NEUTRON SMALL-ANGLE SCATTERING STUDY ON TWO DIFFERENT PRECIPITIN TYPES OF PIG ANTI-DNP ANTIBODIES

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1. Introduction

Anti-Dnp antibodies elicited in pigs at an early phase of the immune response were previously shown to react with polyvalent high molecular weight Dnp-antigens in a different way than antibodies elicited at a later phase [1]. While the early antibodies form mostly insoluble complexes (i.e., precipitates) when interacting with heavily Dnp-substituted proteins, the late antibodies mostly form soluble complexes with the same antigens. It has been suggested that early and late antibodies differ in their shapes and/or the flexibilities of their F_{ab} arms [1]. Neutron small-angle scattering experiments were carried out to test this assumption. The present paper reports that the early and late antibody molecules do differ by their radii of gyration. Moreover, it is shown that the radii of gyration change upon complexing the antibodies with a low molecular weight hapten, the extent of change being approximately the same for both the early and late antibodies.

2. Material and methods

Early and late anti-Dnp antibodies (IgG type) were isolated from sera of immune pigs as described before [1]. Their association constants, K_a, for Dnp-haptens were found to be 6.7×10^5 M^{-1} and 1.8×10^7 M^{-1}, respectively. The hapten, 8-Dnp-5,8-aza-4-oxooctanoic acid was prepared by reacting *N*-Dnp-ethylene diamine (Calbiochem, Luzern, Switzerland) with succinic anhydride (Koch-Light, Colnbrook, England). The reactants were suspended in a mixture of sodium hydroxide, dimethylformamide and methanol. After 2 h, at ambient temperature, the suspension was taken to dryness in vacuo and washed with HCl. The final product was re-crystallized from hot 20% acetic acid and its purity was assessed by thin-layer chromatography, paper electrophoresis and UV spectra. For neutron scattering experiments, 2% (w/v) antibody solutions as well as 2% (w/v) antibody solutions with added hapten (molar ratio of hapten: antibody = 2.2 : 1) were prepared in a deuterium oxide (D_2O) buffer containing 0.1 M sodium phosphate of apparent pH 6.4. The scattering experiments were performed a at D 11 facility, Institut Max v. Laue-Paul Langevin, Grenoble [2]. The radii of gyration were computed from Guinier plots of scattering data as described in detail before [3].

3. Results and discussion

The results of scattering experiments, in a form of Guinier plots (i.e. logarithm of intensity scattered, I, versus square transferred momentum, $\mathcal{H}^2$) are summarized in fig.1. Radii of gyration (R_g) as calculated from these plots are given in table 1. Clearly, the radius of gyration of the early antibody is higher than

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Abbreviations: Dnp = 2,4-dinitrophenyl; F_{ab} = antigen binding fragment of antibody; R_g = radius of gyration; K_a = association constant.

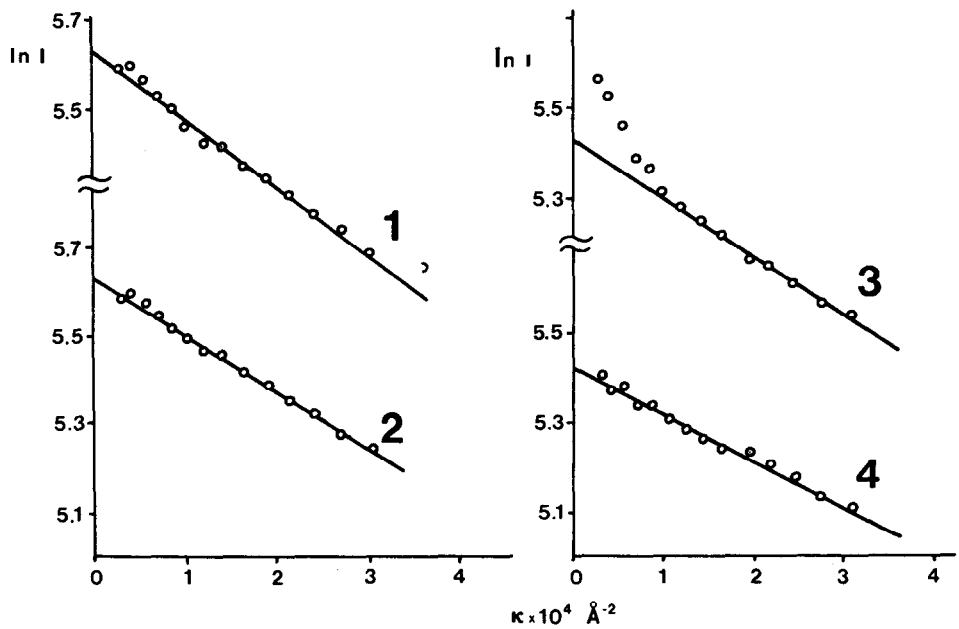

Fig.1. Guinier plots (logarithm of intensity scattered, I, versus square transferred momentum, $\varkappa^2$) of neutron small-angle scattering data. (1) Early anti-Dnp antibody; (2) late anti-Dnp antibody; (3) early anti-Dnp antibody with 8-Dnp-5,8-diaza-4-oxooctanoic acid; (4) late anti-Dnp antibody with 8-Dnp-5,8-diaza-4-oxooctanoic acid.

that of the late antibody. Upon complexing with 8-Dnp-5,8-diaza-4-oxooctanoic acid, the radii of gyration of both antibodies become smaller indicating a change toward a more compact conformation. According to the K_a values (see Material and methods), more than 90% of binding sites in both the antibodies are expected to be occupied by the hapten under the conditions used. A paucidispersity displayed by the Guinier plot of the early antibody–hapten complex (cf. a change of slope of the curve 3 in fig.1) can be ascribed to small amounts of aggregates.

X-ray small-angle scattering data of Pilz and co-workers [4,5] indicate a decrease in R_g of rabbit anti-poly(D-alanyl) and anti-azophenyl-lactoside antibodies after their complexing with tetra-D-alanyl-amide and *p*-azophenyl-β-lactoside haptens, respectively. The changes in R_g observed, namely 7.1% in the case of anti-polyalanyl antibodies (90% of binding sites occupied) and 2.5% in the case of anti-azophenyl-lactoside antibodies (60% of binding sites occupied) compare well with values obtained in the present study, that is, 4.6% for the early antibody and 7.5% for the late antibody. Taken together with results of crystallographic studies [6] both the X-ray and neutron small-angle scattering data suggest that a more compact antibody conformation may be a general feature of liganded antibodies.

Table 1
Radii of gyration derived from scattering curves[a]

Sample	R_g, nm
Early anti-Dnp antibody	6.74 ± 0.08
Late anti-Dnp antibody	6.16 ± 0.07
Early antibody with hapten	6.42 ± 0.09
Late antibody with hapten	5.69 ± 0.08

[a]Data given without correction for resolution

To account for the difference in radii of gyration between the early and the late antibodies, two alternative hypotheses can be raised. First, it can be contended that the late antibody exists in a more compact conformation than the early antibody and, consequently, its overall dimensions are smaller. Alternatively, segmental flexibility of the late antibody is presumed to be higher than that of the early antibody thus resulting in a lower average of the R_g

value. Further experiments employing contrast variation method may provide more detailed information, which could be exploited in deciding between these two alternatives.

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