A STUDY OF BOVINE BLOOD SERUM γ-GLOBULIN AND OF LYMPH GLAND TISSUE PROTEINS WITH CORRESPONDING ISOELECTRIC POINTS BY THE PEPTIDE MAP METHOD

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Comparison of the peptide maps of tryptic digests of bovine serum γ-globulin and lymph gland tissue proteins with corresponding isoelectric points shows considerable similarity between the proteins.

The γ-globulin fraction of blood serum is known to be microheterogeneous in a number of its physicochemical and immunochemical properties [1, 5, 7], and to consist of antibodies which differ in their amino-acid composition [3] and charge [6].

In the investigation described below the peptide map method was used to study the structure of the γ-globulin fractions of bovine and blood serum and lymph gland tissue proteins with corresponding isoelectric points.

EXPERIMENTAL METHOD

Protein fractions with different isoelectric points were isolated in a multimembrane electrodecanter of the authors' design [2]. Several modifications aimed at increasing the purity of the fractions were introduced into the technique as described previously [1]. These were as follows.

The separating chamber of the cooled electrodecanter was filled to one-third of its volume with blood serum or lymph gland tissue extract, dialyzed against phosphate buffer ($\mu = 0.2$) of the required pH value. The mixture of proteins was fed into the separating chamber by a peristaltic pump at the rate of 2 ml/h. The charge on the electrodes was of the order of 2V/cm and the current 0.3 A. Components of the protein mixture migrating in an electric field were concentrated near the membranes of the separating chamber and settled to the bottom. Only protein whose isoelectric point corresponded to the pH of the buffer rose into the top part of the chamber. The solution of this protein was collected through the upper side tube of the separating chamber.

Perihepatic lymph glands isolated from the connective-tissue capsule were frozen, crushed in a mincer, and ground in a mortar with quartz sand. Buffer solution was added to the homogenized tissue in the proportion of 5 : 1. The homogenate was centrifuged for 1 h at 10,000 rpm. The supernatant, which was a tissue protein extract, was used for fractionation by electrodecantation.

The antigenic identity of the tissue protein fractions and serum γ-globulin fractions was demonstrated by the precipitation reaction with these proteins and anti-γ-globulin serum.

γ-Globulin obtained from human blood serum by alcoholic fractionation at the Moscow Research Institute of Epidemiology and Bacteriology also was used for fractionation.

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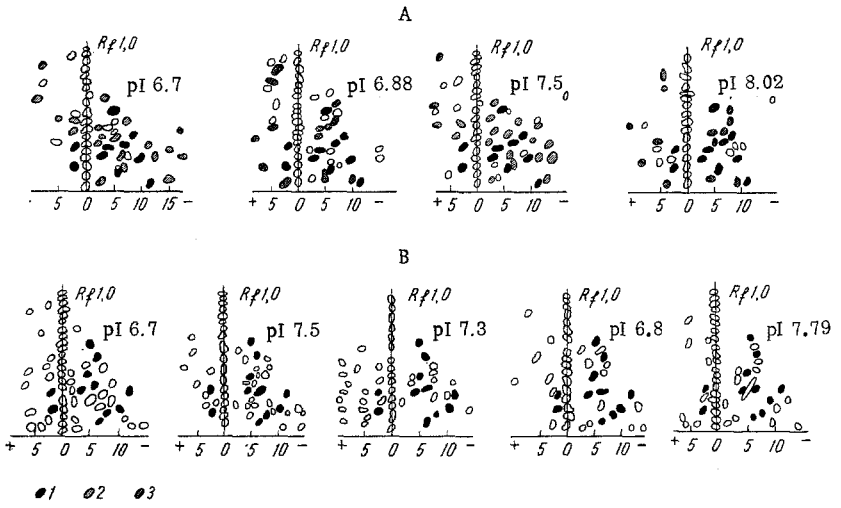

Fig. 1. Diagrams of peptide maps of fractions with different isoelectric points: A) blood serum γ-globulin; B) immunoglobulins from lymph gland tissue. 1) Peptides common to all fractions of serum γ-globulin or tissue immunoglobulins; 2) additional common peptides for serum γ-globulin fractions with pI values 6.7 and 7.5; 3) with pI values 6.88 and 8.02.

Peptide maps were obtained by a combination of high-voltage electrophoresis and chromatography. Hydrolysis of the proteins with trypsin was carried out at 38°C for 24 h, with an enzyme-substrate ratio of 1 : 50. The digests of the protein fractions were separated by high-voltage electrophoresis in pyridine-acetate buffer, pH 6.4 (voltage gradient 50 V/cm), followed by descending chromatography in a butanol–acetic acid–water system (4 : 1 : 5). Ninhydrin-cadmium reagent was used for staining. The peptide maps were analyzed in relation to mobility and R_f value for each spot.

EXPERIMENTAL RESULTS

Fractions of pure serum γ-globulin were isolated within the isoelectric point (pI) range from 6.7 to 8.02. This is in good agreement with the results of electrofocusing of blood serum proteins followed by sectional immunodiffusion [4]. The electrodecantation method in the modification suggested here makes it possible to isolate proteins in a narrow range of isoelectric points, and it appears promising as a method of preparative fractionation. The results of electrofocusing of the proteins belonging to the fractions isolated show that blood serum γ-globulin fractions with pI values of 6.7, 6.88, 7.5, and 8.02 and immunoglobulin fractions from lymph gland tissues with pI values of 6.7, 6.88, 7.3, 7.5, and 7.79 contain no or only a very few protein molecules with isoelectric points found in both fractions simultaneously.

Diagrams of peptide maps of serum γ-globulin fractions are shown in Fig. 1a. The fractions differed significantly from each other, and the differences were greater than those observed previously during fractionation of lyophilized human γ-globulin [1]. This can be partly explained by differences in the original material: in one case fractions of bovine serum γ-globulin were studied, in the other case fractions of human serum γ-globulin. The increase in purity of the isolated fractions in the series of experiments now being described also accounts to a large extent for their significant difference. Diagrams of peptide maps of total lyophilized human γ-globulin and its fractions with an isoelectric point of 7.3 are given in Fig. 2. In one case the fraction was isolated by the method described previously [1], yielding proteins within the pI range of the order of 0.3 pH unit. A similar fraction was also obtained by a modified method as used in the present investigation. Because of the isolation of the protein within a narrower pI interval, it differed significantly from the fraction obtained under less rigid conditions. In the former case the fraction in the anodic part had 11, and in the cathodic part 21 common peptides with the total protein. The fraction isolated under rigid conditions had only eight and 14 common peptides, respectively.

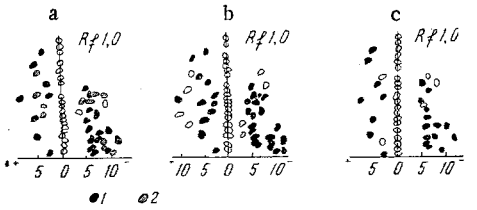

Fig. 2. Diagrams of peptide maps of human serum γ-globulin and fractions with pI 7.3 obtained with the electrodecanter working under different conditions. On the diagram of total γ-globulin: 1) common peptides with fraction isolated under rigid conditions; 2) additional common peptides with fraction isolated under rough conditions.

TABLE 1. Characteristics of Peptide Maps of Immunoglobulin Fractions with Different Isoelectric Points

Protein	Number of peptides			Total
	acid	basic	neutral	
Bovine blood serum γ-globulin				
pI 6.7	11	27	19	57
pI 6.88	13	26	20	59
pI 7.5	12	29	21	62
pI 8.02	12	19	15	46
From bovine lymph gland tissue				
pI 6.7	14	26	21	61
pI 6.88	9	20	17	46
pI 7.3	19	19	15	53
pI 7.5	10	30	18	58
pI 7.79	10	21	22	53

Examination of the peptide maps of lymph gland tissue protein fractions with isoelectric points of 6.7, 6.88, 7.3, 7.5, and 7.79 (Fig. 1b) also revealed substantial differences between the individual fractions.

Despite the mainly very slight differences in content of acid, neutral, and basic peptides in the individual protein fractions (Table 1), their position on the peptide maps was different. Three of the acid and eight of the basic peptides were common to the γ-globulin fraction studied, i.e., about 25% of all the charged peptides of the fraction. The considerable similarity between the individual fractions on comparative analysis is noteworthy. Proteins with isoelectric points of 6.7 and 7.5 had seven common acid and 22 common basic peptides (Fig. 1a), while proteins with pI values of 6.88 and 8.02 had eight and 15 common peptides, respectively. Comparison of the fractions with pI values of 6.7 and 6.88 showed a greater difference in primary structure, definite evidence of the absence of protein with the same isoelectric point simultaneously in their composition.

Diagrams of peptide maps of five immunoglobulin fractions from lymph gland tissues with different pI values are shown in Fig. 1b. Analysis of the peptide maps of all the fractions revealed only a very small number of common peptides (two acid and nine basic). The number of common peptides increased considerably when the individual fractions were compared with each other.

There were many common peptides on the peptide maps of the serum γ-globulin fractions and the lymph gland tissue proteins with identical isoelectric points. For example, there were 9 common acid and 21 common basic peptides for protein fractions with pI 6.7, 5 and 17 common peptides, respectively, for fractions with pI 6.88, and 6 and 18 peptides for fractions with pI 7.5.

These results show that immunoglobulins with identical isoelectric points from blood serum and lymph gland tissue are very similar in their primary structure, and the similarity is often more marked than that between fractions from the same material but with different pI values.

LITERATURE CITED

1. I. F. Kiryukhin, G. V. Troitskii, and G. Yu. Azhitskii, Vopr. Med. Khimii, 15, 610 (1969).
2. I. F. Kiryukhin, Lab. Delo, No. 3, 157 (1970).
3. I. I. Raiker, L. I. Koidan, and N. M. Protserova, Biokhimiya, 34, 1144 (1969).
4. N. Catsimpoolas, Sci. Tools, 16, 1 (1969).
5. R. R. Porter, Biochem. J., 59, 405 (1955).
6. M. Sela, D. Givol, and E. Mozes, Biochim. Biophys. Acta, 78, 649 (1963).
7. E. Valmet, Sci. Tools, 15, 8 (1968).