TRANSGLUTAMINASE IN ENDOTHELIAL CELLS FROM BOVINE CAROTID ARTERY

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ABSTRACT

The transglutaminase activity was found in cytosol and membrane fractions of endothelial cells from bovine carotid artery. The molecular weight was estimated to be approximately 58,000 by gel permeation chromatography. Addition of retinoids (retinol, retinal and retinoic acid) to the culture medium of endothelial cells caused a marked increase in the transglutaminase activity, and also in specific uptake of low density lipoprotein by endothelial cells.

Transglutaminase catalyzes acyl-transfer reaction between primary amines and γ -carboxyamide of glutamyl residues in peptides and proteins. Its activity has been detected in various tissues and organs including blood plasma (4), platelets (16), erythrocytes (11), macrophages (14), hair follicles (15), epidermis (15), and pancreatic islets (7), and the enzyme was isolated from some of the tissues. Although numerous physiological functions have been ascribed to this enzyme, most of them are still unclear, except for the formation of cross-linkages between fibrin molecules by blood coagulation factor XIII (4). One of the most interesting roles seems to be played in receptor-mediated endocytosis (3). Receptor-mediated endocytosis is the process by which cells bind and internalize specific extracellular ligands.

Endothelial cells make up the luminal surface of blood vessel and play important roles against atherosclerosis and thrombosis. Accumulation of low density lipoprotein (LDL) and fibrinogen/fibrin in the arterial wall is responsible for the genesis of atherosclerosis. It is expected that transglutaminase exists also in endothelial cells and is associated with the endocytosis of LDL into the cells. However, no information is available on transglutamin-

ase in endothelial cells and on a possible relation between transglutaminase and uptake of LDL by these cells.

The present paper deals with the transglutaminase activity found in endothelial cells from bovine carotid artery. The activity was enhanced by the addition of retinoids to the culture medium. This enhancement of transglutaminase activity with retinoids is closely associated with uptake of LDL by these cells.

MATERIALS AND METHODS

Materials

Eagle's minimum essential medium, fetal bovine serum and antibiotics (penicillin-streptomycin solution) were purchased from GIBCO, NY. Monodansyl cadaverine, fluorescein isothiocyanate (FITC) and retinoids (retinol, retinal and retinoic acid) were from Sigma, MO. Casein was from E. Merck, Darmstadt, F.R.G. and acetylated by the method of Fraenkel-Conrat (6). LDL was purchased from United States Biochemical, OH. All other reagents were of analytical grade.

Culture of Endothelial Cells

Endothelial cells from bovine carotid artery

Fig. 1 Transglutaminase activities in the cytosol and the membrane extract from endothelial cells. Curves A and C: transglutaminase activities in the cytosol in the presence (●) or absence (○) of 2 mM Ca^{2+} . Curves B and D: transglutaminase activities in the membrane extract in the presence (▲) or absence (△) of Ca^{2+} . In ○ and △, 5 mM EDTA was present.

were cultured in 55-cm² plastic dish by the method of Hagiwara *et al.* (8). The medium consists of Eagle's minimum essential medium containing 10% fetal bovine serum, penicillin (50 U/ml), and streptomycin (50 mcg/ml). The cells which had been passaged 7–19 times (8–20 generations) were used in the present study.

Preparation of Cytosol and Membrane Extract

The cells, which had reached to visual confluency, were scraped from the culture dish. After washing with 10 mM Tris-HCl buffer (pH 7.5) containing 150 mM NaCl and 1 mM ethylenediaminetetraacetic acid (EDTA), the cells were homogenized in the same buffer using a micro-homogenizer, model M-100 (Tokai Rikagaku). The supernatant (cytosol) was obtained by centrifugation of the homogenate at 10,000 *g* for 10 min. The membrane fraction was washed with the above buffer and lysed with 10 mM 3-[(3-cholamidopropyl) dimethyl ammonio]-1-propanesulfonate (CHAPS) to obtain the membrane extract.

Assay of Transglutaminase Activity

Transglutaminase activity was assayed by the method of Takagi *et al.* (17). The cytosol (100 μl) or the extract (100 μl) was added to 2.0 ml of 5 mM Tris-HCl (pH 7.5) containing 5.8 μM monodansyl cadaverine (MDC), 5 mM CaCl_2 and 30 mg/ml acetylcasein. The reaction mixture was incubated at 37°C for 1 h, and 0.1 ml of 1 M NH_4Cl was added to stop the reaction. Fluorescence intensity was measured with a fluorescence spectrophotometer (RF-500, Shimadzu). Excitation and emission wavelengths were 350 and 480 nm, respectively. The activity was expressed as nmol MDC/h/mg protein. Protein concentration was determined by the method of Lowry *et al.* (12), using bovine serum albumin as a standard.

Assay of Specific Uptake of Low Density Lipoprotein by Cells

The specific uptake of LDL by cultured cells was determined as described below. LDL was labelled with FITC to form FITC-LDL

Fig. 2 Gel permeation chromatographic pattern of cytosol from cultured endothelial cells using high performance liquid chromatography. The transglutaminase activity was measured with fractions (500 μ l each) eluted with 50 mM acetate (pH 6.8). Flow rate, 0.5 ml/min using Waters Protein Pak 300 SW column. The reaction mixture was incubated at 37°C for 19 h. The molecular weight of the enzyme was estimated, using bovine serum albumin, ovalbumin and myoglobin as standards. One unit of transglutaminase activity is defined as 1 nmol MDC/19 h/500 μ l fraction. A representative result from three different experiments is shown.

according to the method of Maeda *et al.* (13). Cultured endothelial cells were washed with cold minimum essential medium and were incubated with serum-free medium containing 40 μ g/ml FITC-LDL with or without 7 mg/ml unlabelled LDL for 3 h. After scraping the cells from the culture dish, they were washed with saline and homogenized with 1% Triton X-100 in 0.2 M KCl. The supernatant was obtained by centrifugation at 100,000 g for 30 min, and its fluorescence intensity and protein concentration were measured. Non-specific uptake was measured in the presence of unlabelled LDL, and the total uptake was in the absence of unlabelled LDL. Specific uptake of LDL was then obtained by subtracting nonspecific uptake from the total uptake. Amount of specific uptake of LDL was expressed as fluorescence intensity (FI)/mg protein.

RESULTS AND DISCUSSION

The transglutaminase activity was detected in

both the cytosol and the membrane extract from cultured endothelial cells. Fig. 1 shows the transglutaminase activity as a function of protein concentration. In the presence of 2 mM Ca^{2+} , the enzyme activity was linearly increased with the increase in the protein amount in both the cytosol (curve A) and membrane (curve B). When Ca^{2+} was replaced with 5 mM EDTA, the activity was markedly reduced in both the cytosol (curve C) and membrane (curve D). Sr^{2+} can substitute for Ca^{2+} . The transglutaminase activity was not enhanced by thrombin, and not suppressed by antibodies (raised in rabbits) against blood coagulation factor XIII obtained from plasma (data not shown).

In order to determine the molecular weight of transglutaminase from endothelial cells, the cytosol fraction was subjected to gel permeation chromatography (Fig. 2). A single sharp peak appeared in fractions between 13 and 19. The molecular weight was estimated to be approximately 58,000, which is in good agree-

Fig. 3 Enhancement of transglutaminase activity and specific uptake of LDL by endothelial cells induced by retinol. Endothelial cells, which had reached visual confluency, were cultured for 5 days in the medium with various concentrations of retinol (0–10 μ M) dissolved in 1% ethanol. After the treatment, transglutaminase activity in the membrane extract and the specific uptake of LDL by the cells were measured. Curve A: transglutaminase activity, Curve B: amount of specific uptake of LDL. A representative result from two different experiments is shown.

ment with the values obtained with transglutaminases from hair follicles (M_r , 54,000) (15) and epidermis (M_r , 57,000) (15), but differs from those from liver (M_r , 80,000) (5) and erythrocyte (M_r , 82,000) (1).

We have previously demonstrated that the activity of plasminogen activator in cultured endothelial cells is enhanced by the simultaneous addition of retinoids and L-ascorbic acid (9, 10). We examined in the present experiment whether retinoids stimulate the transglutaminase activity in cultured endothelial cells. Cells were cultured with various concentrations of retinol (0–10 μ M) dissolved in ethanol (final concentration, 1%), and the activity of transglutaminase in the membrane extract was measured 120 h after the addition of retinol. The activity was markedly enhanced by increasing concentration of retinol and reached a constant level (Fig. 3, curve A). The activity was increased 3 times with 10 μ M retinol. Retinal and retinoic acid were similarly effective as retinol, but L-ascorbic acid was not. With retinol, a comparable result was obtained with cells treated for 12 h. The stimulative effect of retinol was also observed

with the transglutaminase in the cytosol fraction (data not shown). The effect of retinoids on transglutaminase activity is not direct, and the detailed mechanism remains to be studied.

It has been reported that transglutaminase may be associated with receptor-mediated endocytosis of ligands (2, 3), and that endothelial cells take up LDL through specific receptor-mediated endocytosis (18). It is thus expected that retinoids enhance the specific LDL-uptake in endothelial cells. After endothelial cells were treated with various concentrations of retinol (0–10 μ M) for 5 days, the specific uptake of LDL by the cells was measured (Fig. 3, curve B). Specific uptake of LDL was found increased with the increase in the transglutaminase activity (curve A) due to retinol. It thus appears that transglutaminase is involved in LDL-uptake in endothelial cells. However, it remains to be studied whether our present observations with endothelial cells from the carotid artery are common to endothelial cells from various arteries.

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