THE DETECTION OF A CONFORMATIONAL CHANGE IN THE ANTIBODY MOLECULE UPON INTERACTION WITH HAPTEN

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SUMMARY

The technique of differential sedimentation is used to detect any change in sedimentation coefficient and in frictional coefficient of the γ-G immunoglobulin molecule upon interaction with hapten. Interaction of anti-lactoside antibody with the univalent haptens, lactose and p-(p-dimethylaminobenzeneazo) phenyl β-lactoside, results in a slight increase in sedimentation coefficient of the antibody molecule, which corresponds to a decrease in frictional coefficient. The interpretation is that the antibody molecule assumes a more compact configuration upon interaction with univalent hapten.

The overall structure of the 7S immunoglobulin G molecule is a problem which still eludes biochemists. Most of the hydrodynamic data seems to support an elongated particle having dimensions of roughly 250A° by 40A°. Noelken *et al.*[1] have argued that the data also are compatible with a more compact, Y-shaped structure. The shape of the antibody molecule has also been studied by electron microscopy. In 1965, Feinstein and Rowe[2] reported that γ-G immunoglobulin, when it was not combined with antigen, was only slightly asymmetric with a maximum dimension of 105A°. Feinstein and Rowe also examined antigen-antibody complexes with the electron microscope. These studies led them to hypothesize that when antibody molecules combine with antigen they "click open," exposing a critical disulfide bond and perhaps the complement-binding site.

Valentine and Green[3] have made a most significant contribution to the understanding of the shape of the antibody molecule with their electron micrographs. They have been able to photograph regular complexes, with each antibody molecule in a Y-shaped configuration, when studying a bivalent hapten-antibody mixture.

Based on the results of these two electron microscopic studies, we hypothesized a simple scheme for the interaction of monovalent hapten and antibody (see Figure 1). The molecule, before interaction with hapten, is assumed to have the legs of the Y closed and upon interaction with hapten to assume the open configuration. If such a conformational change were to occur, one would expect an increase in frictional coefficient of the molecule (C. Warner, unpublished calculations). This would, in turn, be reflected by a decrease in the sedimentation coefficient of the molecule.

It is the purpose of this communication to report the change in frictional coefficient and in sedimentation coefficient of the antibody molecule upon interaction with hapten.

THE INTERACTION OF ANTIBODY WITH UNIVALENT HAPTEN

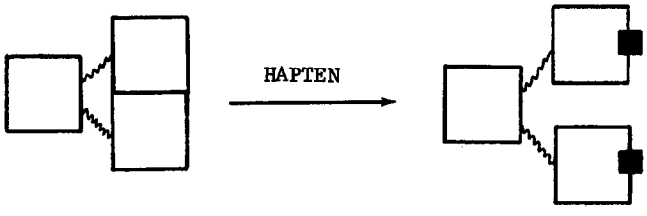

FIGURE 1 LEGEND: The antibody molecule is shown schematically to be Y shaped, with three globular regions. The hapten molecules are depicted by the solid squares. The "click open" hypothesis illustrated in this figure is inconsistent with the data presented in this communication.

MATERIALS AND METHODS

The antibody system used for the experiments was anti-lactoside antibody (Lac Ab) from rabbits. Two univalent haptens were used: lactose and p-(p-dimethylaminobenzeneazo)-phenyl β-lactoside (Lac dye)[4]. The differential sedimentation technique developed by Schumaker and Adams[5] was used to detect small changes in sedimentation coefficient of the antibody molecule compared with the reference protein, a non-specific γ-G immunoglobulin in the same solvent, with or without hapten. All runs were performed in a Beckman-Spinco Model E ultracentrifuge at 20°, as described by Schumaker and Adams[5].

RESULTS AND DISCUSSION

The basic experiment was the measurement of the sedimentation coefficient difference of the antibody molecule before and after treatment with hapten. The results are shown in Table 1. All errors in sedimentation coefficient

TABLE 1

Experiment	Description	Compared to	$\delta S^{\circ}_{20,w}$ [a] (svedbergs)	($\delta f/f \times 100$)
1	Lac Ab plus 10^{-3}M lactose	Lac Ab	$+0.05_6$	-0.43%
2	Lac Ab plus 10^{-4}M Lac dye	Lac Ab	$+0.18_0$	-2.05%

[a] The plus sign indicates that the molecule sedimented faster than the Lac Ab to which it is compared.

differences, $\delta S^{\circ}_{20,w}$, were less than ± 0.016 S. The percent change in frictional coefficient, $\delta f/f \times 100$, corresponding to the observed change in sedimentation coefficient, was calculated by the method of Schumaker[6] taking into account the increase in molecular weight of the antibody molecule due to the binding of hapten. The concentration of hapten was chosen in each experiment to ensure complete saturation of all available binding sites. It is interesting to note that the Lac Ab had a sedimentation coefficient which was approximately 0.3 S less than the sedimentation coefficient of the γ-G immunoglobulin molecule, obtained from Pentex, Inc., and used as the reference solution in all runs.

The results show a small increase in sedimentation coefficient of the Lac Ab molecule when it interacts with hapten. The Lac dye binds more strongly than lactose[4] and also produces a greater change in sedimentation coefficient of the molecule. The observed increases in sedimentation coefficient correspond to a decrease in frictional coefficient of the molecule.

Thus, the interaction of antibody and hapten cannot be described by the

simple model which we proposed. It is possible that the normal configuration of the molecule is an open Y and that hapten binding results in a slight closing of the molecule due to increased hydrophobic interactions from the hydrophobic portion of the Lac dye hapten. Perhaps, Feinstein and Rowe's "click open" hypothesis only applies to large, protein antigens and not to small univalent haptens. It seems that the interaction of Lac Ab with univalent hapten induces the molecule in solution to assume a more compact configuration than before.

REFERENCES

1. Noelken, M. E., Nelson, C. A., Buckley, C. E. and Tanford, C., J. Biol. Chem. 240, 218 (1965).

2. Feinstein, A. and Rowe, A. J., Nature 205, 147 (1965).

3. Valentine, R. C. and Green, N. M., J. Mol. Biol. 27, 615 (1967).

4. Karush, F., J. Am. Chem. Soc. 79, 3380 (1957).

5. Schumaker, V. and Adams, P., Biochemistry 7, 3422 (1968).

6. Schumaker, V., Biochemistry 7, 3427 (1968).