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## Note

# Purification of Fab and Fc fragments from human serum immunoglobulin G by electrophoresis and affinity chromatography

FRANCE DE LA FARGE and PIERRE VALDIGUIE

*Laboratoire de Biochimie, Faculté de Médecine de Rangueil, chemin du Vallon, 31400 Toulouse (France)*

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Since Porter[1] described the isolation of Fab and Fc fragments from human serum immunoglobulin G (IgG), several alternative methods have been reported. These include methods based on ion-exchange chromatography with use of carboxymethylcellulose and diethylaminoethylcellulose[2] and of diethylaminoethylcellulose alone[3]. Methods involving gel filtration on Sephadex have also been reported[4,5].

We describe here two methods for the isolation of IgG sub-fractions; these methods are based on (*i*) electrophoresis on cellulose acetate, and (*ii*) chromatography of a papain digest using cyanogen bromide-activated Sepharose 4B. The purities of the fragments prepared by these two methods were compared by using the classical immunodiffusion technique[6].

We know that most research groups working on antibody fragments have developed similar purification methods. Nevertheless, we feel that our procedure may be of interest, as detailed accounts of the various techniques are not readily available in the literature.

## EXPERIMENTAL

### *Isolation of normal and myeloma-type IgG*

Serum from healthy individuals was pooled and used to prepare 7S IgG. Myeloma proteins were collected from patients with Kahler's disease and subjected to plasma-phoresis[7]; IgG collected from such patients have K-type light chains[8].

Samples (50 ml) of serum were subjected to chromatography on diethylaminoethylcellulose (Whatman DE 22) using the method of Biserte *et al.*[9]; the column dimensions were 50 × 4 cm. The first fraction (40 ml) eluted from the column was collected, dialysed against phosphate buffer for 24 h and then freeze-dried. The purity of the IgG was established by using immunoelectrophoresis against horse antiserum[10] obtained from the Behring-Werke Institute.

### *Papain digestion*

Papain hydrolysis of 200-mg portions of IgG was effected by the procedure of Porter[1], but with an enzyme concentration equivalent to 2% of the IgG; the hydrolysis

was performed for 12 h at 37° and pH 6, with agitation; the pH was monitored and adjusted continuously[11]. The resulting hydrolysate was freeze-dried and tested by immunoelectrophoresis against horse antiserum.

By gel filtration on Sephadex G-150, with use of 0.1 *M* Tris–0.5 *M* sodium chloride–0.002 *M* EDTA (pH 8.2) as buffer, we were able to obtain from the digest Fab and Fc fragments free from IgG[12].

*Electrophoretic separation of isolated fragments*

Electrophoresis was carried out using blocks of cellulose acetate (170 × 60 × 2.5 mm) (Cellogel; Chemetron, Milan, Italy). The buffer used was sodium barbitone–barbitone–Tris (10.3, 1.84 and 7.2 g, respectively, per litre).

To monitor the migration, two thinner strips of the same material were similarly treated; these strips were stained with Ponceau red S, de-stained with 5% aqueous acetic acid and made transparent by treatment with pure methanol for 1 min and then with methanol–acetic acid–glycerol (255:42:3) for 2 min. The transparent strips were kept overnight at 70° in order to dry them for conservation.

Examination of the gels showed that both fragments were distributed on both sides of the deposition point; each of the two fractions was recovered from the blocks by squeezing in a syringe press, then submitted to a second electrophoretic-purification stage under conditions the same as the first. The purity was assessed by immunodiffusion on a glass plate[6].

*Affinity chromatography*

Purification of the Fab and Fc fragments from the papain digest was carried out on cyanogen bromide-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) as described by Litman and Good[13] for IgA; the Sepharose was activated by the manufacturer.

*Purification of the Fc fragment*

1 ml of horse anti-Fc serum (Behring-Werke Institute, Frankfurt, G.F.R.) was added to 25 ml of 0.5 *M* sodium chloride–0.1 *M* sodium hydrogen carbonate buffer, pH 8.7, and the mixture was stirred with 15 g of Sepharose 4B; this coupled the anti-serum to the cyanogen bromide-activated Sepharose, and the activated Sepharose (with bound anti-Fc serum) was placed in a column (21 × 1.6 cm I.D.); the loading was 15 ml. Unbound material was washed from the column with the coupling buffer, and any remaining active groups were allowed to react with 1 *M* ethanolamine at pH 8 for 1–2 h. Three washing cycles were then used to remove non-covalently adsorbed protein, each cycle consisting of a wash at pH 4 (0.1 *M* acetate buffer containing 1 *M* sodium chloride) followed by a wash at pH 8 (0.1 *M* borate buffer containing 1 *M* sodium chloride).

The following procedure usually showed specific uptakes of 80–90% of the Fc fragment on to the Sepharose. A 100-mg portion of the papain digest was dissolved in 0.005 *M* sodium phosphate buffer of pH 8, and this solution was applied to the column; the Fab fragment was immediately and specifically eluted with 150 ml of 0.005 *M* phosphate buffer of pH 8, and the column was then washed with 300 ml of the same buffer. The Fc fragments were subsequently de-coupled and eluted with

0.2 *M* glycine–hydrochloric acid buffer of pH 2.4, the entire chromatographic procedure taking approximately 4 h.

The Fab fragment was isolated by a similar procedure, but with anti-Fab serum bound to the cyanogen bromide-activated Sepharose 4B. Typical elution patterns are shown in Fig. 1, and recovery results are compared with those obtained by using Olin and Edelman's method[3] in Table I.

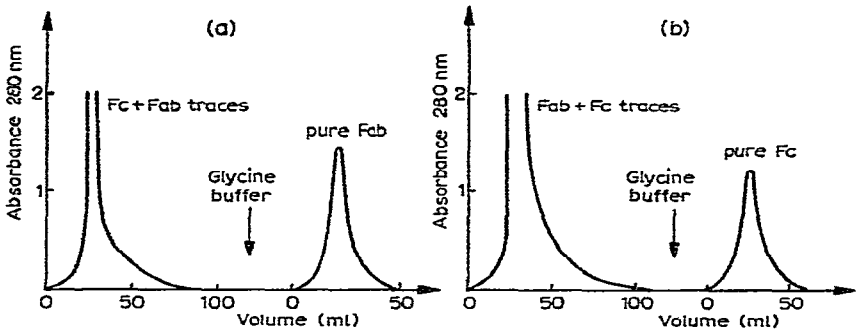


Fig. 1. Affinity chromatography on cyanogen bromide-activated Sepharose 4B. (a) Purification of Fab; (b) purification of Fc.

TABLE I

COMPARATIVE RESULTS BY THREE DIFFERENT METHODS

Protein contents were determined by the method of Lowry *et al*[14]., with Standard Total Protein (Behring-Werke Institute) as standard.

| *Method* | *Hydrolysed immunoglobulins (mg)* | *Fab (mg)* | *Fc (mg)* |
|---|---|---|---|
| Olins and Edelman[3] | 100 | 30 | Not pure |
| Electrophoresis | 100 | 22 | 20 |
| Affinity chromatography | 100 | 40 | 20 |

## CONCLUSION

Fc and Fab fragments, pure by the criterion of immunodiffusion against horse anti-serum, have been obtained by electrophoresis in cellulose acetate blocks and by affinity chromatography on cyanogen bromide-activated Sepharose 4B (Fc fragments against anti-Fab serum show no arc in immunodiffusion gels). The methods described are simple in principle and fairly rapid. Recovery of the pure Fab fragment was superior by the chromatographic method, but either procedure was adequate for isolating the Fc fragment.

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