreover, plasma membranes from non-parenchymal cells account for 26.5% of the total liver plasma membrane content [7]. It is therefore of great importance to ensure that amino acid transport, as studied in the preparation from whole liver, indeed reflects the uptake across the parenchymal cell membrane.

In this paper we report a procedure for the isolation of plasma membrane vesicles from rat liver parenchymal cells. The transport properties with respect to the amino acids studied are identical to those of membrane preparations from intact liver tissue. We conclude that both preparations provide a suitable model system for the study of amino acid transport in rat liver parenchymal cells.

Liver parenchymal cells were isolated from male Wistar rats (200—250 g), fed ad libitum, using a modification [8] of the method of Berry and Friend [9]. Cells were washed twice in homogenization buffer (0.25 M sucrose, 0.2

mM $CaCl_2$ and 10 mM Hepes-KOH, pH 7.5). Structural integrity of the cells was routinely checked by measuring the exclusion of 0.25% trypan blue.

Cells derived from two livers were suspended in homogenization buffer (concentration 150 mg wet wt./ml). The subsequent cell disruption was performed in a tight-fitting Dounce homogenizer (0.06 mm diameter clearance; Braun Melsungen A.G., Melsungen, F.R.G.) with about 20 strokes. Differential and sucrose density centrifugation of the cell homogenate was performed as described previously for homogenates of whole liver [5,6]. The plasma membrane vesicles were stored in liquid nitrogen until use, at a concentration of approx. 10 mg protein/ml. The uptake of amino acids was determined as described previously [5,6].

The activities of ($Na^+ + K^+$)-ATPase (EC 3.6.1.3), cytochrome *c* oxidase (EC 1.9.3.1), glucose-6-phosphatase (EC 3.1.3.9) and alkaline phosphatase (EC 3.1.3.1), as well as the protein concentrations were determined as described in Ref. 5. The Mg^{2+}-ATPase activity (EC 3.6.1.3) was determined parallel to that of ($Na^+ + K^+$)-ATPase [5], but in the absence of KCl, and in the presence of 2.1 mM ouabain. Leucine aminopeptidase activity (EC 3.4.11.1) was determined by using the method of Nagel et al. [10].

Isolated liver parenchymal cells can be satisfactorily disrupted in a tight-fitting Dounce homogenizer. The precise number of strokes needed for an adequate disruption varies for each batch of cells. The degree of disruption can be followed by light microscopy using the dye trypan blue: disruption is continued until no cells, but solely intact nuclei are observed. This control should be performed for each membrane isolation. Analysis of marker enzyme activities in the cell homogenate, and the final membrane preparation is shown in Table I. It can be seen that the specific activities of typical plasma membrane marker enzymes are several-fold enriched, as compared to the cell homogenate. As in the plasma membrane preparation from intact liver tissue, the final preparation is contaminated by endoplasmic reticulum (glucose-6-phosphatase). Also a slight contamination with mitochondrial inner membranes is observed (cytochrome *c* oxidase). It should be noted that both en-

TABLE I

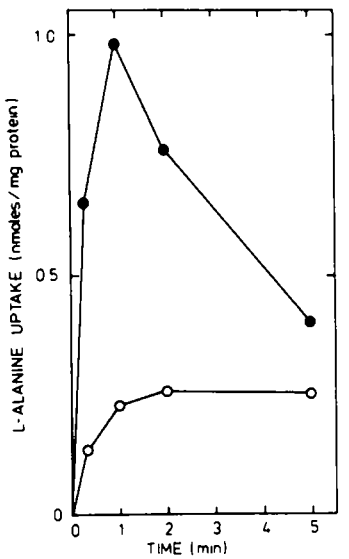
ENZYME ACTIVITIES IN HOMOGENATE AND PLASMA MEMBRANE PREPARATION FROM ISOLATED RAT LIVER PARENCHYMAL CELLS

Specific activities are expressed in μmol of substrate/h per mg of protein. Relative specific activities refer to the homogenate taken as unity. Means of three experiments are given with standard errors.

	Homogenate specific activity	Plasma membranes	
		Specific activity	Relative specific activity
($Na^+ + K^+$)-ATPase	0.14 ± 0.02	3.29 ± 0.63	24.0 ± 5.7
Mg^{2+}-ATPase	2.56 ± 0.28	12.07 ± 2.49	4.7 ± 1.1
Alkaline phosphatase	1.01 ± 0.07	10.92 ± 1.16	10.8 ± 1.4
Leucine aminopeptidase	0.37 ± 0.03	3.26 ± 0.27	8.8 ± 1.0
Glucose-6-phosphatase	0.12 ± 0.01	0.34 ± 0.01	2.8 ± 0.1
Cytochrome *c* oxidase	15.42 ± 2.16	1.72 ± 0.17	0.11 ± 0.02

zymes characteristic for the blood sinusoidal ($Na^+ + K^+$)-ATPase [11] and the bile canalicular (alkaline phosphatase, leucine aminopeptidase [12]) region of the plasma membrane of the liver parenchymal cell are present. This indicates that both membrane types are present in this membrane preparation.

The transport capacity of these plasma membrane vesicles is demonstrated in Fig. 1. In the presence of an Na^+ concentration gradient, a transient accumulation of L-alanine is observed. This overshoot phenomenon is identical to that which has been demonstrated in plasma membrane vesicles from whole liver [5,6]. Kinetic analysis of the L-alanine transport (data not shown) reveals an apparent K_m of about 3 mM, which is in good agreement with the apparent K_m of 2 mM observed in plasma membrane vesicles from whole liver. Fig. 2 illustrates that amino acid transport in these vesicles is not restricted to L-alanine. Also other amino acids, such as L-glutamine and L-serine, are accumulated in the presence of an Na^+ concentration gradient. In contrast, other amino acids, such as L-leucine, are not accumulated. These observations are also in complete agreement with the results obtained with membrane vesicles from intact liver [13].



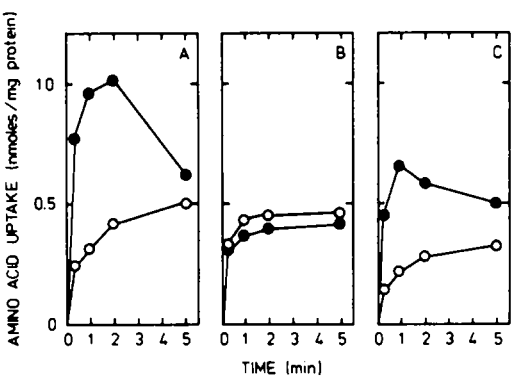


Fig. 1. L-Alanine uptake by plasma membrane vesicles from isolated rat liver parenchymal cells. Uptake of 0.2 mM L-[2,3-^{3}H] alanine was determined in the presence of 100 mM NaSCN (●—●), or 100 mM KSCN (○—○). At the time indicated, the uptake was stopped as described previously [5,6].

Fig. 2. Uptake of L-glutamine, L-leucine and L-serine by plasma membrane vesicles from isolated rat liver parenchymal cells. Uptake of 0.2 mM L-[G-^{3}H] glutamine (A), L-[4,5-^{3}H] leucine (B) and L-[3-^{3}H] serine (C) was determined in the presence of 100 mM NaSCN (●—●), or 100 mM KSCN (○—○). At the times indicated, the uptake was stopped as described previously [5,6].

From the data presented we conclude that the transport properties of these vesicles with respect to amino acids are identical to those observed in preparations from intact liver. Thus, the assumption that the preparation from whole liver is suited for the study of amino acid transport across the parenchymal cell membrane is tenable. Transport of amino acids is, a priori, expected to occur across the parenchymal cell plasma membrane, since

gluconeogenesis is restricted to the parenchymal liver cells and absent in non-parenchymal liver cells [14,15]. The question as to whether transport systems specific for non-parenchymal liver cells exist remains a point for further studies.

Several lines of evidence indicate that the plasma membrane of the liver parenchymal cell consists of three different domains. This heterogeneity is reflected in both structural and functional differences (for a review see Ref. 16). Recent studies in this laboratory revealed that the alanine transport activity is located in the blood sinusoidal part of the parenchymal cell plasma membrane [17]. At present, studies are in progress in order to elucidate whether the domain heterogeneity with respect to amino acid transport is conserved in plasma membranes from isolated parenchymal cells.

This study was in part supported by a grant from the Netherlands Organization for the Advancement of Pure Research (Z.W.O), under the auspices of the Netherlands Foundation for Chemical Research (S.O.N.).

References

1 Le Cam, A. and Freychet, P. (1977) J. Biol. Chem. 252, 148—156
2 McGivan, J.D., Bradford, N.M. and Mendes-Mourão, J. (1977) FEBS Lett. 80, 380—384
3 Joséph, S.K., Bradford, N.M. and McGivan, J.D. (1978) Biochem. J. 176, 827—836
4 Edmondson, J.W., Lumeng, L. and Li, T.K. (1979) J. Biol. Chem. 254, 1653—1658
5 Van Amelsvoort, J.M.M., Sips, H.J. and van Dam, K. (1978) Biochem. J. 174, 1083—1086
6 Sips, H.J., van Amelsvoort, J.M.M. and van Dam, K. (1980) Eur. J. Biochem. 105, 217—224
7 Blouin, A., Bolender, R.P. and Weibel, E.R. (1977) J. Cell Biol. 72, 441—455
8 Meijer, A.J., Gimpel, J.A., De Leeuw, G.A., Tager, J.M. and Williamson, J.R. (1975) J. Biol. Chem. 250, 7728—7738
9 Berry, M.N. and Friend, D.S. (1969) J. Cell Biol. 43, 506—520
10 Nagel, W., Willig, F. and Schmidt, F.H. (1964) Klin. Wochenschr. 42, 447—449
11 Blitzer, B.L. and Boyer, J.L. (1978) J. Clin. Invest. 62, 1104—1108
12 Holdsworth, G. and Coleman, R. (1975) Biochim. Biophys. Acta 389, 47—50
13 Sips, H.J., van Amelsvoort, J.M.M., de Graaf, P.A. and van Dam, K. (1979) Abstr. 11th Int. Congr. Biochem., Toronto, p. 463
14 Crisp, D.M. and Pogson, C.I. (1972) Biochem. J. 126, 1009—1023
15 Van Berkel, T.J.C. and Kruijt, J.K. (1977) Eur. J. Biochem. 73, 223—229
16 Evans, W.H. (1977) Trend Biochem. Sci. 2, 169—171
17 Van Amelsvoort, J.M.M., Sips, H.J., Apitule, M.E.A. and van Dam, K. (1980) Biochim. Biophys. Acta 600, 950—960