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Electron microscopy study of non-precipitating anti-dinitrophenyl antibodies

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Non-precipitating anti-dinitrophenyl pig immunoglobulins G have been studied by negative staining, freeze-drying and high-resolution shadow casting. The general morphology of the molecules is described. The predominant conformation of antibody molecules is a tripod-like one.

Immunoglobulin, non-precipitating; Electron microscopy; (Pig)

1. INTRODUCTION

Immunoglobulins G (IgG) of different animal species and of different subclasses within the same species differ markedly in their biological properties: the ability to bind the complement, to fix on the Fc receptors of various cells, and to induce precipitation and agglutination of antigens. The main differences in their primary structure are concentrated in the hinge region connecting the Fab and Fc subunits. This region can be from 15 to 56 amino acid residues long in various IgGs and contains from 1 to 11 disulfide bonds. One of the first papers on the electron microscopy of rabbit IgG antibodies [1] reported that the angle between the Fab and Fc subunits can vary due to flexibility of the hinge region. Therefore, it is reasonable to assume that differences in the structure of the hinge region can cause a different degree of molecule flexibility, which, in its turn, can give rise to distinctions in biological properties. Pig antibodies to the DNP group can be obtained as two functionally different types, presumably subclasses of pig IgG. While the antibodies isolated from sera collected at an early stage of antibody response are typically precipitating ones, the sera collected upon decline of the immune response can serve as a source of non-precipitating antibodies, i.e. antibodies that form exclusively soluble complexes with multivalent ligands [2,3]. The non-precipitating pig IgG antibodies differ significantly in a number of biological properties and physical parameters [2-7] from the rabbit IgG investigated previously by electron microscopy. In particular, they are characterized by a lower degree of segmental flexibility [4,7]. Here, we have aimed at revealing structural peculiarities of non-precipitating pig antibodies using both the techniques of freeze-drying and high-resolution shadow casting [8] and negative staining.

We have found that the non-precipitating pig IgG molecule has a tripod-like shape.

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2. MATERIALS AND METHODS

Non-precipitating antibodies to the dinitrophenyl group were isolated from sera of pigs immunized with dinitrophenylated bovine serum albumin according to Franěk et al. [2,3]. The antibodies belonged to immunoglobulins of class G (150 kDa). The constant of association with ϵ-(2,4-dinitrophenyl)-L-lysine was $1.8 \times 10^7\ M^{-1}$. According to immunoelectrophoresis data and in SDS-polyacrylamide gel electrophoresis, the immunoglobulin preparations were homogeneous [2,3].

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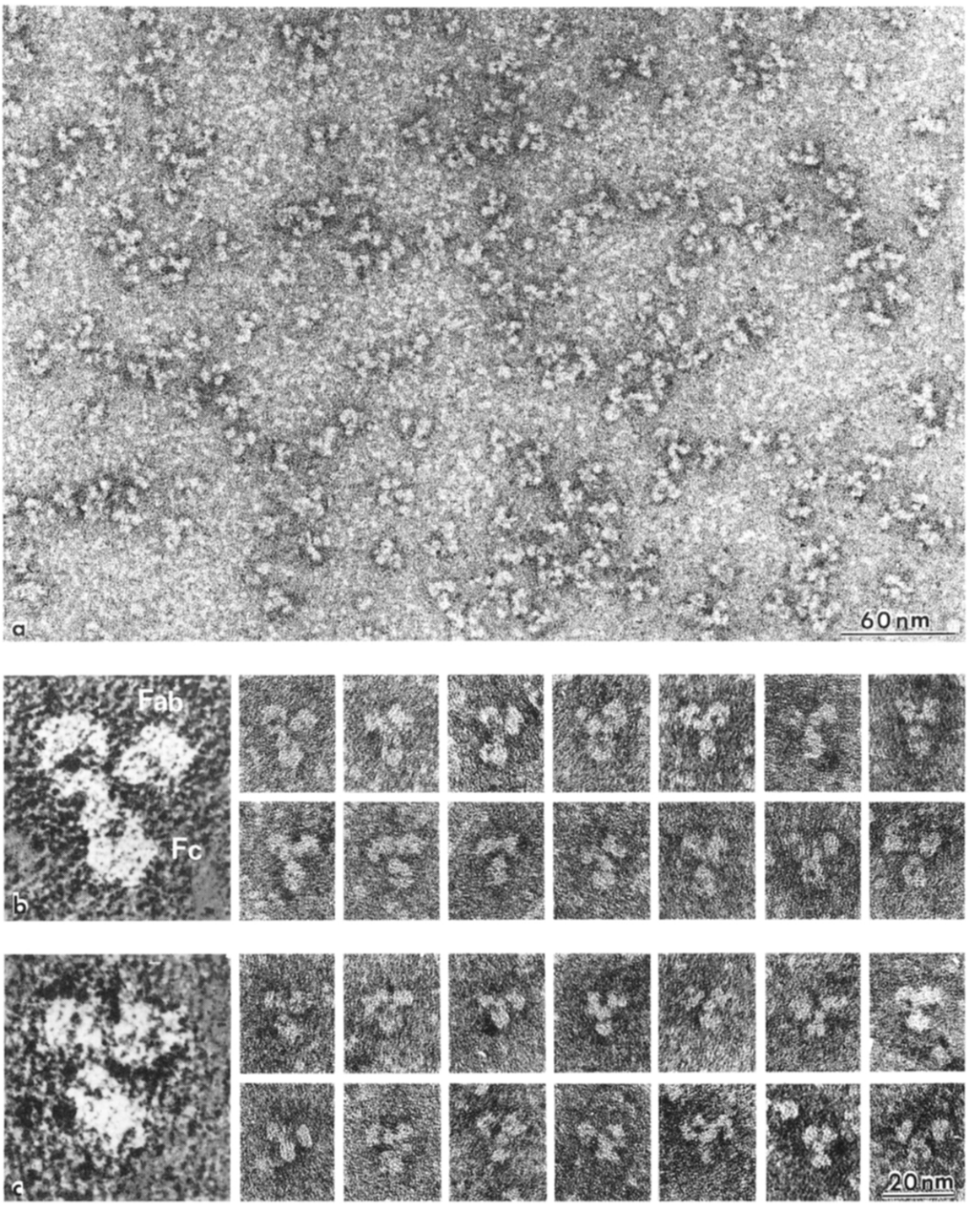

Fig.1. Negatively stained intact non-precipitating antidinitrophenyl pig IgG. A field of preparations (a) and two types of images of IgG molecules: type 1 (b) and type 2 (c). For details see text. The images of each type are shown at higher magnification on the left. The shape and fine structure of the Fab and Fc subunits are clearly seen.

The immunoglobulin preparation was dialyzed vs 0.01 M ammonium acetate, pH 7.8. Just before use, the preparation was centrifuged at 1200 × *g* for 15 min and diluted with ammonium acetate to a concentration of $A_{280}=0.01$ U/ml. Negative staining was carried out according to Valentine et al. [9] with 1% aqueous uranyl acetate. The carbon film was mounted on a grid with holed plastic film coated with carbon. All procedures were carried out at 4°C.

Freeze-drying and high-resolution shadow casting were performed according to Vasiliev and Koteliansky [8]. Grids with adsorbed immunoglobulins were rapidly cooled to liquid N_2 temperature and then inserted through the air lock into the vacuum chamber. After lyophilization under ultrahigh oil-free vacuum, specimens were shadowed with tungsten-rhenium (3 : 1) at an angle of 26° and a rate of 10 Å/min. Evaporation of tungsten-rhenium was performed using an electron gun. The thickness of the metal layer measured by a quartz oscillator was 10 Å.

Specimens were examined with a JEM-100C electron microscope (JEOL, Japan) at 80 kV, 25 μm objective aperture and magnification of 80000. An anticontamination liquid N_2 trap was used. Magnification was calibrated with a cross-grating replica (21 600 lines/mm; Balzers Union, Liechtenstein).

3. RESULTS

Fig.1 shows micrographs of the negatively stained pig IgG. In almost all images, the molecules are seen to have a Y-like shape. Three visible subunits can be easily classified as Fab and Fc subunits by the 2-fold axis of the molecule. A cursory examination shows that all images of the IgG molecules can be subdivided according to size and shape into two groups. This is clearly seen in the histogram presented in fig.2. The first group includes larger molecules (type 1), the second comprising smaller species (type 2). Separate images of molecules of the first type are shown in fig.1b. These are large Y-shaped molecules, each consisting of two equal, symmetrically located subunits conventionally identified as Fab, and the third as Fc. The Fab subunits can be approximated by a spheroid of 50 Å diameter. At a certain orientation the Fab subunit is seen to consist of two parts subdivided by a groove or a cleft. The Fc subunit is usually pear-shaped with a length of about 80 Å and a maximum width of 60 Å. The Fc subunit is also subdivided into two parts, which are clearly visible in almost all micrographs presented. The maximum length of the type 1 IgG molecules is 150 Å.

Separate images of molecules of the second type are shown in fig.1c. The images of the Fab subunits are the same as described above. Those of the Fc

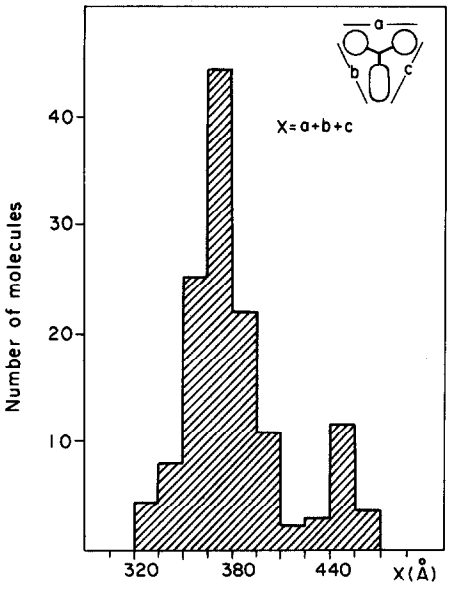

Fig.2. Size distribution of IgG molecules. The sum of dimensions of the molecule in three directions is used as a parameter (*X*).

subunits have a somewhat different appearance, resembling a sphere rather than a pear. This is clearly seen in the micrographs presented. The overall dimensions of type 2 IgG molecules do not exceed 120 Å. The histogram in fig.2 shows that the predominant images of the molecules in the IgG preparation are those of type 2 (about 80%).

We have assumed that type 1 images refer to those of the flat molecules adsorbed on the supporting carbon film (the long axes of their Fc subunits are parallel to the film). Type 2 images refer to those of the rigid 'convex' molecules (the long axes of their Fc subunits are not parallel to the film).

We have applied freeze-drying and high-resolution shadow casting [8] to verify this assumption. Shadowing allows us to estimate the dimensions and shape of the molecule in the direction perpendicular to the supporting carbon film using the shape and length of the shadow. The outlines of the shadow depend on the orientation of the molecule relative to the direction of shadowing. The differences between the flat and convex molecules are revealed best for an orientation such as that shown in the micrographs in fig.3b, c. The shadow of the flat molecule has an obtuse apex and

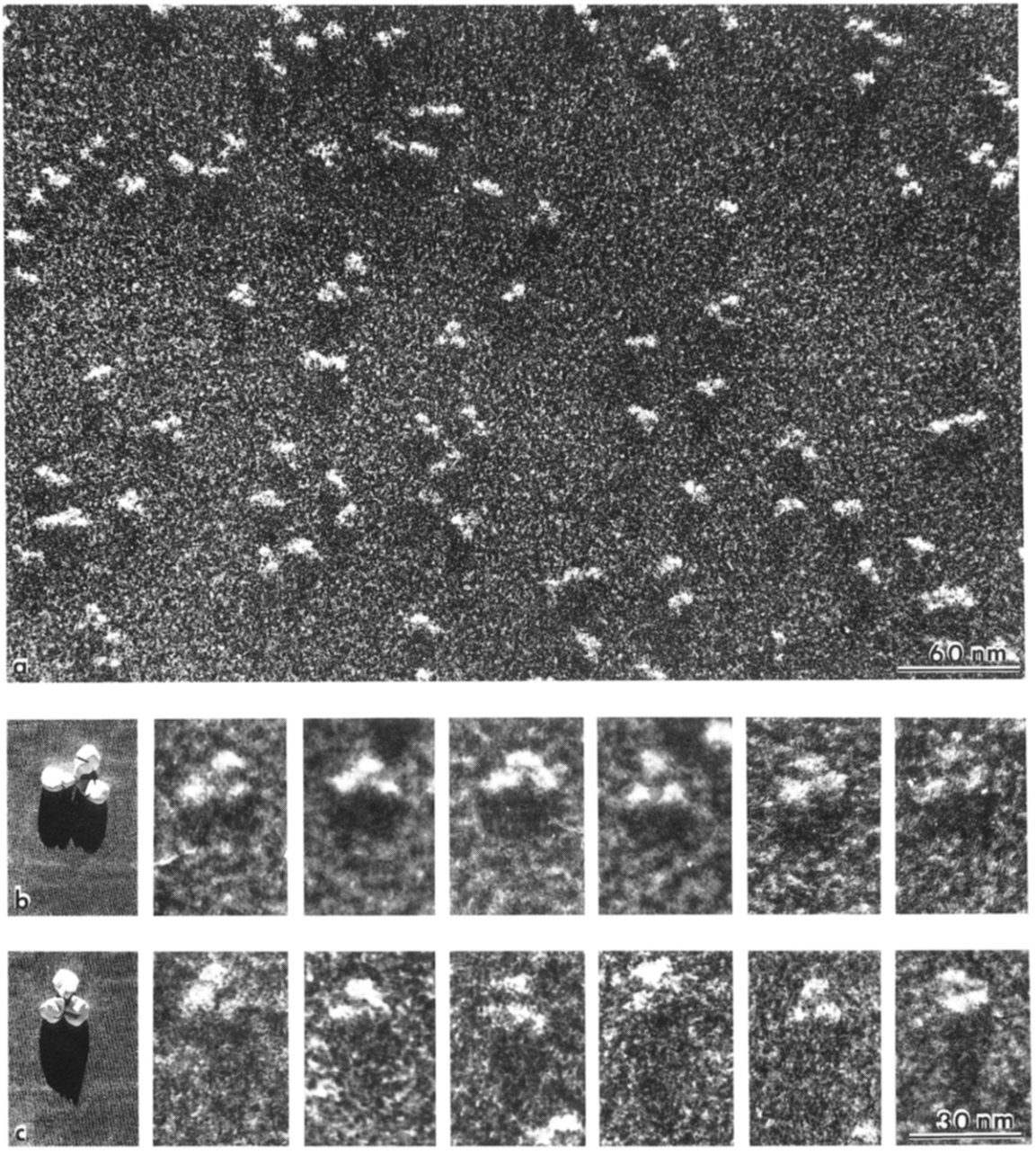

Fig.3. Freeze-dried and high-resolution shadowed intact non-precipitating antidinitrophenyl pig IgG. A field of preparations (a) and two types of images of IgG molecules: type 1 (b) and type 2 (c). For details see text.

its length coincides with the shadow length of an individual Fab subunit. The shadow of the convex molecule has an acute apex and its length exceeds the shadow length of the subunit. The histogram of the shadow length distribution of non-precipitating anti-dinitrophenyl pig IgG molecules is presented in fig.4. The ratio of the shadow length of the whole molecule to that of the individual Fab subunit (L/L_0) lying nearby is taken as a parameter for estimation of the molecule's height in-

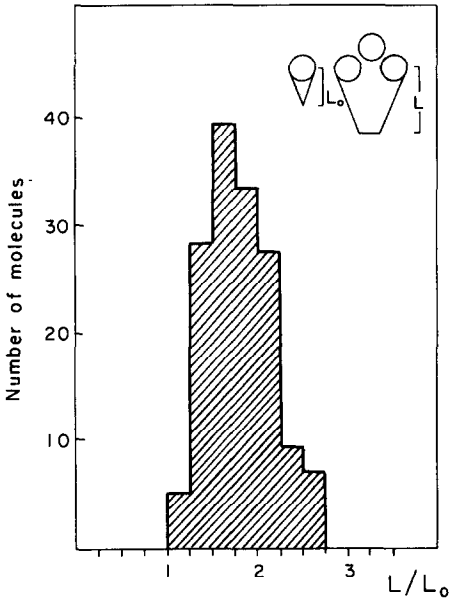

Fig.4. Height distribution of IgG molecules. The shadow length of the molecules (L) was measured for an orientation of their shadowing direction as shown schematically in the upper part of the figure. L_0, shadow length of the individual Fab subunit lying nearby, so that L/L_0 is not dependent on the angle of the shadowing.

dependently of the local inclination of the supporting film. The histogram shows that more than 80% of the molecules have a convex conformation where the Fc long axis is inclined to the supporting film and the Fab and Fc subunits are drawn together. We designate such molecules tripod-like. The histogram has a maximum at an L/L_0 ratio of 1.5.

4. DISCUSSION

A distinctive feature of anti-dinitrophenyl pig antibodies is their inability to precipitate polyvalent antigens. It is reasonable to assume that this property is due to structural features of non-precipitating antibodies.

Polarization of fluorescence [4] and spin label [7] studies have shown a substantially lower flexibility of pig IgG molecules compared to IgG of other animal species e.g. human and rabbit. Small-angle neutron scattering experiments [5] suggest that in a pig IgG molecule the Fab and Fc subunits are drawn together and the molecule has the shape of a 'shirt'. Our data do not contradict the conclusion on drawing together of the Fab and Fc in the molecule of pig IgG, but show that the molecule is convex, i.e. it has a tripod-like shape.

Such a conformation of the molecule is in good correspondence with its biological properties. In the rigid tripod-like molecule the Fc subunit seems to be fixed in such an orientation that its first complement factor binding center is exposed and accessible for interaction with C1q [10]. The fixation of the Fab subunits prevents the formation of precipitates which requires a certain flexibility of the molecule [1]. At the same time there remains the possibility of binding dinitrophenyllysine (association constant: $1.8 \times 10^7\ M^{-1}$).

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