ISOLATION AND CHARACTERIZATION OF A PROLINE-RICH POLYPEPTIDE FROM OVINE COLOSTRUM

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1. Introduction

During our comparative studies on serum and colostral immunoglobulins we have obtained serum and colostral IgG1 and IgG2 immunoglobulins [1]. The preparations of IgG1 obtained after chromatography on DEAE-cellulose were immunoelectrophoretically pure and only one protein band was observed in disc electrophoresis. After gel filtration on Sephadex G-200 also one peak was observed. Although ovine colostral IgG2 immunoglobulins were immunoelectrophoretically pure, two protein bands were found in disc electrophoresis, and two protein peaks were observed on gel filtration on Sephadex G-200. The first fraction obtained was pure IgG2 immunoglobulin. The second fraction was not precipitated by antiserum against ovine colostral immunoglobulins. Preliminary studies on the second fraction obtained after chromatography on Sephadex G-200 revealed that it was a polypeptide with a mol. wt of approx. 40 000 containing exceptionally large proportion of proline, glutamic acid and valine residues. The proline-rich polypeptide (PRP) was soluble at 4°C but reversibly precipitated upon warming to room temperature. To our knowledge, a polypeptide with so unusual amino acid composition was never found in sera or colostra. Although the fact that PRP is eluted from DEAE-cellulose (at low ionic strength) together with ovine colostral IgG2 cannot be taken as a proof that PRP is an extra secretory chain, nevertheless it will be very interesting to determine the properties of PRP and find out possible biological functions.

In this report preparation and some properties of PRP are described.

2. Materials and methods

Ovine colostral IgG immunoglobulins were prepared according to the procedure of Janusz et al. [2].

Immunoelectrophoresis was carried out according to the method of Scheidegger [3] modified by Hirschfeld [4] : 1.5% agar gel was used in 0.05 M veronal buffer, pH 8.6, and for electrophoresis 1% solutions of proteins were applied.

Disc electrophoresis was carried out in 0.1 M phosphate, pH 7.2, according to the procedure described by Kickhöfen et al. [5].

Hexoses were determined by the phenol method of Dubois et al. [6] using galactose as a standard. Hexosamines were determined according to the method of Ludowieg and Benmaman [7]. Sialic acid content was measured by the method of Warren [8].

Protein content was measured by the method of Lowry et al. [9], using dialyzed and lyophilized ovine IgG as a standard, and spectrophotometrically at 280 nm. The value of extinction coefficient found for PRP, $E_{280}^{1\%}$, was 7.82.

For determination of amino acid content, protein samples were dialyzed against deionized water and then hydrolyzed with 6 N HCl for 24 hr at 110°C in ampoules sealed in vacuo. The hydrolysates obtained were analyzed in the automatic amino acid analyser of Locarte, England. The amino acid content was expressed as per cent of an amino acid recovered in

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respect to the total content of amino acids determined.

Mol. wt was determined by thin-layer gel filtration in Sephadex G-100 (super-fine) in 0.1 M Tris–HCl buffer containing 0.2 M NaCl, pH 7.4. As standards 2% solutions of Blue Dextran, chymotrypsinogen, bovine serum albumin, ovalbumin and bovine IgG immunoglobulins were used.

Studies of the optical rotatory dispersion (ORD) and circular dichroism (CD) were performed in Jasco Model ORD/UV-5 instrument with the circular dichroism attachment. Spectra in the range 320–200 nm were recorded at 28°C in 2 and 10 mm cuvettes, and protein concentrations were 0.005–0.01% in 0.01 M phosphate buffer containing 0.1 M NaCl, pH 7.2. The absorbancy of solutions was below 1.0 in the range studied, and values of rotation and elipticities were proportional to protein concentrations. Data are presented as reduced mean residue rotation,or mean residue elipticity, $[\theta]$, in degrees X cm^2/decimol, using a mean residue weight of 110.

3. Results

Ovine colostral IgG2 immunoglobulin obtained after chromatography on DEAE-cellulose was filtered through Sephadex G-200. The second fraction

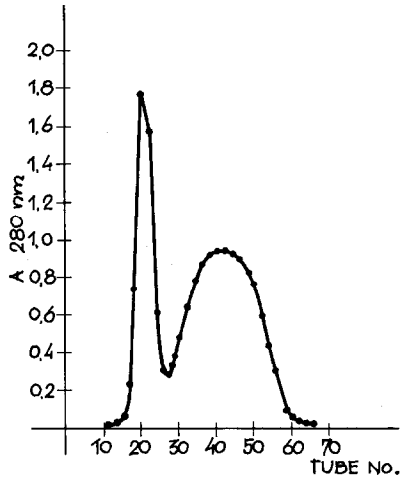

Fig. 1. Gel filtration of ovine colostral IgG2 immunoglobulin on Sephadex G-200 in 0.1 M phosphate buffer, pH 7.2. To the column of 3 X 40 cm, 95 mg of protein were applied. The flow rate was 12 ml/hr and fractions of 4 ml were collected.

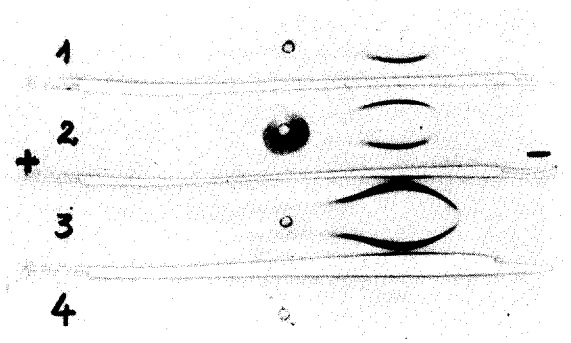

Fig. 2. Immunoelectrophoresis of ovine colostral IgG2 preparates. 1 and 2: IgG2 after chromatography on DEAE-cellulose; 3: first fraction after chromatography on Sephadex G-200; 4: second fraction after chromatography on Sephadex G-200. For precipitation, rabbit antiserum against ovine colostral proteins was used.

Fig. 3. Disc electrophoresis of PRP in 0.1 M phosphate buffer, pH 7.2.

containing PRP was rechromatographed on Sephadex G-200 (fig.1). In immunoelectrophoresis only the first fraction containing IgG2 gave precipitation line with rabbit anti-ovine colostrum antiserum (fig.2). PRP obtained after rechromatography showed in disc electrophoresis in phosphate buffer only one protein band (fig.3).

The polypeptide obtained was soluble at 4°C but reversibly precipitated upon warming to room temperature. Therefore, preparation and all manipulations were carried out at 4°C.

The mol. wt determined by thin-layer gel filtration was 38 000. No neutral carbohydrates and hexosamines were found in PRP. The amino acid composition of PRP is presented in table 1. CD

Table 1
Amino acid composition of PRP

Amino acid	%	Amino acid	%
Lys	7.16	Ala	1.38
His	1.94	Val	12.85
Arg	1.80	Met	3.93
Asp	2.56	Ileu	2.48
Thr	6.55	Leu	9.60
Ser	5.27	Tyr	1.62
Glu	14.90	Phe	4.72
Pro	22.90	Cys	1.05
Gly	2.32		

The results are expressed as per cent of amino acids recovered in respect to the total content of amino acids determined. The values presented in the table are the average of three determinations.

and ORD spectra of PRP are presented in fig. 4a and b.

4. Discussion

PRP described in this report does not resemble any known protein. The main amino acid components are proline, glutamic acid, valine, leucine, lysine, threonine and serine. The first three amino acids comprise 51% of the total amino acids determined. Comparatively low content of glycine makes PRP different from collagen-like proteins. The only other protein showing some similarity to PRP is cuticlin, a structural protein isolated from Ascaris lumbricoides by Fujimoto and Kanaya [10,11], which contained about 30% of proline residues and relatively low content of glycine.

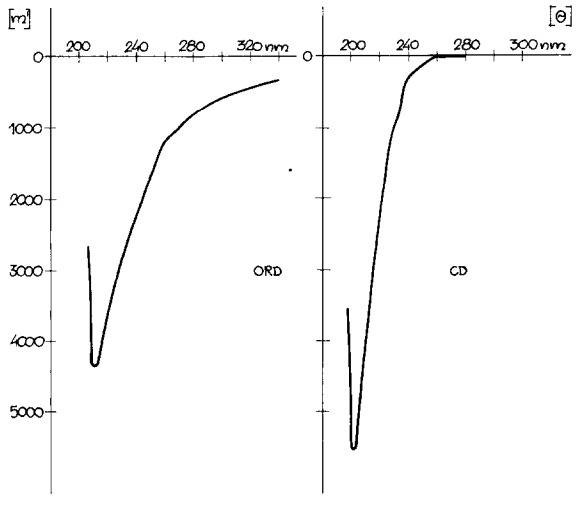

Fig. 4. Optical rotatory dispersion (a) and circular dichroism (b) of PRP.

PRP could be also prepared by sulfitolysis of PRP-containing ovine colostral IgG2 and separation of products on Sephadex G-100 in 6 M guanidine hydrochloride. The properties of PRP thus obtained were similar to properties of PRP obtained by the procedure described in this report.

Relatively low electrophoretic mobility of PRP in disc electrophoresis at pH 7.2 might suggest that large proportion of glutamic acid residues is in the form of glutamine.

Solutions of preparates of PRP-containing ovine colostral IgG2 obtained after chromatography on DEAE-cellulose kept for 2 hr at room temperature showed the presence of a precipitate which dissolved when the solutions were cooled to 4°C. The effect was reversible. No reversible precipitation was observed in case of PRP-free IgG2. However, reversible precipitation was still observed in case of solutions of purified PRP, when the concentrations of solutions were higher than 0.2%.

The general shape of the ORD curve is similar to that of poly-L-proline II. The position of the minimum is at 212 nm which is slightly shorter wavelength than poly-L-proline II (216 nm; Blout et al. [12]). The circular dichroism spectrum of PRP has a minimum at 202 nm, which is almost the same wavelength as in case of poly-L-proline II (205 nm; Carver et al. [13]) However, the values of mean residue rotations and elipticities in case of PRP are much lower than in case of poly-L-proline II. This could be due to the fact that the composition of PRP, especially high content of hydrophobic amino acids, will influence the environment of each residue and, consequently, change the conformation of each residue from that found when the residue is located in a long sequence of the same residues, i.e. in homopolymers. This was confirmed by results of Sakai and Isemura [14] who studied the conformation of copolymers of proline and glutamic acid.

The problem of a possible biological function of PRP is very intriguing and is currently under study.

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