RATIO OF THE FAB FRAGMENTS I AND II FROM GOAT ANTIBODIES AND NORMAL γ-GLOBULINS

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Received 3 December 1969

1. Introduction

It was found by Porter [1] that rabbit antibodies or γ-globulins on digestion by papain were degraded to three fragments which could be separated from each other by chromatography on CM-cellulose. The more acidic fragments Fab I and Fab II are split products of two different types of antibody, of the composition (Fab I) $_2$Fc and (Fab II) $_2$Fc. Sela and coworkers [3] succeeded in separating the two types of antibody by means of DEAE-Sephadex, and Sela and Mozes [4] found that antibodies of the type (Fab I) $_2$Fc are predominantly formed in response to immunization by basic antigens, whereas acidic antigens induce principally the formation of antibodies of the type (Fab II) $_2$Fc.

In this work we present evidence that goat γ-globulins can be similarly cleaved in three fragments, which could be separated on chromatography on CM-cellulose. The distribution of the Fab I and Fab II fragments in antibodies directed against a basic antigen (lysozyme) and in normal γ-globulins of goat and rabbit are compared.

2. Materials and methods

Lysozyme (three times crystallized) was obtained from Sigma Chemical Co. Mercuripapain (twice crystallized) was obtained from Worthington Biochemical Corp. Lyophilized *Micrococcus lysodeikticus* cells were freeze-dried vials from Worthington Biochemical Corp.

2.1. *Immunochemical methods*

Goat antisera were prepared against lysozyme by repeated weekly injections of 2 ml of an enzyme solution containing 20 mg per ml NaCl 0.15 M. Rabbit antilysozyme antisera were prepared as described previously [5].

Antibodies were separated from the other serum proteins by an immunoadsorbent prepared according to the procedure of Avrameas and Teirnynck [6]. Normal γ-globulins were prepared from the non-immunized animals by two successive ammonium sulfate precipitations at 35% saturation followed by chromatography on DEAE-cellulose according to Sober et al. 1956 [7].

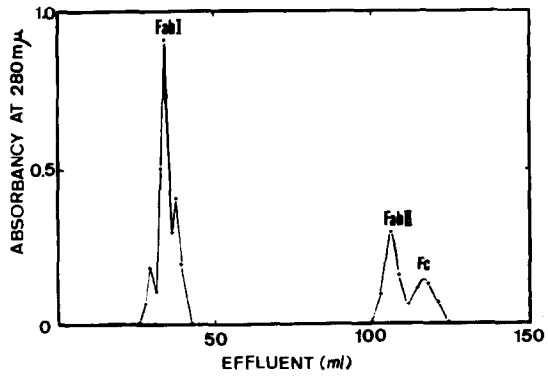

Fig. 1. Chromatography of papain-digest of goat anti-lysozyme antibody on carboxymethylcellulose. Gradient from 0.01 M sodium acetate, pH 5.5 to 0.9 M sodium acetate, pH 5.5, commencing at 90 ml eluate volume.

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Table 1
Ratios of the concentrations of fragment Fab I to Fab II in normal γ-globulins and anti-lysozyme antibodies from goat and rabbit antisera.

	Fab I (%)	Fab II (%)	R
Normal goat CI γ-globulins	36	64	0.56
Anti-lysozyme goat CI antibodies	79	21	3.76
Normal rabbit 118 γ-globulins	60	40	1.50
Anti-lysozyme rabbit 118 antibodies	87	13	6.69

2.2. *Analytical methods*

Protein concentrations were determined photometrically assuming $E^{280\ m\mu}_{1\ mg/ml}$ to be 2.60 for lysozyme, and 1.40 for rabbit as well as for goat antibodies and 1.50 for rabbit as well as for goat fragments.

Analytical centrifugations were performed on the Spinco Model E centrifuge and sedimentation constants were determined using Schlieren Optics.

2.3. *Enzymic activity*

Enzymic activity of lysozyme was based on the rate of lysis of *Micrococcus lysodeikticus* as described in a previous publication [8].

3. Results and discussion

Papain digestion of goat γ-globulins causes splitting into three fragments. The sedimentation constant of these fragments is 3.5 S. Non-cleaved globulin has a sedimentation constant of 6.5 S.

When submitted to gel filtration on a Sephadex G-100 column the fragments obtained by papain cleavage of goat γ-globulin have the same elution volume as cleaved rabbit γ-globulins. This tends to indicate that the molecular weight of the papain fragments from goat and rabbit γ-globulin are similar.

The three fragments could be separated from each other on CMC-cellulose. Fragment Fc, the least acidic of the three fragments was recovered with poor yields, indicating high susceptibility to papain cleavage. The more acidic fragments were called Fab I and Fab II by analogy with the fragments obtained from rabbit γ-globulin. In one of the two studied goats it was observed that the fraction containing Fab I could be resolved in three components. This was observed with many different preparations of serum of this animal (fig. 1).

Goat Fab fragments seem to bear the active sites of the antibody γ-globulins. Lysozyme activity was inhibited in the presence of the Fab fragments obtained from cleavage of goat anti-lysozyme antibodies. For complete inhibition four moles Fab per mole lysozyme were necessary.

When a mixture of goat Fab (9 mg/ml) and lysozyme (0.6 mg/ml) was subjected to ultracentrifugation at 20°C, pH 7, two peaks were observed, one with a sedimentation constant of 3.5 S and the other of 7.0 S. This last peak corresponds probably to a complex containing 3 Fab per lysozyme. A very similar result was obtained with rabbit anti-lysozyme antibody fragments.

The distribution of Fab fragments in the goat anti-lysozyme antibodies was studied and was found to be similar to the distribution observed for rabbit and anti-lysozyme antibodies (table 1).

The data obtained with goat Fab fragments are in agreement with the observations of Sela and coworkers [3] and [4]. It appears that the correlation between the net electrical charge of the immunogen and the type of antibody elicited in the animal is a common feature of the immune systems of rabbit and goat.

Acknowledgements

This research was aided by a grant from EURATOM: 016-61-10. A.D.S. is a predoctoral fellow of the "Institut pour l'encouragement de la recherche Scientifique dans l'Industrie et l'Agriculture".

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