MACROMOLECULAR PROTEIN ANTIGENS OF BURNED SKIN

FRACTIONATION AND PROPERTIES

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Macromolecular protein fractions, with a relatively high content of specific antigenic components, were isolated from the burned skin of Wistar rats by gel filtration and ion-exchange chromatography. Their composition and the impurities present were determined by electrophoresis in polyacrylamide gel and by the double immunodiffusion test. None of the antigens studied were found in fractions of normal skin.

An important aspect of the problem of noninfectious immunology of burns is the study of products present in burned skin as possible sources for autoimmunization. Antigenic differences between normal and burned skin were demonstrated for the first time by N. A. Fedorov et al. [8, 9] and subsequently confirmed experimentally and clinically by means of various immunological methods [3-5, 11, 12].

Modern methods of protein chemistry have resulted in considerable progress toward the isolation of a "burn antigen." Burned skin has been shown to have a much higher content of certain macromolecular proteins and, in particular, of proteins not found in normal, unburned skin [6, 7].

The object of the investigation described below was to continue the study of fractionation of burned skin antigens in order to isolate them and study their properties.

EXPERIMENTAL METHOD

The skin of 350 Wistar rats 46-48 h after burning (total mass of fresh tissue 1.45 kg) was used in the experiments. After homogenization, removal of the lipids, extraction with 0.14 M NaCl solution, pH 7.2, and lyophilization, about 35 g of dry protein powder was obtained. From 1.5 to 2.5 g protein was dissolved in 0.05 M tris-chloride buffer, pH 8.0, centrifuged for 25 min at 30,000 g, and applied to a column measuring 3.6×147 cm containing Sephadex G-200 (Pharmacia, Sweden). Elution with the same buffer, containing 0.1 M NaCl and merthiolate (1:20,000) was carried out in the ascending direction under an effective pressure of 10 cm water. The rate of elution was 3 ml/h/cm^2. Fractions with an elution volume of 0.35-0.42 v_t were pooled and dialyzed against 0.05 M acetate buffer, pH 4.0, and centrifuged for 25 min at 30,000 g. The supernatant was dried from the frozen state and kept at 4°C. This was called the pH 4-1 fraction [6].

The pH 4-1 fraction from normal skin (0.39 kg) was obtained in the same way.

Ion-exchange chromatography was carried out on DEAE-Sephadex A-50 (Pharmacia, Sweden), equilibrated with 0.1 M tris-chloride buffer, pH 8.3, or on DEAE-cellulose (Reanal, Hungary), equilibrated with 0.01 M tris-chloride buffer, pH 8.0. The preparation, dialyzed against the corresponding buffer solution, was applied to the column of ion-exchanger measuring 2.5×42 cm and eluted with a linear or stepwise NaCl gradient in the same buffer. The protein concentration in the samples was measured with a type SF-4A spectrophotometer from the optical density at 278 nm or by Lowry's method.

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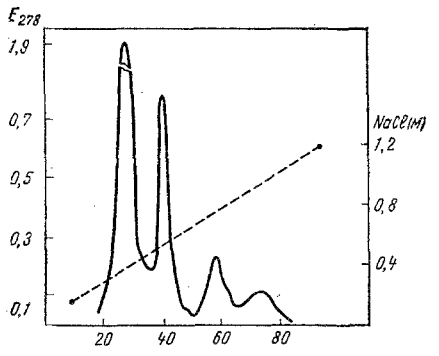

Fig. 1

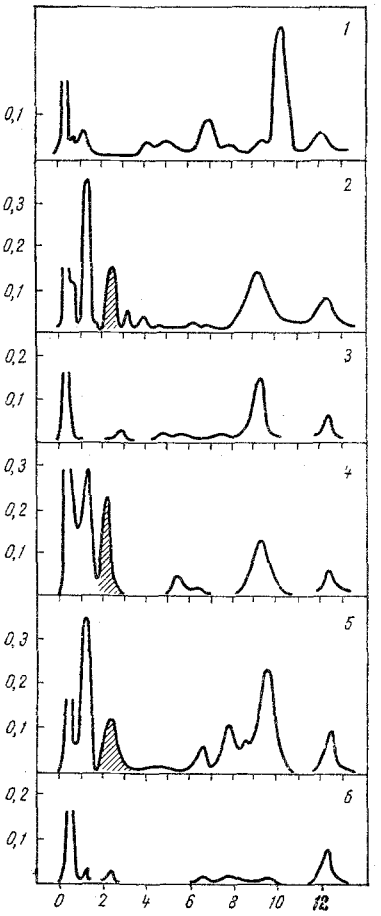

Fig. 2

Fig. 1. Fractionation of pH 4-1 preparations of burned skin on DEAE-Sephadex. Abscissa, sample No.; ordinate, optical density (left), NaCl gradient (right).

Fig. 2. Densitometry of preparations of normal and burned skin after electrophoresis in polyacrylamide gel. Abscissa, distance (in mm); ordinate, optical density (in O. D. units); 1) pH 4-1 fraction of normal skin; 2) pH 4-1 fraction of burned skin; 3-6) fractionation products of pH 4-1 preparation (burn) on DEAE-Sephadex, eluted by stepwise NaCl gradient of 0.1, 0.2, 0.3, and 0.4 M respectively; specific antigen is shaded.

Gel-electrophoresis was carried out by Raymond's method [13]. A 7% polyacrylamide gel in tris-glycine buffer, pH 8.3, was used. A current of 4 mA was applied to the tube and the separation time was 100 min. The gels were removed from the tubes, stained with amido black 10B, decolorized electrically and photographed on Mikrat-300 film, with reduction of the image to 1 : 2.7. Densitometric examination of the film was carried out with a type MF-2 microphotometer. The counting interval was 0.2 mm, the magnification of the apparatus 30×, and the slit width 3.00 mm (half of the counting interval).

Fractions containing specific antigen were determined from the results of the double immunodiffusion test [1]. Immune antisera were prepared and weak precipitation lines detected as described previously [6].

Chromatography was carried out at room temperature and the other stages of isolation and fractionation of the proteins at 0 to 4°C.

EXPERIMENTAL RESULTS

In 21 experiments the quantity of the pH 4-1 preparation of burned skin obtained by gel chromatography was equivalent to 419 mg protein. About 250 mg protein was used for immunization and preparation

of antisera and the rest for fractionation and analysis. The pH 4-1 fraction of normal skin was obtained in significantly lower yield, and as a result this was used only for comparing the properties of the original preparations.

Chromatography on ion-exchanges was carried out in five experiments with high reproducibility. The results of one experiment are shown in Fig. 1.

The proteins of the pH 4-1 fraction were practically completely adsorbed on the anion-exchange resin irrespective of the nature of the carrier (cellulose, Sephadex). If a linear NaCl gradient was used, four peaks were eluted from the column, the first with the highest protein content. Electrophoresis in polyacrylamide gel revealed that this fraction contains up to 7 different components, including the zone corresponding to the specific antigen. The relative mobility of a protein whose presence in the pH 4-1 preparation from burned skin distinguished it most completely from the corresponding preparation of normal skin was 0.20 (the mobility of the fastest component, the acid skin protein described by Adelmann [10], was taken as 1.00).

Compared with the initial pH 4-1 preparation fractionated on the ion-exchange resin, the content of specific antigen in the first peak was increased by 19%.

If a stepwise gradient was used most components of this fraction were eluted from DEAE-Sephadex by a 0.2-0.3 M NaCl solution (Fig. 2). Gel electrophoresis and the precipitation test revealed that this fraction contains, besides the specific antigen, an α_2-macroglobulin, a small quantity of transferrin, and the acid skin protein.

An attempt to purify the specific antigen of burned skin further by gel filtration on Biogel P-300 was unsuccessful. Despite the use of columns measuring 180-200 cm, with low VETT [2], the macromolecular proteins of the original pH 4-1 preparation and its fractions were eluted as a single peak corresponding to the elution volume of the column. The results of gel electrophoresis indicate that this peak contains several different proteins, and no significant increase in the relative proportion of the specific antigen was observed.

The results are regarded as a prelude to the isolation and comprehensive study of the biological properties of the specific burned skin antigen and also of its pathogenetic role in the dynamics of burns.

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