Carbohydrate Research, 72 (1979) 315–319

Note

The affinity of a linear, α-D-(1→6)-linked D-glucopyranan (dextran) for homogeneous immunoglobulin A W3129

LARRY G. BENNETT AND CORNELIS P. J. GLAUDEMANS

National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014 (U.S.A.)

(Received September 11th, 1978; accepted for publication, September 23rd, 1978)

Bivalent immunoglobulins bind ligands in their combining sites, each one of which can vary from 1.4 to 3.0 nm in length[1,2]. Polysaccharide antigens bearing multiple, identical determinants generally bind many immunoglobulin molecules per single polysaccharide chain, each determinant of ~2.0 nm being potentially capable of association with an immunoglobulin combining-site. Indeed, the resulting crosslinking of polysaccharides by immunoglobulins, leading to precipitation of the complex, has been extensively investigated[3]. Thus, it is unusual for serum immunoglobulins to bind to a polymeric, multideterminant antigen while not precipitating with it. Cisar *et al.*[4] observed that immunoglobulin W3129 maximally binds to isomaltopentaose, but that the immunoglobulin does not precipitate with a synthetic, linear α-D-(1→6)-linked dextran of molecular weight 36,500 (degree of polymerization 255), whereas it does precipitate with more highly branched (1→6)-α-D-dextrans. The results were interpreted by the authors to mean that protein W3129 binds *only* to the nonreducing end of the dextran, and not to any intercatenary segments of five D-glucopyranosyl residues.

We have now investigated this problem by using a different approach. Our results are equally compatible with either of two possibilities: (*a*) IgA W3129 binds strongly to the nonreducing-terminal D-glucopentaose segments of the (1→6)-α-D-dextran, or (*b*) the IgA binds *weakly* to intercatenary segments of D-glucopentaose in the (1→6)-α-D-dextran. Combined with the precipitin data previously obtained[4], our results fully confirm the conclusions of Cisar *et al.*[4].

When any (bivalent) immunoglobulin is cleaved to yield the monovalent Fab′ fragment[5], it can no longer precipitate, even with antigens bearing multiple determinants, as it lacks the ability to crosslink these antigens. It has been shown that Fab′ fragments show with their ligands binding constants that are identical to those of the intact immunoglobulin and that ligand[6]. Thus, using the method of ligand-induced, altered fluorescence[7], it becomes meaningful to study immunoglobulin binding to *whole* antigens by measuring the affinity of whole antigens with Fab′ fragments[8].

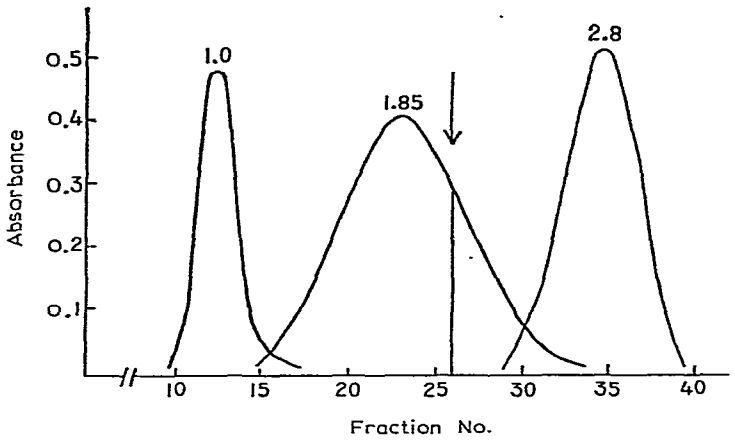

Fig. 1. Elution of synthetic (1→6)-α-D-dextran from a column (85 × 1.5 cm) of Sephadex G-200. [Standards chromatographed separately were Blue Dextran (1.0 void volume) and D-glucose (2.8 void volumes).]

By use of this technique, we have measured the affinity constant (K_a) for the association between protein W3129 Fab′ and a linear, α-D-(1→6)-linked dextran of molecular weight[9] ~36,000. The dextran was first purified by chromatography on Sephadex (see Fig. 1), and the central (high molecular weight) part of the elution peak (fractions 16–26) was taken, in order to avoid any possibility of contamination with oligosaccharides, as it had already been shown that the immunoglobulin is capable of maximally binding a pentasaccharide fragment[4].

We wished to determine whether or not a (nonreducing) terminal D-glucosyl group is a requirement for binding of W3129 to a (1→6)-α-D-dextran. If such is the case, then *equimolar* amounts of dextran of high molecular weight (M.W. 36,000; 500 μM = 18 mg/mL) and pentasaccharide necessary to saturate the available combining-sites of immunoglobulin W3129 (pentasaccharide M.W. 828; 500 μM = 0.41 mg/mL) have equal numbers of nonterminal D-glucosyl residues per unit volume, and therefore should exhibit the same, concentration-dependent, site saturation of Ig W3129 despite the fact that the dextran solution contains 44 times as much D-glucose as an equal volume of the pentasaccharide solution.

TABLE I

EQUILIBRIUM-BINDING CONSTANTS BETWEEN W3129 Fab′ AND SPECIFIC LIGANDS

Ligand	K_a			
	FT[a]	*FT*[a,b]	*ED*[a,b]	*PI*[a,b]
Methyl α-D-glucopyranoside	1.8×10^3	1.4×10^3	9.4×10^2	8.0×10^2
Isomaltopentaose	1.65×10^5	1.7×10^5	1.9×10^5	1.7×10^5
Dextran (mol. wt. ~36,000)	2.0×10^5			

[a]Abbreviations: FT, fluorescence titration; ED, equilibrium dialysis; and PI, precipitation inhibition. Equilibrium-binding constants have the units M^{-1}. [b]Data from Cisar *et al.*[4] for intact immunoglobulin W3129.

The equilibrium constants (K_a) in Table I were calculated by using a molecular weight of 36,000 for the dextran and of 828 for the pentaose, yielding almost identical K_a values. This shows that the concentration of available ligand *is* the same for the solution containing 0.41 mg of pentasaccharide per mL and the dextran solution containing 18 mg of polysaccharide per mL.

Another way of stating this problem would be as follows: if the protein could bind pentaose segments all along the polysaccharide chain, each pentasaccharide repeating-unit would become a potential ligand. However, as each such segment is ~3.0 nm long and the antibody itself has a dimension of ~4.0 nm across its binding face, the protein could never bind to each sequential pentasaccharide. Separate molecules of immunoglobulin could, however, interact *a priori* with every other sequential-pentasaccharide arrangement, and this has, in fact, been experimentally shown to be possible[8]. Therefore, if protein W3129 can bind to any penta-[(1→6)-α-D-glucopyranose] sequence in the dextran, including intercatenary segments, a titration of the dextran with Ig would yield the known K_a value for the pentasaccharide[4] only if the concentration of the dextran was defined as the concentration (C) of *available pentaglucose segments*:

$$C = \frac{\text{wt. of dextran. L}^{-1}}{\text{mol.wt. of } n \text{ pentasaccharide fragments}}$$

where n is $\geqslant 2$. For $n = 2$, the determinant would become 10 D-glucosyl residues, ~6.0 nm long, sufficient to allow a 4.0-nm wide, Ig combining-site to interact with such sequential determinants. Based on $n = 2$, the concentration of dextran used in the calculation of K_a would become 11mM, and the K_a for the dextran would become 9×10^3 M^{-1}, considerably less for the whole antigen than for the pentaose fragments (1.7×10^5). If however, protein 3129 binds to the nonreducing end of *each* polysaccharide molecule *only*, the concentration of the dextran has to be defined as the true molar concentration of the *polymer*, namely, c = wt. of dextran. L^{-1}/mol.wt. of dextran, and use of this concentration in the calculation of the K_a value will then yield the correct value (2.0×10^5).

Thus, it appears that the chain of α-D-(1→6)-linked glucosyl residues beyond the 5 units at the nonreducing end of the dextran provides few or no additional binding sites for Ig W3129 Fab', and thus, that this protein is only capable of binding terminal D-glucosyl groups. This confirms the data[4] of Cisar *et al.* In addition, it may be seen from Table I that the results from the method of ligand-induced, fluorescent titration agree well with those from other methods of determination of K_a, and we have also reconfirmed that Fab' fragments bind to their ligands with the same affinity as that of the intact immunoglobulin.

EXPERIMENTAL

Mouse-myeloma, IgA immunoglobulin W3129 was obtained from Litton Bionetics. The protein was purified from ascites fluid by affinity chromatography on

Sephadex G-75, and eluted from the column with 0.2M NH_4HCO_3–5M guanidine. The antibody was dialyzed against 0.15M Tris buffer, pH 8.0, containing 0.15M NaCl and 2mM EDTA to remove guanidine and to equilibrate it with the appropriate buffer in which to reduce and alkylate in order to break up immunoglobulin polymers. Following reduction and alkylation[10], the protein was digested with pepsin to obtain monomeric, Fab′ antibody fragments. The course of enzymic hydrolysis was monitored by removing aliquots periodically and determining the relative proportions of intact immunoglobulin and Fab′ by molecular-sieve, high-pressure liquid chromatography on μBondagel E-300 (Waters Associates). The digest was applied to a column of Sephadex G-200, and the Fab′ was eluted from the affinity column with 0.1M methyl α-D-glucopyranoside. The Fab′ was dialyzed against phosphate-buffered saline (PBS, pH 7.4), and the final concentration of Fab′ in PBS was adjusted to A_{280} 0.07.

Three ligands were assayed for their binding affinity for W3129 Fab′, namely, methyl α-D-glucopyranoside, isomaltopentaose [*i.e.*, (1→6)-(α-D-glucopyranosyl)$_4$-D-glucopyranose], and poly[(1→6)-α-D-glucopyranosyl]-D-glucopyranose (dextran, molecular weight ~36,000). Isomaltopentaose was obtained by mild hydrolysis of dextran with acid, followed by chromatography on a column (120 × 1.2 cm) of Bio-Gel P-2 (minus 400 mesh), fractions being monitored for their carbohydrate content by the phenol–sulfuric acid assay[11]. Mono-, di-, tri-, tetra-, and pentasaccharides were separated from one another, essentially free from contamination by other oligosaccharides. The poly[(1→6)-α-D-glucopyranosyl]-D-glucopyranose of mol. wt. ~36,000 was prepared synthetically, and was generously donated for this study by Dr. C. Schuerch[9]. The dextran was subjected to chromatography on a column (85 × 1.5 cm) of Sephadex G-200 (see Fig. 1). Fractions 16–26 were used, in order to ensure that a small amount of tailing material, possibly consisting of oligosaccharides of low molecular weight, was excluded from the sample. These fractions were combined, and concentrated by ultrafiltration, using an Amicon UM2 membrane.

Fluorescence titrations were performed according to the general procedure described[7], in PBS (pH 7.4) at 25°; the excitation wavelength was 295 nm, and emission was monitored at 340 nm.

Equilibrium-binding constants of the three ligands for W3129 Fab′ are summarized in Table I, together with binding constants reported in an earlier study.

ACKNOWLEDGEMENT

We are grateful to Dr. C. Schuerch for a gift of synthetic dextran prepared under a grant (GM 06168) from the Division of General Medical Sciences, NIH.

REFERENCES

1 E. A. KABAT, *J. Cell. Comp. Physiol.*, 50 [Suppl. 1] (1957) 79–102.
2 D. G. STREEFKERK AND C. P. J. GLAUDEMANS, *Biochemistry*, 16 (1977) 3760–3765.

3 M. HEIDELBERGER, *Bacteriol. Rev.*, 3 (1949) 49–95.
4 J. CISAR, E. A. KABAT, M. M. DORNER, AND J. LIAO, *J. Exp. Med.*, 142 (1975) 435–459.
5 L. A. STEINER AND R. R. PORTER, *Biochemistry*, 6 (1967) 3957–3970.
6 M. E. JOLLEY, S. RUDIKOFF, M. POTTER, AND C. P. J. GLAUDEMANS, *Biochemistry*, 12 (1973) 3039–3044.
7 M. E. JOLLEY AND C. P. J. GLAUDEMANS, *Carbohydr. Res.*, 33 (1974) 377–382.
8 C. P. J. GLAUDEMANS, B. N. MANJULA, L. G. BENNETT, AND C. T. BISHOP, *Immunochemistry*, 14 (1977) 675–679.
9 E. R. RUCKEL AND C. SCHUERCH, *Biopolymers*, 5 (1967) 515–523.
10 D. INBAR, M. ROTMAN, AND D. GIVOL, *J. Biol. Chem.*, 246 (1971) 6272–6276.
11 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350–356.