Biochimica et Biophysica Acta 817 (1985) 190–192
Elsevier

BBA 70230

BBA Report

Presence and characterization of acetylcholinesterase in brush-border and basolateral membranes of rabbit enterocytes

Jean-Pierre Sine and Bernard Colas *

Laboratoire de Biochimie, Centre de Recherche de Biologie et Physico-Chimie Cellulaires, Université de Nantes, 2 rue de la Houssinière, 44072 Nantes Cedex (France)

(Received April 2nd, 1985)

Key words: Acetylcholinesterase; Membrane enzyme; Brush-border membrane; Basolateral membrane; (Rabbit enterocyte)

Acetylcholinesterase is found in the brush-border and basolateral membranes purified from rabbit enterocytes. The sedimentation coefficients of the enzymes solubilized from two types of membrane are identical (5.5 ± 0.2 S) and the apparent molecular weights are not significantly different ($154\,000 \pm 8000$ for the brush-border and $145\,000 \pm 8000$ for the basolateral membrane enzyme). These results suggest a unique G_2 molecular form for acetylcholinesterase from brush-border as well as from basolateral membranes.

Acetylcholinesterase (EC 3.1.1.7) involved in cholinergic synaptic transmission is found highly concentrated in nervous tissue and neuromuscular junctions [1] where its essential role is to catalyze the hydrolysis of acetylcholine. The enzyme has also been detected in membranes of non-neuronal cells (for reviews, see Refs. 1, 2) like erythrocyte [3,4] and lymphocyte [5,6]. In the gastrointestinal tractus, the presence of acetylcholinesterase might be related to that of acetylcholine identified as an excitatory enteric neurotransmitter involved in the control of gastrointestinal motility [7]. However, at the present time, its localization has not been investigated.

The intestinal epithelium is predominantly coated by a monolayer of absorbing cells or enterocytes. The plasma membrane of these highly polarized cells is composed of at least two domains: the brush-border membrane facing the intestinal lumen and the basolateral membrane on the contra-luminal faces of the cells. These two specialized membrane domains which are separated by the tight junctions differ not only by their morphological aspect but also in their enzymatic equipment well adapted to their specific functions [8–11].

In this report, acetylcholinesterase is shown to be present in both plasma membrane types of enterocytes and the characterization of the enzyme is undertaken.

The intestinal mucosa from rabbits was isolated by scraping. Brush-border and basolateral membranes were separated from each other by differential centrifugation followed by purification of each membrane type on sucrose gradients as described previously [10]. Acetylcholinesterase activity determined by the method of Ellman et al. [12] with acetylthiocholine as substrate was found in both membrane types. As seen in Table I, the apparent kinetic parameters for acetylcholinesterase activity were found to be comparable in magnitude for the brush-border and basolateral membranes. Precedently it has been demonstrated by using immunological techniques [10,14] that the basolateral membrane contains small amounts (about 5–8%) of all the characteristic digestive

* To whom correspondence should be addressed.

TABLE I

APPARENT KINETIC PARAMETERS FOR THE HYDROLYSIS OF ACETYLTHIOCHOLINE BY ACETYLCHOLINESTERASE BOUND TO PLASMA MEMBRANES OF RABBIT ENTEROCYTES

Acetylcholinesterase activity was determined by the spectrophotometric method of Ellman et al. [12] at pH 7.4 and 25°C in a 20 mM Tris-HCl buffer with acetylthiocholine as substrate. Control experiments without acetylthiocholine were always performed to account for thiol groups eventually present in the preparations and susceptible to react with the Ellman's reagent. The enzymatic unit was defined as the amount of protein which catalyzes the hydrolysis of 1 nmol substrate per min at 25°C. Protein concentration was determined by the method of Spector [13]. Michaelis constants (K_m) and maximal velocities (V) were derived by extrapolation from the linear segments of the plots obtained for the low substrate concentrations (in due to the characteristic property of excess substrate inhibition) by the double-reciprocal method of Lineweaver-Burk and also by the Dixon method.

Acetylcholinesterase	K_m (mM)	V (units/mg)
Brush-border membrane	0.10	55
Basolateral membrane	0.06	80

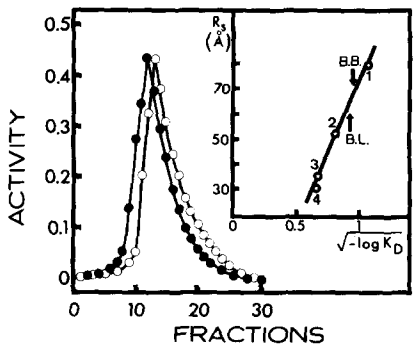

Fig. 1. Molecular weights and Stokes radii of acetylcholinesterase from brush-border (●——●) and basolateral (○——○) membranes of rabbit enterocytes. The plasma membranes were washed with a 20 mM Tris-HCl buffer (pH 7.4) and, after a 105000 × g centrifugation, detergent soluble acetylcholinesterase was solubilized from pellet by incubation for 30 min at 25°C in a 20 mM Tris-HCl buffer (pH 7.4) containing 1% Triton X-100. Gel filtration was performed by HPLC. TSK-G-3000 SW column was equilibrated with the buffer used for solubilization of acetylcholinesterase prior to injection of 0.2 ml sample. Elution was run at a flow rate of 1 ml/min with fractions of 0.25 ml. Molecular weights were determined from hydrodynamic properties according to Siegel and Monty [19] using a linear relationship between the Stokes radius (R_S) and the elution parameter (K_D). The column was calibrated by using as markers: (1) ferritin (R_S = 79 Å); (2) catalase (R_S = 52.2 Å); (3) bovine serum albumin (R_S = 35.5 Å); (4) ovalbumin (R_S = 30.5 Å). The positions of brush-border membrane acetylcholinesterase (BB) and basolateral membrane acetylcholinesterase (BL) are indicated by arrows on the graph.

hydrolases (aminopeptidases, disaccharidases, alkaline phosphatase) of the brush-border membrane. It cannot be excluded that these hydrolases come off the brush-border membrane either by some slight contamination despite the good separation of the two types of membrane or by diffusion during cell homogeneization. Indeed, redistribution of the brush-border hydrolases over the whole cell surface often occurs immediately after disruption of the tight junctions [15–17]. Here, the assumption of a contamination can be reasonably ruled out in consideration of the rather identical amount of acetylcholinesterase found to be present in two membrane types.

The molecular state of acetylcholinesterase is known to be dependent on its distribution in the tissues and its cellular localization [1]. A nomenclature proposed by Bon et al. [18] distinguishes two types of forms corresponding to asymmetric forms (A_n) and globular forms (G_n) and identifies each form by its number (n) of catalytic subunits. Therefore we have determined the molecular size and shape of acetylcholinesterase extracted by 1% Triton X-100 from brush-border and basolateral membranes of enterocytes. The apparent molecular weights (M_r) and Stokes radii (R_S) determined by gel filtration in HPLC (Fig. 1) were found to be very close for acetylcholinesterase solubilized from brush-border membranes (M_r, 154000 ± 8000; R_S, 65 ± 3 Å) and basolateal membranes (M_r, 145000 ± 8000; R_S, 62 ± 3 Å). The molecular forms were analyzed by centrifugation in linear sucrose gradients. Under the conditions indicated in Fig. 2, a symmetrical and unique peak corresponding to a sedimentation coefficient of 5.5 ± 0.2 S was observed for acetylcholinesterase from brush-border as well as from basolateral membranes. Thus, it is possible to identify these enzymes as detergent-soluble G_2 (D-S) forms by reference to other acetylcholinesterase species described [20]. This molecular form (G_2) has been found in the plasma membranes of non-neuronal cells such as erythrocyte [3,4] and lymphocyte [5,6].

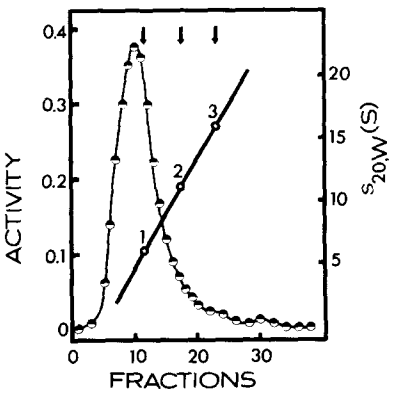

Fig. 2. Sucrose gradient centrifugation of acetylcholinesterase from brush-border (●———●) and basolateral (○———○) membranes of rabbit enterocytes. Acetylcholinesterase extracted (under the conditions given in Fig. 1) from plasma membranes by a 20 mM Tris-HCl buffer (pH 7.4) containing 1% Triton X-100 was layered on 0–30% linear sucrose gradients prepared with the same buffer. Isokinetic centrifugation was performed at 4°C and 119000 × *g* (calculated at the middle of the tubes) for 16 h in a Spinco-Beckman centrifuge equipped with a swinging bucket rotor SW 41. Apparent sedimentation coefficients of acetylcholinesterase were obtained from the migration relative to internal sedimentation standards (indicated by arrows): (1) alkaline phosphatase (6.1 S); (2) catalase (11.3 S) and (3) β-galactosidase (16 S).

The presence of acetylcholinesterase in enterocyte plasma membranes raises the question about its physiological role. The only well defined function of the enzyme is its ability to hydrolyze extracellular acetylcholine. Recently it has been suggested that the widespread distribution of acetylcholinesterase might reflect the location of all its natural substrates and not only acetylcholine [21]. Besides its esterase activity, acetylcholinesterase can act as a peptidase and thus hydrolyze a number of peptides including enkephalins and substance P [21,22]. In the case of the enterocyte plasma membrane, acetylcholinesterase might also possess a peptidase activity and thus be able to regulate the rate of various neuropeptides (e.g. somatostatin, substance P) found to be present in the intestinal tissue [23–25]. Further studies are now in progress to verify such a hypothesis.

References

1 Massoulié, J. and Bon, S. (1982) Annu. Rev. Neurosci. 5, 57–106
2 Rosenberry, T.L., Barnett, P. and Mays, C. (1982) Methods Enzymol. 82, 325–339
3 Ott, P. and Brodbeck, U. (1978) Eur. J. Biochem. 88, 119–125
4 Rosenberry, T.L. and Scoggin, D.M. (1984) J. Biol. Chem. 259, 5643–5652
5 Kutty, K.M., Chandra, R.K. and Chandra, S. (1976) Experentia 32, 289–291
6 Sine, J.-P. and Caye-Vaugien, C. (1984) Biochimie 66, 203–214
7 Catalán, R.E., Martinez, A.M., Aragonés, M.D., Miguel, B.G. and Blazquez, J. (1983) IRCS Med. Sci. 11, 1060–1061
8 Miller, D. and Drane, R.K. (1961) Biochim. Biophys. Acta 52, 293–298
9 Fujita, M., Ohta, H., Kawai, K., Matsui, H. and Nakao, M. (1972) Biochim. Biophys. Acta 274, 336–347
10 Colas, B. and Maroux, S. (1980) Biochim. Biophys. Acta 600, 406–420
11 Kenny, J. and Maroux, S. (1982) Physiol. Rev. 62, 91–128
12 Ellman, G.L., Courtney, D.K., Andres, V. and Featherstone, R.M. (1961) Biochem. Pharmacol. 7, 88–95
13 Spector, T. (1978) Anal. Biochem. 86, 142–146
14 Carlsen, J., Christiansen, K. and Bro, B. (1982) Biochim. Biophys. Acta 689, 12–20
15 Ziomek, C.A., Schulman, S. and Edidin, E. (1980) J. Cell. Biol. 86, 849–857
16 Feracci, H., Bernadac, A. Hovsépian, S., Fayet, G. and Maroux, S. (1981) Cell Tissue Res. 221, 137–146
17 Maroux, S. and Feracci, H. (1983) Methods Enzymol. 96, 406–423
18 Bon, S., Vigny, M. and Massoulié, J. (1979) Proc. Natl. Acad. Sci. USA 76, 2546–2550
19 Siegel, L.M. and Monty, K. J. (1966) Biochim. Biophys. Acta 112, 346–362
20 Massoulié, J., Bon, S. and Vigny, M. (1980) Neurochem. Int. 2, 161–184
21 Chubb, I.W., Ranieri, E., White, G.H. and Hodgson, A.J. (1983) Neuroscience 10, 1369–1377
22 Chubb, I.W., Hodgson, A.J. and White, G.H. (1980) Neuroscience 5, 2065–2072
23 Leeman, S.E. and Mroz, E.A. (1974) Life Sci. 15, 2033–2044
24 Catalán, R.E., Martinez, A.M. and Aragonés, M.D. (1984) Mol. Physiol.5, 149–158
25 Vinik, A.I., Gaginella, T.S., O'Dorisio, T.M., Shapiro, B. and Wagner, L. (1981) Endocrinology 109, 1921–1926