ANTIGENIC ANALYSIS OF SUBMOLECULAR STRUCTURE OF BOVINE γ-GLOBULIN

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After proteolysis of bovine γ-globulin with papain, 3.6S- and 2.4S- fragments were isolated. Two types of 3.6S-fragments were found, differing in their electrophoretic mobility, solubility, and antigenic structure. The crystallizable F_c fragment is formed by parts of the heavy polypeptide chains of γ-globulin, and the soluble F_{ab} fragment by parts of the heavy and light chain.

The 2.4S-fragment is partly identical antigenically with the F_{ab}-fragment and reacts with antisera against the light and heavy chains.

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Immunochemical analysis of the polypeptide chains and 3.5S papain fragments of human and rabbit immune γ-globulins [3, 5, 12-14] have made possible the construction, in its general form, of a scheme of the structure of this class of serum proteins (see below, Fig. 3) [4, 13]. The universality of this schematic structure may be confirmed by investigating γ-globulins arising from a different species.

The object of the present investigation was to perform an immunochemical analysis of the submolecular structure of bovine γ-globulin. Choice of this particular globulin was determined by the fact that, compared with human and rabbit γ-globulins, this protein is noticeably resistant to proteolysis by papain, and its hydrolysate obtained with high concentrations of the enzyme contains, besides 3.6S-fragments, products with a sedimentation constant of 2.4 Svedberg units [2].

EXPERIMENTAL METHOD

Isolation of γ-globulin from normal bovine serum, its purification on a DEAE-cellulose column, proteolysis with papain, and subsequent separation of the hydrolysis products on a Sephadex G-100 column were carried out as described earlier [2]. Immunoelectrophoresis and double diffusion in agar were performed in a micromodification [1]. To separate the polypeptide chains of bovine γ-globulin after reduction of the protein with 0.1 M mercaptoethanol and alkylation with monoiodoacetate [7], gel-filtration was performed on a Sephadex G-200 column (120 × 2.2 cm) in 1 M propionic acid. To analyze the peptide composition of the papain fragments of bovine γ-globulin, antisera to the L- and H-chains of bovine γ-globulin were obtained by selective exhaustion of a rabbit antiserum against bovine γ-globulin with pure preparations of L- and H-chains used in excess. Completeness of exhaustion of the sera was assessed by direct and cross titration in a double diffusion reaction in agar with L- and H-chains.

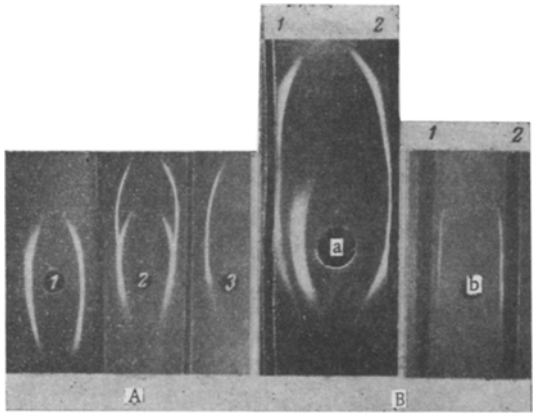

Fig. 1. Immunoelectrophoresis of 3.6S- and 2.4S-fragments of bovine γ-globulin. A: 1) F component; 2) initial 3.6S-fragment; 3) S component. B: a) 3.6S-fragment; b) 2.4S-fragment; 1) antigen-H serum; 2) anti-L serum.

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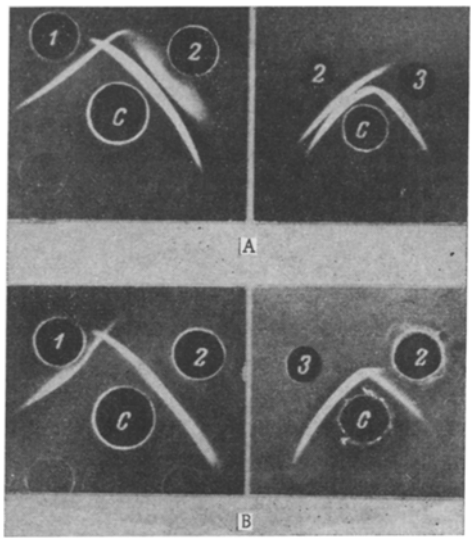

Fig. 2. Comparison of 3.6S- and 2.4S-fragments with F and S components in double diffusion reaction in agar. A: 1) F component; 2) 3.6S-fragment; 3) S component; c) rabbit bovine antiserum; B: 1) F component of 3.6S-fragment; 2) 2.4S-fragment; 3) S component of 3.6S-fragment; c) rabbit bovine antiserum.

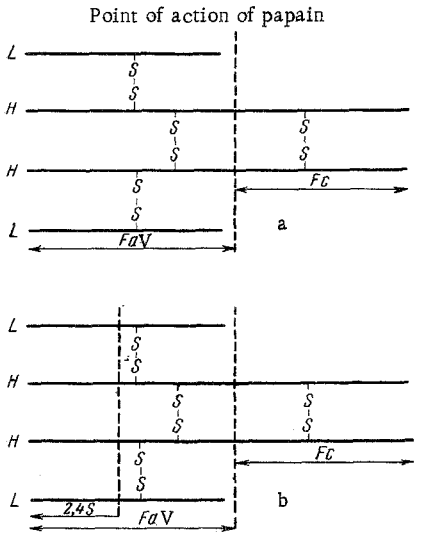

Fig. 3. Scheme of structure of immune γ-globulin. a) After Porter [13]; b) probable scheme of structure of bovine γ-globulin.

EXPERIMENTAL RESULTS

After proteolysis of the bovine γ-globulin with papain, with an enzyme–protein ratio of 1:16 and filtering the hydrolysate through a Sephadex G-100 column (180 × 1.6 cm), equilibrated with 0.15 M NaCl, fractions of 3.6S-fragments and 2.4S-fragments [2] were obtained.

Investigation of the fraction of 3.6S-fragments by immunoelectrophoresis with antiserum against bovine γ-globulin showed that it contained two components differing in electrophoretic mobility and antigenic structure (Fig. 1, A), designated fast (F) and slow (S). During dialysis against 0.01 M phosphate buffer in the cold, a precipitate of crystals, composed of thin, rectangular plates, was thrown down from the solution of the fraction of 3.6S-fragments [2]. The supernatant fluid after dialysis against 0.02 M acetate buffer, pH 5, was filtered through a column of carboxymethyl-cellulose (30 × 2.2 cm), equilibrated with the same buffer. The filtrate obtained from the column corresponded in immunoelectrophoresis to the S component, while the crystals, which dissolved on alkalinization, were analogous to the F component (Fig. 1, A, 1 and 3). During double diffusion in agar, the fraction of 3.6S-fragments gave two precipitation lines, one of which merged with the precipitation line of the solution of crystal (F component), while the other merged with the precipitation line of the pure S component (Fig. 2, A).

During double diffusion in agar the fraction of the 2.4S-fragments was shown not to be identical antigenically to the F component, but to be partially identical to the S component (Fig. 2, B).

Antiserum against bovine γ-globulin, exhausted with L-chains of γ-globulin (anti-H serum), revealed the presence of both F and S components in the fraction of 3.6S-fragments (Fig. 1, B). Antiserum against bovine γ-globulin, exhausted by H-chains of γ-globulin (anti-L serum), revealed the presence of the S component only in the fraction of 3.6S-fragments (Fig. 1, B). The fraction of 2.4S-fragments reacted during immunoelectrophoresis with both (anti-H and anti-L) sera (Fig. 1, B).

The results of antigenic analysis of the fragments of the bovine γ-globulin molecule described above are further evidence in support of the earlier hypothesis [2] of a resemblance between the submolecular structures of this protein and those of human and rabbit γ-globulins. As in these other proteins [5, 6, 8-10, 13], the crystallizable F component of the fraction of 3.6S papain fragments of bovine γ-globulin was antigenically different from the soluble S component and was formed only by segments of the H-chains; meanwhile the S component was formed by both H- and L-chains. In turn, on the basis of the modern nomen-

clature [11] and of the results described above, we can designate the F and S components of the papain hydrolysate of bovine γ-globulin as F_c and F_{ab} fragments respectively (Fig. 3).

Bearing in mind that the 2.4S-fragments of bovine γ-globulin are related antigenically to its S fragment (the F_{ab} component) and react with anti-L and anti-H sera, they must be regarded as cleavage products of the F_{ab} fragment. According to our preliminary data the 2.4S-fragments isolated from antibodies against the dinitrophenyl group possess specific activity, and their position in the bovine immune γ-globulin molecule may therefore be conventionally defined as in Fig. 3, b.

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