HIGH AFFINITY ANTI-CARBOHYDRATE ANTIBODIES IDENTIFIED IN ANTI-A-VARIANT STREPTOCOCCAL ANTISERA

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1. Introduction

Studies of the interactions of anti-polysaccharide antibodies with carbohydrate moieties have led to important contributions to the understanding of the size and fine specificity of combining sites (e.g. [1–3]), maturation of the immune response, and structural changes accompanying hapten and antigen binding. Where quantitative data are available, an interesting generalization is apparent. The intrinsic affinities of antibody combining sites for fully complementary polysaccharide units lie in the range 10^5–10^6 M^{-1} [2,4,8]. In the course of screening rabbit anti-streptococcal group A-variant carbohydrate antibodies for antigen binding detectable by antibody fluorescence changes, several preparations were found to contain anti-polysaccharide antibodies of higher than average affinity ($K_a \sim 10^9$ M^{-1}). Of specific interest also, was the coexistence of antibodies of lower affinity (K_a 10^5–10^6 M^{-1}). These observations are interesting in terms of the nature of immune responses and the nature of protein–polysaccharide interactions. Additionally, the demonstration of a very strict correlation between the antigen binding affinities of antibodies from different sera and a number of immunochemical properties, has fostered attempts to elucidate the reasons for this behavior. We document here the existence of these high-affinity antibodies.

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2. Materials and methods

The preparation and isolation of the various antibody fractions has been detailed [11].

Antibody purified by isoelectric focusing was contaminated by a fluorescent impurity derived from the ampholines which was removed by treating antibody solutions with washed, activated charcoal and by extensive dialysis against a suspension of charcoal in 0.2 M sodium phosphate buffer, pH 6.0. The antibody solutions were then dialyzed against 0.2 M sodium phosphate buffer, pH 7.4. Before titrating, samples were centrifuged at 40 000 × g for 15 min. The group A-variant polysaccharide was dissolved in 0.2 M sodium phosphate buffer, pH 7.4.

Fluorescence titrations were performed on a Schoeffel fluorimeter equipped with a 1000 W Xe lamp and double monochromators in the exciting beam. The fluorescence of antibody solutions (0.1–0.5 μM) was digitalized and averaged to improve the signal-to-noise ratio. Individual measurements taken in quadruplicate had a reproducibility of 0.1–0.3%. The quenching data were evaluated by means of a Scatchard plot [12]. The maximal fluorescence quenching was estimated from double reciprocal plots of fluorescence changes versus antigen concentration. Sufficient antigen was added to ensure that 80–90% binding sites were saturated. In the calculations 2 antigen binding sites/antibody molecule were assumed and mol. wt 5000 for the antigen was employed (W. S., D. G. R., unpublished).

F_{ab} fragments were isolated from a papain digest of reduced and alkylated antibody [13].

3. Results

The binding of the streptococcal group A-variant polysaccharide antigen to rabbit antibody K151-748 was accompanied by a quenching of antibody fluorescence. This spectroscopic signal permitted the binding of antigen to be quantitated by means of a Scatchard plot (fig.1). When unfractionated antibody was titrated, the binding curve was biphasic disclosing the presence of a high affinity component ($K_a = 4.3 \times 10^8\ M^{-1}$) and a low affinity component ($K_a = 2 \times 10^5\ M^{-1}$). This biphasic behavior in the binding curve was due to the presence of two distinct antibody populations of different affinity. They were separated from one another by means of affinity chromatography. In the case of a protein which is bivalent, a high overall affinity constant can be ascribable to the binding of the two antibody combining sites to the same antigen molecules (monogamous bivalence) or the presence of sites of intrinsically high affinities which bind monovalently to the antigen. The hot formamide extracted group A-variant polysaccharide is a L-rhamnose homopolymer [14] est. 15–20 nm length (W. S., D. G. B., unpublished), while the distance between the combining sites in the F_{ab} segments has been estimated to lie between 10–20 nm by a variety of physical measurements (see [15–18]) being of course, dependent on the angle between the F_{ab} arms. To discriminate between the above possibilities the binding of intact antibody and its papain-produced F_{ab} fragments to antigen were compared for a number of high affinity antibody fractions freed of low affinity antibody by affinity chromatography [11]. The results summarized in table 1 clearly demonstrate that the observed binding constants of the high-affinity antibodies represent intrinsic binding constants of the antibody combining sites and consequently that combining sites of a single antibody molecule were unable to bind to the same antigen molecule. At the low concentrations of antigen and antibody employed in these measurements the antibody did not precipitate although large soluble antigen–antibody complexes were formed and could be detected by analytical centrifugation ($s_{20,w} \leq 30$ S, data not shown).

Table 1
Binding of streptococcal group A-variant carbohydrate to intact antibodies and their F_{ab} fragments

Serum	K_a (M^{-1})	
	IgG	F_{ab}
K151-748[a]	4.3×10^8	5.4×10^8
K116-700[b]	2.9×10^8	6.7×10^8
K 81-543[c]	1.02×10^9	6.6×10^8

[a] Homogeneous antibody isolated by preparative isoelectric focusing
[b] High-affinity component eluted from affinity column at pH 3
[c] High-affinity component of mixture of high and low affinity antibody mixture

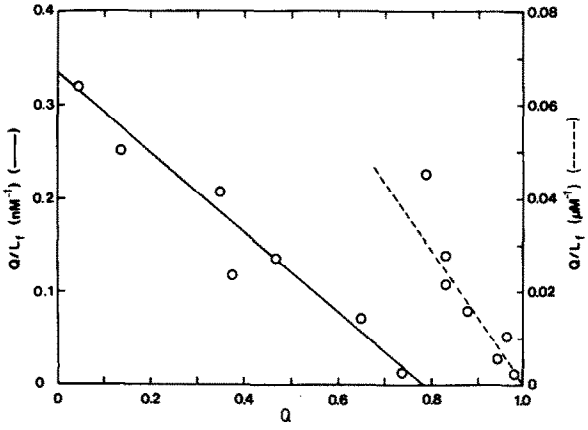

Fig.1. Fluorescence titration of rabbit anti-streptococcal group A-variant polysaccharide antibody 151-748. The binding of the group A variant polysaccharide $(-rh-rh-)_{16}$ to this antibody preparation was accompanied by a quenching of antibody tryptophan fluorescence (excitation and emission wavelengths 280 nm and 332 nm, respectively). The antigen concentration dependence of this fluorescent quenching was biphasic, indicative of the presence of antibodies of radically different affinities. Antibody was titrated to a first endpoint of 41 nM antigen and then to an endpoint of 89 mM antigen using different standard solutions of antigen. Analysis of this data according to the Scatchard method yielded the biphasic binding isotherm plotted as Q/L_f vs Q, where Q is the fraction of total quenching and L_f is the concentration of free antigen. Because of the great difference in the dissocation constants of these two components, the scale for the data on the right has been expanded to show the details of the binding to the lower affinity antibody. The dissociation constants for (o———o) high- and (o– – –o) low-affinity antibodies are 2.3×10^{-9} M and 5×10^{-6} M, respectively.

The small size of the antigen (mol. wt 5000) and the low concentrations of antigen and antibody led to conditions where the kinetics of the dissociation of extremely large complexes was more rapid than the growth of these complexes to insoluble species. Similar behavior was observed in the interaction of anti-SIII pneumococcal polysaccharide antibodies and SIII oligosaccharides of intermediate molecular weight (3000–5000) ([7]; J. K. W., unpublished data).

4. Discussion

We have detected antibodies of high intrinsic affinity ($K_a \sim 10^9$) in various rabbit anti-streptococcal group A-variant polysaccharide antisera. Since monovalent F_{ab} fragments exhibited likewise a high affinity for antigen, the measured values represent the intrinsic affinities of the combining sites for antigen. The different strategies on the molecular level leading to strong binding between polysaccharide antigens and their corresponding antibodies are instructive to compare. The solution to this problem in the present case is simply to design combining sites of high intrinsic affinity. An alternative solution requires that an antibody binds twice to the same antigen molecule. The inability of an antibody to enter into the latter type of binding interaction may be occasioned by the structure of the antigen. This can be illustrated by a specific example. The anti-SIII pneumococcal polysaccharide antibody 45-394 possesses binding sites of intrinsic affinity $0.9–3.0 \times 10^5\ M^{-1}$ [8] with a dissociation rate constant of $11–12\ s^{-1}$. From the stability of its soluble antigen–antibody complexes, a dissociation rate constant for the complexes of $\sim 10^{-1}\ s^{-1}$ can be estimated [9] yielding an estimate of the overall association constant for antibody in the complex of $10^{10}\ M^{-1}$. The SIII polysaccharide has mol. wt 350 000 and a radius of gyration of 46 nm (H. Fuchs, personal communication). The size of the antigen certainly permits the binding of both antigen combining sites of a single molecule to the same antigen molecule resulting nearly in a doubling of the free energy of binding. The A-variant polysaccharide is estimated to possess a maximum extended length of 20 nm and is apparently too small to permit bivalent binding of antigen to a single antigen molecule. The distinctive immunochemical properties of the high-affinity antibodies studied here, in contrast to those of the coexisting low-affinity, are the subject of a subsequent publication.

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