# COMPLEMENT FIXATION BY PIG IMMUNOGLOBULIN M AND ITS ENZYMIC FRAGMENTS

D. BEALE, D. G. DUNHAM and C. M. KENT
*Agricultural Research Council, Institute of Animal Physiology, Babraham, Cambs., UK*

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## 1. Introduction

It is now well established that the binding of antigen to the Fab regions of immunoglobulins can induce biological activity in the distant Fc regions of the molecules. The mechanism whereby this is achieved is an intriguing problem which has been reviewed and discussed by Metzger [1].

Plaut et al. [2] have reported that the proteolytic removal of Fab regions from human IgM leaves an $(Fc)_5$ fragment that has a significantly enhanced complement fixing activity relative to that of the parent molecule. This result suggested that the Fab arms might be sterically hindering the complement fixing site of the Fc region. Indeed, electron micrographs of IgM antibody bound to flagella [3] show that the Fab arms move away from the plane of the disc-shaped Fc region, thereby possibly uncovering a complement fixing site.

Preliminary studies on $(Fc)_5$ fragment from pig IgM failed to show any enhancement of complement fixing activity [4]. This result suggested that removal, or movement, of Fab arms was not necessarily sufficient to activate complement fixation and that an accompanying comformational change in the Fc region might be required.

Unfortunately these $(Fc)_5$ fragments had been prepared by different methods using conditions that are likely to cause conformational changes. Further, different complement systems were used in the fixation assays. It therefore seemed desirable to study the effect of different methods of preparation and the efficiency of different complement systems.

## 2. Materials and methods

Pig IgM was prepared as previously described [5] and digested with pepsin at pH 4.6 and 37°C for 12 and 18 h [5,6]; with trypsin at pH 8.0 and 60°C for 3 h [7]; and with thiol free papain at pH 6.0 and 37°C for 3 h [8]. All three methods produced Fab and $(Fc)_5$ fragments which were separated and purified by gel filtration on Sephadex G-200 and characterised by sedimentation, immunoelectrophoresis and SDS polyacrylamide gel electrophoresis as previously described [5].

Most pig IgM preparations had natural haemolytic activity which was removed by absorption with erythrocyte membranes or erythrocytes. One preparation that had a particularly high level of activity was used as a source of pig haemolytic IgM antibody.

Heat aggregates of pig IgM and human IgG were prepared by the method of Augener et al. [9]. Pig IgM and $(Fc)_5$ fragment were reduced and alkylated as previously described [5].

Complement fixation assays were carried out by the methods of Kabat and Mayer [10] and Levine [11] as modified by Lachmann et al. [12]. Serum from pig, rat, rabbit, guinea pig and man was used as a source of complement. Only sera devoid if haemolytic activity were employed. Assays were carried out at 37°C for 30 min and at 4°C for 16 h, and 2 or 20 $CH_{50}$ units of complement were used. Aggregated human $IgG_1$, known to fix complement, and Fab fragment, known not to fix complement, were included as controls in all assays.

## 3. Results

The properties of pig $(Fc)_5$ prepared by digestion of IgM with pepsin have already been fully described [5,6]. The $(Fc)_5$ fragments prepared by trypsin or papain digestion appeared to be identical with the pepsin $(Fc)_5$ fragment as regards sedimentation coefficient, antigenic determinants revealed by specific antisera, and molecular size on SDS polyacrylamide gels. All three fragments were seen as disc-shaped molecules in the electron microscope.

Table 1 gives the amounts of fragments, IgM and various aggregates required to fix 1 out of 2 $CH_{50}$ units of different species of complement at 37°C for 30 min. Fab fragment had no measurable activity. Pig $(Fc)_5$ fragment, irrespective of its method of preparation, had only weak activity which was less than that of non-aggregated pig IgM on a molar basis. Only with rabbit complement did pig $(Fc)_5$, and IgM, display significant activity but there was still no enhancement of the fragment. These activities may be due to some peculiarity of rabbit complement or of the particular serum used.

Assays carried out at 4°C for 16 h, and assays using 20 $CH_{50}$ units of complement did not give significantly different overall results from those shown in table 1.

As shown in table 1 heat aggregation of pig IgM and human IgG increased their complement fixing activities in accordance with the observations of Augener et al. [9]. It will also be seen that pig haemolytic IgM–erythrocyte membrane complexes were highly efficient at fixing pig complement. However, reduction and alkylation of the antibody significantly decreased the activity of complexes.

## 4. Discussion

The removal of Fab from pig IgM by three different proteolytic methods employing large differences in pH, temperature and time, failed to produce an $(Fc)_5$ that had enhanced complement fixing activity relative to that of the parent molecule. The use of different species of complement and variations in the assay conditions did not significantly influence this result.

It would seem that removal of Fab arms, mimicking the movement seen in electron micrographs of IgM antibody attached to flagella [3], is not necessarily sufficient to enhance complement fixation. An accompanying change in the conformation of the Fc region might also be required. In the case of human $(Fc)_5$, which does show enhanced activity relative to the parent molecule [2], the method of preparation may have induced a conformational change. Species differences may make such a change more likely to occur during the preparation of human $(Fc)_5$ than

Table 1
Fixation of complement by immunoglobulin aggregates and fragments

| Sample | μg to fix 1 out of 2 $CH_{50}$ units C′ from | | | | |
|---|---|---|---|---|---|
| | Pig | Rat | Guinea pig | Human | Rabbit |
| Haemolytic pig IgM + membranes | 0.4 | – | – | – | – |
| Reduced haemolytic IgM + membranes | 2.4 | – | – | – | – |
| Absorbed pig IgM heat aggregates | 20 | 40 | 20 | 40 | 4 |
| Absorbed pig IgM | 120 | 120 | 240 | 240 | 12 |
| Reduced absorbed pig IgM | 300 | – | – | – | – |
| Fab fragment | >850 | >850 | >850 | >850 | >850 |
| Trypsin pig $(Fc)_5$ fragment | 500 | – | – | – | – |
| Papain pig $(Fc)_5$ fragment | 400 | – | – | – | – |
| Pepsin pig $(Fc)_5$ fragment | 450 | 450 | >900 | >900 | 9 |
| Reduced pig $(Fc)_5$ | >900 | >900 | >900 | >900 | >900 |
| Human $IgG_1$ heat aggregates | 4 | 8 | 4 | 1 | 4 |
| Human $IgG_1$ | 120 | 180 | 180 | 60 | 120 |

of pig $(Fc)_5$. Indeed pig IgM and human IgM have been reported to differ in their behaviour towards reduction and fragmentation [4–6] in ways which suggest that the Fc region of pig IgM may be more stable than that of human IgM.

Pig haemolytic IgM antibody–erythrocyte membrane complexes were highly efficient at fixing pig complement but their activity was significantly depressed when the inter chain disulphide bridges of the antibody were reduced. Reduction of antibody would not be expected to influence affinity but it might affect avidity. Evenso, the loss of activity on reduction of inter chain bridges might indicate that they influence flexibility or conformational changes in the IgM molecule.

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