OBTAINING A HIGHLY PURIFIED PREPARATION OF CLASS I HLA ANTIGENS

E. V. Pankratova and A. V. Chervonskii

UDC 612.124.017.1:017.1:547.962.4].08

KEY WORDS: HLA; affinity chromatography; proteins; incompatibility.

Proteins coded by genes of the major histocompatibility complex (MHC) play an exceptionally important role in immune reactions. They are the central comonent in the process of antigen recognition by T lymphocytes. The solution to many problems of cellular and molecular immunology necessitates the obtaining of a pure molecule of histocompatibility protein uncontaminated by other proteins. Parham and co-workers [3, 4] developed and described a method of isolating MHC proteins by affinity chromatography on monoclonal antibodies. A disadvantage of this method is that in most cases besides MHC proteins, minor contamination with other proteins is present in most cases in the eluate from the affinity column, and these are revealed during electrophoresis under denaturing conditions. The reasons for the presence of these minor proteins may be as follows: 1) specific and nonspecific interaction of membrane proteins with the constant part of the immunoglobulin (Ig) molecule; 2) the presence of contaminating Ig with different specificity in the ascites fluid, which often cannot be separated from the monoclonal antibodies (McAb); 3) specific interaction of some membrane proteins with histocompatibility molecules (interaction of this kind has already been demonstrated for the CD8 molecule [2], for the heavy chain of the insulin receptor [1], and certain other proteins); 4) denaturation and destruction of McAb during the use of acid and alkaline eluting buffers, leading to the appearance of light and heavy Ig chains is the eluate; 5) additional proteolysis of some HLA proteins, leading to the appearance of separate fragments of the histocompatibility molecule in the eluate.

The causes listed above make it difficult to obtain histocompatibility proteins in the pure form. We have suggested additional purification of human histocompatibility proteins (HLA), obtained by affinity chromatography on a column with hydroxyapatite. By this method of purification it is possible to remove most of the minor impurities and to isolate the HLA molecule in a pure form.

EXPERIMENTAL METHOD

MHC proteins were isolated by the method described previously [3, 6]. Human spleen was used as the source of HLA proteins. After freezing and thawing twice the splenic tissue (200 g) was cut into small pieces and then homogenized in a Waring blender with 300 ml of 0.05 M Tris-HCl buffer, pH 7.4, containing 0.14 M NaCl. The homogenate was centrifuged at 105,000g for 1 h. The residue (the coarse membrane fraction) was washed with buffer and recentrifuged at 105,000g for 1 h.

The membrane residue was suspended in 0.05M Tris-HCl buffer, pH 7.4, containing 0.05 M Na_2-EDTA with the addition of 3 mg/ml of papain ("Sigma"), previously activated at 37°C in the presence of 0.05 M cysteine and 0.5 M EDTA for 5 min. The suspension was then incubated for 1 h at 37°C and centrifuged for 1 h at 105,000g. The supernatant was dialyzed against 0.05 M Tris-HCl buffer, pH 7.4, with 0.14 M NaCl.

The extracellular parts of the HLA proteins were isolated from the supernatant on McAb, fixed to sepharoze 4B ("Pharmacia"). For this purpose the supernatant was passed successively through three columns: 1) with sepharose 4B, 2) with sepharose 4B on which human IgG were fixed, 3) with sepharose 4B, on which W6/32 McAb against the α_3-domain of class I HLA proteins were fixed. After rinsing to remove unbound proteins, the attached proteins were eluted from column 3 with 0.05 M diethylamine, pH 11.5. All the experimental procedures

Institute of Molecular Biology, Academy of Sciences of the USSR. Institute of Carcinogenesis, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Trapeznikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 10, pp. 401-402, October, 1990. Original article submitted August 10, 1989.

0007-4888/90/0010-1389$12.50

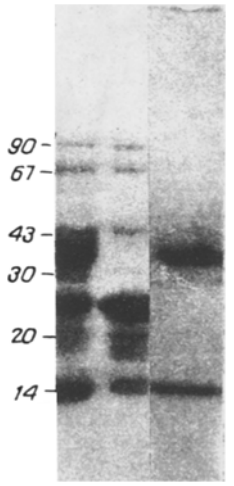

Fig. 1. Electrophoresis of proteins on PAG slabs in the presence of SDS. A) Proteins eluted from affinity column with W6/32 McAb; B) proteins eluted from HAP column by 0.5 M phosphate buffer, pH 6.8; C) proteins not bound to HAP column (extracellular parts of molecules of class I histocompatibility protein molecules). Gel gradient 7-22%.

were conducted at 4°C. Before elution, 0.2 ml of 3 M TRis-HCl buffer, pH 7.4, was introduced into the test tubes. The eluate (1-2 ml) was dialyzed against 0.1 M phosphate buffer, pH 6.8, samples being taken for electrophoresis.

The dialyzed eluate was passed through a membrane ("Millipore" with pore diameter of 0.2 μ to remove aggregates, after which it was applied to a column with hydroxy-apatite (HAP; BioGel HT, Bio-Rad, USA), calibrated with 0.01 M phosphate buffer, pH 6.8. The column was washed with 2 volumes of 0.01 M phosphate buffer, pH 6.8. All the material not bound with HAP was collected. Proteins bound to HAP were eluted with 0.5 M phosphate buffer, pH 6.8. The eluate was collected and used for electrophresis. Electrophoresis was carried out in slabs of gradient (7-22%) acrylamide gel, 0.8 mm thick, in the presence of SDS, by Laemmli's method.

EXPERIMENTAL RESULTS

The human MHC proteins were isolated on W6/32 McAb. Alkaline elution (0.05 M diethylamine, pH 11.5) was most effective. A solution with pH 11.5 damaged McAb, and for that reason light and heavy mouse Ig chains were always present in the eluate. The eluate from the affinity column was tested by PAG electrophoresis in the presence of SDS (Fig. 1A). Preparations of HLA proteins gave two principal bands with apparent mol. mass of 35 and 12 kD and, as a rule, a number of minor bands.

To remove the Ig impurities the resulting proteins were purified on an HAP column, equilibrated with 0.01 M phosphate buffer, pH 6.8. The HLA proteins passed without delay under these conditions through the HAP column, and all the impurities were retained on the column and were eluted with 0.5 M phosphate buffer, pH 6.8. Preparations obtained by the use of HAP also were tested by PAG electrophoresis (Fig. 1B, C). The results of electrophoresis show that only two polypeptide chains with apparent mol. mass of 35 and 12 kD (which corresponds to the light and heavy chains of class I HLA proteins) were not retained on the HAP. Consequently, class I HLA proteins can be effectively purified on HAP, and the method, moreover, is very simple. Chromatography of proteins on HAP was used previously to purify McAb [5]. Since the authors cited worked with a large set of alleles of class I HLA proteins (the hybridoma used reacted with the nonpolymorphic region of the HLA molecules), the possibility cannot be ruled out that certain HLA-allelic variants can bind to HAP and can be eluted with 0.5 M phosphate buffer. For a final explanation of this problem further experiments are needed, using McAb with specificity for different alleles of class I HLA proteins.

LITERATURE CITED

1. G. Due, M. Simonsen, and L. C. Olsson, Proc. Natl. Acad. Sci. USA, 83, 6005 (1986).
2. A. M. Norncent, R. D. Salter, P. Parham, et al., Nature, 336, 79 (1988).
3. P. Parham, B. N. Alpert, H. T. Orr, and J. L. Strominger, J. Biol. Chem., 252, 7555 (1977).
4. P. Parham, Meth. Enzymol., 92, 110 (1983).
5. L. H. Stanker, M. Vanderlaan, H. Juares Sulines, J. Immunol. Meth., 76, 157 (1985).
6. L. Tragardh, B. Curman, K. Wilnan, et al., Biochemistry, 18, 2218 (1979).

ACTION OF THE IMMUNOMODULATOR T-ACTIVIN ON ELECTRICAL PROPERTIES OF THE THYMUS PLASMA CELL MEMBRANE STUDIED BY FLUORESCENT PROBES

V. A. Petrov, A. V. Sinegubov,
E. V. Sokolova, N. V. Glukhova,
V. V. Smeyanov, and L. V. Koval'chuk

UDC 615.362.438.017:615.275.4/.015.4:/
612.438.014:576.314/.076.9

KEY WORDS: immunomodulators; thymus cell membrane; fluorescent probes.

Thymic hormones, synthesized by the epithelial cells of the thymus, are known to have a definite influence on the formation of many thymus-dependent functions of T cells [1, 2, 10, 14]. An important role in the activation, differentiation, and maturation of lymphocytes is played by their membrane [7]. However, the direct action of thymic hormonal factors on the structure of the lymphocyte membrane has received only little study.

It was accordingly decided to study the action of T-activin on the electrical properties of membranes of a suspension of thymus cells. For this purpose we used the method of membrane fluorescent probes [14], which has been used with success for these purposes [5].

EXPERIMENTAL METHOD

Thymus cells were obtained from (CBA × C57Bl) mice aged 2 months in colorless Hanks' solution. Refraction of intact cells (as shown by the test of their permeability to 0.2% trypan blue) amounted to 94% of the total.

The thymocytes were stained with the following fluorescent probes: 1) aniline-naphthalene-8-sulfonate (ANS), 2-(p-dimethylaminostyryl)-4-methylpyridinium (DSM), 4-dimethylaminochalcone (DMC), and 3-methoxybenzanthrone (MBA). To 0.2 ml of a thymocyte suspension (2×10^7 cells/ml) were added 1 mM solutions of ANS or DSM in water and of DMC or MBA in dimethylformamide to a final concentration of 10 μM. The intensity of fluorescence was measured on an ML-4 microfluorometer (LOMO, USSR).

The wavelengths of exciting light for probes MBA, DMC, and DSM were 405-436 nm and for ANS 365 nm; the wavelength of emission of MBA and LMC was 520 nm, of ANS 450 nm, and DSM 550 nm, distinguished with the aid of interference filters. Measurements were made on single cells in a field of the microscope 10 μ in diameter. Apochromatic 40 × objective and 7 × ocular were used. In each preparation the intensity of fluorescence of 100 individual cells was measured. To evaluate the action of T-activin, a solution of T-activin was added to a suspension of thymocytes in Hanks' solution up to a final concentration of between 1 and 10 μg per $1 \cdot 10^6$ cells, and the suspension was incubated at 37°C for 1 h with periodic shaking. After the end of incubation the thymocytes were washed with Hanks' solution with centrifugation at 200 g for 10 min. The residue was resuspended in Hanks' solution, and the number of cells in the suspension thus obtained was counted and their viability determined.

The thymocytes were incubated with neuraminidase as follows: to a suspension of thymic lymphocytes in Hanks' solution a solution of neuraminidase was added up to a final concen-

Department of Biophysics, and Department of Immunology, Medico-Biological Faculty, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR R. V. Petrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 10, pp. 402-404, October, 1990. Original article submitted December 20, 1989.

