MONOCLONAL ANTIBODIES TO ANGIOTENSIN CONVERTING ENZYME FROM HUMAN LUNG

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Angiotensin converting enzyme (ACE), located chiefly on the surface of the endothelial cells of blood vessels, catalyzes the formation of vasoactive angiotensin II from angiotensin I, and also the inactivation of bradykinin by removal of C-terminal peptides [1, 4]. By forming a hypertensive peptide and inactivating the hypotensive, ACE changes the concentration of these peptides in the blood and tissues, and it is evidently a key enzyme in the regulation of vascular tone [2]. This enzyme also hydrolyzes other biologically active substances, such as Na-uretic factor [8], substance P, and enkephalins [6]. Recent publications have described the existence of a certain structural and functional heterogeneity of ACE isolated from different organs [7, 14]. However, attempts to discover immunologic differences between such ACE have been undertaken with the use of polyclonal antibodies and have not proved particularly successful [5].

Monoclonal antibodies (MCA), because of their narrow specificity, are a much more delicate instrument for the detailed study of the tissue distribution of ACE and differences in the structure and function of this enzyme in different organs. MCA also can be used to develop simple and technological methods of isolation and quantitative determination of ACE.

This paper describes the preparation and brief characterization of one of several MCA against ACE, its specificity, and its antigen-binding properties.

EXPERIMENTAL METHOD

ACE from human lung tissue was purified to the homogeneous state by affinity chromatography [12] with certain modifications. The ACE inhibitor carboxyaminopentylglycyl-glycine (CA-Gly-Gly) was used as the immobilized ligand. The enzyme preparation after affinity purification, was subsequently used for immunization and for selection of hybridoma clones. Mice were immunized by intraperitoneal injection of 80 μg of the enzyme in Freund's complete adjuvant, followed by injections at intervals of 3 weeks for 2 months. An injection of 40-50 μg of antigen was given into the caudal vein 3 days before hybridization. Hybridization was carried out by the method in [10] with certain modifications. Murine myeloma P_3O_1 was used as the starting cell line. Primary screening was carried out by ELISA [11]. Primary hybridoma populations producing monoclonal antibodies against ACE were tested by a second independent method, namely immunosorption of ACE from solution. For this purpose, rabbit antibodies against mouse immunoglobulins were introduced into microtitration wells (2 μg per well) and incubated for 12-16 h at 4°C. Culture media of the test hybridomas were then incubated in the wells in a volume of 100 μl for 1 h at 37°C. ACE in phosphate-buffered saline (PBS, 7 μg in 1 ml) was then added to the wells. The quantity of adsorbed enzyme was determined fluorometrically. All washing procedures were done with 0.2% albumin in PBS. The selected populations were cloned by the terminal dilution method in 96-well culture plates. To obtain ascites fluid, $2 \cdot 10^6$-$5 \cdot 10^6$ hybrid cells were injected intraperitoneally into mice previously treated with pristane. After 2-3 weeks the ascites fluids were collected and immunoglobulins precipitated with sodium sulfate (50% saturation). The residue was dissolved in 0.2 M borate buffer, pH 8.1, with 0.15 M NaCl, dialyzed against the same buffer, and frozen at −70°C.

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TABLE 1. Screening Culture Fluids from Hybridomas by ACE Binding and Immunosorption

Sample tested	ELISA (optical density at 492 nm), relative units	ACE activity in well (fluorescence rel. units
Culture medium	0,08	0,32
Control (MCA to low-density lipoproteins	0,09	0,34
Primary population		
5F1	0,970	1,43
9B9	0,656	7,82
3G8	0,400	3,07

Legend. Hybridoma cells were cultured in 96-well plate and culture fluids were tested after 9 days for binding with ACE by ELISA and immunosorption tests.

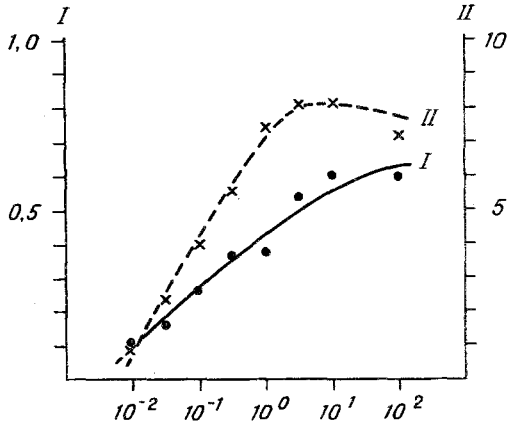

Fig. 1. Binding of ACE and immunosorption activity of 9B9F1 antibodies against ACE. Abscissa, immunoglobulin concentration (in μg/ml); ordinate: I) immunosorption activity (absorbance at 492 nm), II) ACE activity (fluorescence, relative units).

The class of immunoglobulins to which the MCA belonged was determined by the radioimmunosorption inhibition method [9].

EXPERIMENTAL RESULTS

The use of the ACE inhibitor CA-Gly-Gly as immobilized ligand for affinity chromatography led to the isolation of 3 mg of purified ACE from 800 g of human lung tissue, with a yield of 28% and with 4200-fold purification. The purified preparation had specific activity of about 13 units/mg protein and was a polypeptide with mol. wt. of 150 kilodaltons.

After fusion of $5 \cdot 10^7$ mouse myeloma P_3O_1 cells and $15 \cdot 10^7$ spleen cells from BALB/c mice immunized with the purified ACE preparation, growth of the hybrid cells after 9 days in culture in selective HAT medium was found in 95% of the total number of wells in 10 96-well culture plates. The ability of the hybrid cells to secrete antibodies was determined by binding with ACE adsorbed on the plate (by ELISA). Primary populations which were positive in this test (127 populations) were later tested for their ability to bind ACE from solution (immunosorption test). The results of double screening of some of these populations are given in Table 1.

Cells of the primary population, designated 9B9, were selected for cloning, subcloning, and conversion into the ascites form. The hybridoma line obtained as a result of cloning was given the index a-ACE-9B9F1. The remaining hybridoma populations were frozen for later analysis. MCA isolated from the ascites fluid belonged to the IgG1 subclass. They have high anti-

gen-binding activity in the enzyme immunoassay and immunoadsorption tests (Fig. 1), but they do not affect the enzymic activity of ACE either in solution or in the immobilized state.

The MCA product thus obtained can be used for immunoassay of ACE in biological fluids and tissue extracts and also for immunologic purification of ACE from different sources by affinity chromatography on a-ACE-9B9F1 antibodies bound with sepharose (unpublished results). This particular monoclonal antibody binds with ACE isolated from various human organs (lungs, kidneys, brain, liver, and heart) and it cannot be used, like the anti-ACE MCA obtained by Auerbach et al. [3], to study the tissue distribution of possible ACE isozymes.

We hope that among the wide range of MCA to ACE in our possession it will be possible to find antibodies capable of recognizing both organotypical forms of ACE and functionally important regions of the enzyme molecule.

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