Journal of Supramolecular Structure 8:79–88 (1978)
Cell Surface Carbohydrates and Biological Recognition 515–524

Dimensions and Specificities of Recognition Sites on Lectins and Antibodies

Elvin A. Kabat

Departments of Microbiology, Human Genetics and Development, and Neurology, College of Physicians and Surgeons, Columbia University, The Neurological Institute, Presbyterian Hospital, New York, and the National Cancer Institute, National Institutes of Health, Besthesda, Maryland 20014

A comparison is made of the specific combining sites of a number of lectins and of antibodies with emphasis on those reacting with blood group A, B, and H determinants. The ranges of site sizes and specificities of both groups are similar both from immunochemical studies and from the limited x-ray diffraction data available.

Key words: plant hemagglutinins, carbohydrate binding site

INTRODUCTION

During the past 10 years there has been an extraordinary burst of activity in the study of plant and animal lectins stimulated largely by the findings that they have specific receptor sites for carbohydrate (1–6) and react with glycoproteins in solution or on cell membranes, are involved in the removal of asialoglycoproteins from the circulation (7), and may hold specific nitrogen fixing bacteria in root nodules (8, 9). In many instances they are mitogenic producing blast transformation (2–6) and also react to cause movement of receptors in cell membranes producing patching and capping (2–6).

At the same time there has been extensive interest in antibodies and immunoglobulins and especially in myeloma immunoglobulins for many of these are homogeneous and have specific receptor sites (10, 11, cf.6) so that they may be sequenced and, if crystallized, may be studied by x-ray diffraction. In addition, some antibodies have been obtained in a relatively homogeneous form suitable for sequence and x-ray diffraction studies (12–18, cf.6). To date only very few myeloma antibodies and one lectin but a fair number of enzymes have been studied.

Immunochemical investigations, most frequently by quantitative precipitin and quantitative hapten inhibition assays along the lines developed by the Heidelberger School (19, 6) but more recently by radio (20, 21) and enzyme (22) immunoassay, have made it possible to explore the nature of the specific combining sites of antibodies, myeloma

Received March 4, 1977; accepted August 1, 1977.

0091-7419/78/0801-0079$02.00

globulins, and lectins and to obtain estimates of their dimensions, specificities, and shapes. In the few instances in which x-ray diffraction studies (12–18) are available, these have been in general agreement and the overall range of site sizes of enzyme sites studied crystallographically and of antibody combining sites from immunochemical estimates has been similar (6). For instance the lysozyme site has been shown to accommodate a hexasaccharide composed of alternating N-acetylglucosamine and N-acetylmuramic acid residues (23) while the upper limit for the antidextran site has been shown to be a chain of 6 (34 Å in most extended form) or 7 α1→6 linked D-glucose residues (6). The lysozyme site is a cleft at the surface of the molecule and combining sites of myeloma antidextrans have been shown to be either grooves or cavities in shape (24); human antidextrans although generally restricted in specificity have been shown to be mixtures with both kinds of sites (24).

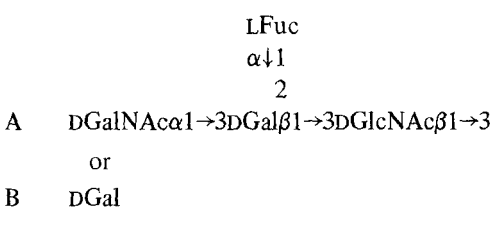
The lower limit for an antibody combining site has been estimated to be between 1 and 2 glucoses (about 6 Å) (25, 6) and the only lectin studied thus far by high resolution x-ray diffraction, Concanavalin A (Con A), has a carbohydrate binding site comparable in size as determined by quantitative inhibition assays (26, 27), nuclear magnetic resonance (28–30), and x-ray diffraction (31–33). This range of dimensions allowing for shape and conformational differences may well include receptor sites on all antibodies and lectins and a large number of enzymes. Con A also has a hydrophobic pocket located in a cleft between the 2 subunits distinct from the carbohydrate binding site and about 25 Å from it. The combining sites of Bence Jones dimers and Fab fragments studied directly by x-ray diffraction (12–18) fall into the same range of site sizes. Many lectins unlike antibodies are metalloproteins and the metal is necessary for binding activity (1–6).

These 3 classes of substances also show other similarities. Lectins (1–5) and enzymes (34) may exist in multiple forms and antibodies (6) may be mixtures of molecules with combining sites of different sizes even to a single antigenic determinant (6, 19). The former 2 classes are generally paucidisperse being limited to 4 or 5 species while antibodies may be extremely heterogeneous populations but in some instances may show also paucidispersity or even homogeneity (6).

Since specific receptor sites on antibodies and on lectins are directed toward carbohydrate, we are dealing largely with sequential rather than conformational determinants of protein antigens and it becomes possible to explore their nature by using a variety of oligosaccharides to inhibit the precipitin reaction between the glycoprotein or polysaccharide antigens and the antibody or lectin. The site which inhibits at the lowest concentration is considered to be most complementary to the oligosaccharide (19, 6).

A number of lectins, notably from Lens culinaris (lentil) and Robinia pseudoacacia have combining sites of specificity similar to Con A reacting best with mannose but also with fructose, glucose, and DGlcNAc and especially well with glycopeptides of IgG (35–37).

The existence of a large number of lectins with combining sites specific for the blood group A, B, and H antigenic determinants makes possible some comparisons of these receptor sites with antibody combining sites both in size and specificity. The blood group A, B, and H determinants (38–43) have each been found to be of 4 kinds, 2 of which are:



LFuc
α↓1
2
A DGalNAcα1→3DGalβ1→4DGlcNacβ1→6
or
B DGal

The other 2 A and B determinants have a second LFuc linked to the DGlcNAc on carbons 4 and 3 respectively. The 4 H determinants have the same structures but lack nonreducing DGalNAc or DGal. Anti-A and anti-B with specificities complementary to each of these structures including the second LFuc and the DGlcNAc have been obtained. The oligosaccharides isolated from blood group substances, the substances themselves, and other structurally similar oligosaccharides and polysaccharides have made possible the gathering of substantial amounts of data on a considerable number of lectin sites.

It has been possible to purify these lectins readily using an insoluble adsorbent, polyleucyl hog A+H substance made by addition of long polyleucyl chains on to the lysines of the blood group A+H glycoproteins from hog gastric mucosa (44, 45). This insoluble adsorbent has blood group A and blood group H determinants as well as determinants with terminal nonreducing DGlcNAcα1→DGalβ1→4 and in addition smaller numbers of determinants from incompletely synthesized chains. It affords considerable versatility for purifying different lectins.

Two lectins which specifically agglutinate human A but not B or O erythrocytes have been shown to vary substantially in the sizes of their specific receptor sites and also in their ability to accommodate related sugars. The receptor site of the lectin of *Helix pomatia* (46–48) appears to be relatively small. Indeed methyl αDGalNAc is the best inhibitor found thus far. Oligosaccharides isolated from blood group A substance are all somewhat less active on a molar basis. Even more surprising in view of its strict specificity for A erythrocytes is the finding that methyl αDGlcNAc is quite a good inhibitor being about one tenth as active and that melibiose (DGalα1→6DGlc) and methyl αDGal also inhibit but are very much less active. The site is thus capable of accommodating portions of these other sugars but the fit is poor. The molecule has a molecular weight of about 79,000 and is composed of 6 subunits of $K^a = 5 \times 10^3$ liter/mole (47). In addition to A substances with which it reacts best, it precipitates with B,H,Le^a and precursor I blood group substances most probably due to the terminal nonreducing DGlcNAc residues and with R_a and R_b rough mutants of *Salmonella typhimurium* having terminal DGalα1→6DGlc determinants (48). Its ability to precipitate is a consequence of its multivalence and the multivalence of the antigen despite the low binding constant of each site. Indeed its high specificity for A erythrocytes is surprising since B erythrocytes have a terminal nonreducing DGalα1→3.

However, methyl αDGal was 1/250 as potent as methyl αDGalNAc in inhibiting precipitation of the lectin by R_a lipopolysaccharide (48). This might not be sufficient to agglutinate B erythrocytes. Since methyl αDGlcNAc is only 1/10 as active as methyl αDGalNAc, it may be that B and O erythrocytes do not have many terminal nonreducing α-linked DGlcNAc accessible at their surface or one might expect agglutination not to be blood group A specific.

By contrast, however, the lectin of *Dolichos biflorus* (49) shows more specificity for the A pentasaccharide,

LFucα1
↓
2
DGalNAcα1→3DGalβ1→4DGlcNAcβ1→6R,

being about 1/3 more active than methyl α-DGalNAc, DGalNAcα1→3DGal, and DGalNAcα1→3DGalβ1→3DGlcNAc, all of which were of equal potency. Thus the site is probably specific for DGalNAcα1→3DGal with the LFuc side chain stabilizing a more favorable conformation of the other 2 sugars or involves the DGalNAcα1→3DGal plus some hydrophobic contribution of the fucose to binding. Unlike the *Helix pomatia* site the Dolichos lectin does not react with methyl αDGlcNAc or methyl αDGal and precipitates only with blood group A_1 and A_2 substances and with streptococcal group C polysaccharide which also contains terminal nonreducing α-linked DGalNAc residues. Enzymic de-N-acetylation of the terminal nonreducing DGalNAc of blood group A substances destroys reactivity and re-N-acetylation restores it.

Both the snail and the Dolichos lectins have homogeneous sites as evidenced by the findings that different fractions eluted from the insoluble adsorbent by increasing concentrations of DGalNac all exhibit the same ratio of inhibition of different inhibitors to one another (45, 48); the snail lectin is homogeneous by equilibrium dialysis (46). Although the Dolichos lectin exists in two forms distinguishable by chromatography on Con A-Sepharose these have been shown by Carter and Etzler (50) not to differ in specificity.

Two lectins from the lima bean (51), *Phaseolus lunatus,* have been purified and show blood group A specificity, methyl αDGalNAc being more active than DGalNAc which is better than methyl αDGal. One of the lectins, component III, was more extensively studied, the o- and p-nitrophenyl αDGalNAc being as active as methyl αDGalNAc. Replacement of the N-acetamido group of methyl αDGalNAc by a p-amino or p-nitrobenzamido group gave increased activity; this is probably an indication of a hydrophobic region in the protein capable of reacting with the aromatic moiety on carbon 2.

The soybean agglutinin (52) does not show blood group specificity. It agglutinates all human erythrocytes and also agglutinates mouse, rat, and human cell lines transformed by viral or chemical carcinogens. The purified protein has a K^a of 3×10^4 liter/mole for DGalNAc, a molecular weight of 120,000, and is composed of 4 subunits. It is precipitable by blood group substances with terminal nonreducing α-linked DGalNAc or α- or β-linked DGal residues.

The soybean agglutinin site (52) differs from the others in that both α and β methyl or ethyl DGalNAc inhibit very well, the α being slightly better than the β but both are more potent than DGalNAc itself; the corresponding α- and β-glycosides of DGal are considerably less active but both are more active than DGal. DGalNAcα1→3DGal was identical in inhibiting power to methyl αDGalNAc while the trisaccharide DGalNAcα1→3DGalβ1→3DGlcNAc, the best oligosaccharide inhibitor, was about 2–3 times more potent suggesting that the β1→3-linked DGlcNAc moiety contributes to the binding site. An important finding with soybean lectin is that phenyl αDGalNAc was more potent even than the trisaccharide. This type of finding, namely that phenyl or nitrophenyl glycosides of sugars are often more potent inhibitors than the methyl compounds reported earlier for Con A (27) and *Sophora japonica* (53, 54) lectin, suggests that some of these lectins have a hydrophobic region which may react with the aglycone portion of these glycosides. This

may complicate determination of site size. It is of interest that with antibodies to tobacco mosaic virus protein, addition of an octanoyl group to the C terminus of the tripeptide or tetrapeptide making up a portion of the pentapeptide determinant also increased binding substantially (55). An understanding of this finding in terms of site structure would be of great interest. These hydrophobic-binding regions must be reasonably contiguous to the sugar-binding sites. With Con A the binding region contains 2 tyrosines at positions 12 and 100 which are considered to account for the increased activity of the p-nitrophenyl as compared with methyl α-D-mannoside (33).

The soybean agglutinin precipitated poorly with blood group B substances despite the presence of terminal nonreducing DGalα1→3DGal and this was attributable to steric effects of the fucose substituents on the subterminal DGal, the P1 fractions, obtained by mild acid hydrolysis which removes fucose, showing greatly increased activity. Blood group A substances reacted well and thus the reduced activity due to substitution of fucose is less with terminal nonreducing DGalNAc than with DGal.

The *Sophora japonica* lectin which agglutinates A and B cells also resembles soybean in that DGalNAc is a better inhibitor than DGal and methyl α- and β-glycosides of DGal are more active than DGal; methyl glycosides of DGalNAc were not studied. It differs in that β-glycosides are more potent than α-glycosides and in that it does not agglutinate transformed cells.

The anomalous finding that β-linked oligosaccharides of DGal were somewhat more active than α-linked oligosaccharides despite the B specificity was explained (54) when it was found that removal with coffee bean α-galactosidase of terminal αDGal from B substance and periodate oxidation and Smith degradation of B substance to expose I determinants left substantial precipitating capacity of these substances for the *Sophora japonica* lectin. Thus the intact blood group substances were reacting with the lectin by virtue of the terminal α-linked DGal B determinants as well as with βDGal determinants, probably on incomplete chains.

The peanut agglutinin (56), built of subunits of molecular weight 27,500 also agglutinates all human erythrocytes, binds to neuraminidase-treated human, rat, mouse, and guinea pig lymphocytes but stimulates DNA synthesis only in human and rat lymphocytes. It resembles the T agglutinin present normally in human serum which agglutinates neuraminidase-treated erythrocytes. It has a binding site most complementary to DGalβ1→3DGalNAc which is 14, 55, and 90 times as active as DGalβ1→4DGlcNAc, DGal, and DGalβ1→3DGlcNAc, respectively, and is 25 times more active than DGalβ1→3 N-acetyl D-galactosaminitol. Methyl αDGal and methyl βDGal are more active than DGal. The peanut agglutinin precipitates to different extents with various A, B, and H substances and reacts with determinants in the interior of the blood group substances most probably accessible because of incomplete biosynthesis. This is supported by the findings that reactivity is strikingly increased by one stage of periodate oxidation and Smith degradation of the blood group substances exposing DGalβ1→3DGalNAc and DGalβ1→4DGlcNAc determinants and also that fractions of blood group substances more soluble in ethanol precipitated to a greater extent. The best precipitant of peanut agglutinin is antifreeze glycoprotein containing repeating units of DGalβ1→3DGalNAcα1 →O-Thr-Ala-Ala (57). The peanut agglutinin resembles certain human anti-I and anti-i sera of certain groups (58) in many respects and further studies on this relationship are needed.

Ricinus communis seeds contain 2 proteins (59) RCA_I and RCA_{II}, the former being a hemagglutinin, molecular weight 120,000; the latter is highly toxic, molecular weight

60,000. Both react best with lactose and are specific for terminal nonreducing β-linked DGal or with sugars having the DGal conformation on carbons 2,3, and 4 (60). The toxin binds DGalNAc while the hemagglutinin does not (59). With RCA_{II} methyl βDGal and methyl αDGal are better inhibitors than DGal, the β form being slightly more active than the α form. The hemagglutinin is a tetramer with 2 kinds of subunits, called α and β, and has 2 DGal specific binding sites of K^a 3.8×10^3 and 1.2×10^{-3} liter/mole for lactose and galactose, respectively. The toxin is a dimer of an α and a β′ subunit.

Con A precipitates with RCA_I, the mannose-containing carbohydrate of RCA_I being specific for the Con A site. The complex is RCA_I-Con A_4 and can be dissociated by DMan (61).

The H-specific lectins of *Lotus tetragonolobus* (62–64) have proven especially valuable in establishing structures of blood group oligosaccharides. They all have identical sites reacting very strongly with LFucα1→2DGalβ1→4DGlcNAc but not at all

or

DGlc

with LFucα1→2DGalβ1→3DGlcNAc (64) and thus have proven of great value in elucidating structures of compounds in which the DGlcNAc had LFuc on positions 3 or 4 respectively (65), such compounds not being distinguishable by methylation. Substitution of DGalNAcα1→3 or DGalα1→3 on the DGal to form the blood group A or B determinants completely abolishes activity of the active compound.

Bandeiraea simplicifolia seeds contain 2 lectins one of which is blood group B specific (66) while the other is specific for terminal nonreducing DGlcNAc (67). The B-specific lectin is a glycoprotein of molecular weight 114,000 with 4 identical subunits. It agglutinates B and AB erythrocytes strongly. A_1 weakly, and A_2 and O not at all. Blood group A substance precipitates about 20% of the amount precipitated by B substance. Methyl αDGal, DGalα1→6DGlc, and p-nitrophenyl αDGal were equally effective and the best inhibitors; p-nitrophenyl βDGal was slightly less active. Thus with this lectin a hydrophobic contribution of the p-nitrophenyl group is not seen (66). It is of interest that methyl αDGalNac gives an inhibition curve with a slope very different from that found for the methyl αDGal and the other oligosaccharides; if the basis for this difference were understood it might contribute materially to elucidating site structure.

Another lectin, from *Euonymus europeus* (68, 69), shows blood group B and H specificity. It precipitated with B and H substances but not with A_1 substances. B and H specificity are associated with a single molecule since absorption on and elution from an H immunoadsorbent give a purified protein with both B and H specificity. The site is unusual in that it is complementary to the blood group B tetrasaccharide but unlike anti-B does not distinguish between the subterminal DGal linked β1→3 or β1→4 to the DGlcNAc:

LFuc
α↓1
2
DGalα1→3DGalβ1→3 or 4DGlcNAcβ1→

Molecular models show that placing the N-acetamido group on opposite sides in the 1→3 and 1→4 linked compounds produces a striking similarity in contour.

Recently our laboratory has been engaged in a study of lectins produced by the sponges *Axinella polypoides* (70, 71) and *Aaptos papillata* (72). Both of these sponges

produce three lectins; two have been purified from each and their sites characterized. Axinella lectin I is a strong mitogen for human peripheral blood T and is less mitogenic for B lymphocytes (71): Axinella lectin II is not mitogenic. Mitogenic response correlated with degree of lymphocyte agglutination and was specifically inhibited by DGal, DFuc, 2 deoxy DGal, and raffinose if added up to 5 h after the lectin (71). Axinella lectin I had a site reacting best and equally with terminal nonreducing p-nitrophenyl βDGal and with DGal-linked β1→6 to DGlc, DGlcNAc, and DGal while lectin II reacted better with p-nitrophenyl βDGal than with these 3 compounds all of which were equally active; methyl βDGal and α-linked compounds showed much lower activity. The site thus involves terminal nonreducing DGal linked β1→6 to a second sugar but some hydrophobic contribution is involved. p-Nitrophenyl αDGal is as active as methyl αDGal. Thus the hydrophobic contribution to binding is intimately associated with the β linkage.

The two Aaptos lectins (72) which have been purified show extraordinary differences in their sites. Like the wheat germ lectin they are specific for β1→4-linked DGlcNAc residues. Aaptos lectin II is inhibited best and equally by N, N′, N″-triacetylchitotriose and N,N′,N″,N‴-tetraacetylchitotetraose which were 13 times more active than DGlcNAc; it thus has a site complementary to a trisaccharide. With lectin I, N,N′,N″,N‴-tetraacetylchitotetraose is better than the trimer establishing the site as complementary to a tetrasaccharide; the tetramer was 2,000 times more active than DGlcNAc.

Hydrophobic contributions to the binding of Aaptos lectin I are very striking. It precipitated with the monofunctional hapten p-nitrophenyl αDGalNAc while the β anomer did not precipitate and was a good inhibitor; both were weak inhibitors of Aaptose lectin II and did not precipitate. Even more interesting was the finding that inhibition of precipitation by a human blood group A substance, MSS 10% 2x, which was reacting because of its content of β-linked DGlcNAc residues, required much larger amounts of the β1→4 DGlcNAc oligomeric inhibitors at 37° than at 4°C.

Wheat germ agglutinin, molecular weight 36,000 with 4 binding subunits, has a site most complementary to the trisaccharide N,N′,N″ triacetylchitotriose. It also binds N-acetylneuraminic acid, reacts with a population of mouse leukocytes lacking immunoglobulin and T cell surface markers, which occurs in highest proportion in bone marrow, spleen, and peripheral blood and is found in less than 0.5% of lymph node and thymus cells. It has an insulin-like effect reacting with insulin receptors or fat cells and blocks fertilization (73–76).

This survey of lectin sites as determined by immunochemical methods even for those which react with blood group A,B,H,Le^a,Le^b, I, and i substances shows them to be quite different in site sizes and specificities. As a group they are analogous to antibody-combining sites except that they are distributed more or less individually among different plant and animal species, while among vertebrates each species as far as is known may form the entire repertoire of antibody specificities. It is evident that further understanding of site structure will come only when a sufficient number of sites have been defined crystallographically to make possible understanding of the nature of the bonds by which these sites interact with their specific oligosaccharide determinants and comparison of lectin sites with antibody sites of similar specificities. The mechanisms by which the characteristic biological functions are affected may or may not be related to site structure, but the specific interactions of the site are the *sine qua non* of their action. Table I summarizes the data on various lectin combining sites and their properties.

TABLE I. Specificities and Properties of Sites on Various Lectins

Lectin	Best estimate of site size and complementarity	Hydrophobic forces (H) or conformation (C) important	Relationship of activity of glycosides and to free sugar[a]				Blood group specificity
			α > Free sugar > β / β > Free sugar > α	Methyl α / Methyl β > Free sugar	p-Nitrophenyl or Phenyl > Methyl	pNO_2 Phenyl or Phenyl = Methyl	
Concanavalin A	DManα1→[b]	H	Yes	No	Yes[c]		
Helix pomatia	DGalNAcα1→	C	Yes	No			A
Dolichos biflorus	DGalNAcα1→3[LFucα1→2]DGalβ1→	C	Yes	No			A
Lotus tetragonolobus	LFucα1→2DGalβ1→4[LFucα1→3]DG1cNAcβ1→	C or H					H
Aaptos lectin II	DGlcNAcβ1→4DGlcNAcβ1→4DGlcNAc	H	Yes	No	Yes		
BS I[d]	DGalα1→		Yes	No	No	Yes	B
BS II[d]	DGlcNAc	H	Yes	No	Yes	No	
Axinella lectin I	DGalβ1→6		No	Yes?	Yes	No	
Axinella lectin II	DGalβ1→6	H	No	Yes	Yes	No	
Aaptos lectin I	DG1cNAcβ1→4DGlcNAcβ1→4DGlcNAcβ1→4DGlcNAc	H	No	Yes	Yes	No	
Peanut	DGalβ1→3DGalNAc		No	Yes	No	Yes	
RCA I[e]	DGalβ1→4		Yes[f]	No			
RCA II[e]	DGalβ1→4		No	Yes			
Soybean	DGalNAcα1→3DGalβ1→3DGlcNAc	H	No	Yes	Yes	No	
Euonymus europeus	DGalα1→3[LFucα1→2]DGalβ1→3 or 4DGlcNAc						BH
Lima bean III	DGalNAcα1→	H				Yes	A
Sophora japonica	DGalNAcα and DGalβ1→			Yes			A and B

[a]With antibodies and myeloma proteins with specificity for carbohydrates for either α or β linkages. The relationship is to my knowledge always α > free sugar $\geq \beta$ or β > free sugar $\geq \alpha$.

[b]Concanavalin A also reacts with terminal nonreducing α-linked DGlc, DGlcNAc, DFru to a lesser degree than DMan.

[c]The 4-methylumbelliferyl αDMan is more active than the p-nitrophenyl glycoside (Clegg RM, Loontiens FG, Jovin TM: Biochemistry 16:167, 1977).

[d]*Bandeiraea simplicifolia*

[e]*Ricinus communis*

[f]DGal and methyl αDGal equal as inhibitors

[g]DGalNAc also bound

ACKNOWLEDGMENTS

This work was supported in part by National Science Foundation grants BMS-72-02219-AO4 and PCM 76-81029.

REFERENCES

1. Mäkelä O: Ann Med Exp Biol Fenn (Suppl II)35:144, 1957.
2. Sharon N, Lis H: Science 177:949, 1972.
3. Lis H, Sharon N: Annu Rev Biochem 42:541, 1973.
4. Nicolson GL: Int Rev Cytol 39:89, 1974.
5. Cohn E (ed): Ann NY Acad Sci 234:5, 1974.
6. Kabat EA: "Structural Concepts in Immunology and Immunochemistry." 2nd Ed. New York: Holt Rinehart and Winston 1976.
7. Ashwell G, Morell AG: Adv Enzymol 41:99, 1974.
8. Bohlool BB, Schmidt EL: Science 185:269, 1974.
9. Wolpert JS, Albersheim P: Biochem Biophys Res Commun 70:729, 1976.
10. Potter M, Mushinski EB, Rudikoff S, Appella E: Third International Convocation on Immunology," Buffalo, New York. Basel, Switzerland: S. Karger, 1972, p 270.
11. Cohn M: Cold Spring Harbor Symp Quant Biol 32:211, 1967.
12. Poljak RJ, Amzel LM, Avey HP, Chen BL, Phizackerley RP, Saul F: Proc Natl Acad Sci USA 70:3305, 1973.
13. Schiffer M, Girling RL, Ely KR, Edmundson AB: Biochemistry 12:4620, 1973.
14. Segal DM, Padlan EA, Cohen GH, Rudikoff S, Potter M, Davies DR: Proc Natl Acad Sci USA 71:4298, 1974.
15. Epp O, Colman P, Fehlhammer H, Bode W, Schiffer M, Huber R: Eur J Biochem 45:513, 1974.
16. Edmundson AB, Ely KR, Girling RL, Abola EE, Schiffer M, Westholm FA, Fausch MD, Deutsch HF: Biochemistry 13:3816, 1974.
17. Fehlhammer H, Schiffer M, Epp O, Colman PM, Lattman EE, Schwager P, Steigemann W, Schramm HJ: Biophys Struc Mech 1:139, 1975.
18. Edmundson AB, Ely KR, Abola EE, Schiffer M, Panagiotopoulos N: Biochemistry 14:3953, 1975.
19. Kabat EA: "Kabat and Mayer's Experimental Immunochemistry." 2nd Ed. Springfield, Illinois: Charles C. Thomas, 1961.
20. Berson SA, Yalow R: Clin Chem Acta 22:51, 1968.
21. Parker CW: "Radioimmunoassay of Biologically Active Compounds." Englewood Cliffs, New Jersey: Prentice-Hall, Inc., 1976.
22. Engvall E, Perlmann P: Immunochemistry 8:871, 1971.
23. Phillips DC: Sci Am 215: 11, 78, 1966.
24. Cisar J, Kabat EA, Dorner MM, Liao J: J Exp Med 142:435, 1975.
25. Arakatsu Y, Ashwell G, Kabat EA: J Immunol 97:858, 1966.
26. Goldstein IJ, Hollerman GE, Smith EE: Biochemistry 4:876, 1965.
27. Poretz RD, Goldstein IJ: Biochemistry 9:2890, 1970.
28. Brewer CF, Sternlicht J, Marcus DM, Grollman A: Biochemistry 12:4448, 1973.
29. Alter GM, Magnuson JA: Biochemistry 13:4038, 1974.
30. Villafranca JJ, Viola RD: Arch Biochem Biophys 160:465, 1974.
31. Hardman KD, Ainsworth CF: Biochemistry 12:4442, 1973.
32. Becker JW, Reeke GN Jr, Cunningham BA, Edelman GM: Nature (London) 259:406, 1976.
33. Hardman KW, Ainsworth CF: Biochemistry 15:1120, 1976.
34. Markert CL (ed): "Isozymes." New York: Academic Press, 1975, vol 1.
35. Kornfeld S, Rogers J, Gregory W: J Biol Chem 246:6581, 1971.
36. Howard IK, Sage H: Biochemistry 8:2436, 1969.
37. Leseney AM, Bourrillon R, Kornfeld S: Arch Biochem Biophys 153:831, 1972.
38. Kabat EA: "Blood Group Substances." New York: Academic Press, 1956.
39. Marcus DM: N Engl J Med 280:994, 1969.
40. Watkin WM: In Gottschalk A (ed): "Glycoproteins." New York: Elsevier, 1972, p 830.

41. Kabat EA: In Isbell HS (ed): "Carbohydrates in Solution." Washington DC: American Chemical Society, Advances in Chemistry Series No. 117, 1973, p 334.
42. Lloyd KO: In Aspinall GO (ed): "International Review of Science, Organic Chemistry Series Two." London: Butterworths, 1976, vol 7, p 251.
43. Hakomori S, Kobata A: In Sela M (ed): "The Antigens." New York: Academic Press, 1974, vol 2, p 79.
44. Kaplan MH, Kabat EA: J Exp Med 123:1061, 1966.
45. Lloyd KO, Kabat EA, Beychok S: J Immunol 102:1354, 1969.
46. Hammarström S, Kabat EA: Biochemistry 8:2696, 1969.
47. Hammarström S, Kabat EA: Biochemistry 10:1684, 1971.
48. Hammarström S, Lindberg AA, Robertsson ES: Eur J Biochem 25:274, 1972.
49. Etzler ME, Kabat EA: Biochemistry 9:869, 1970.
50. Carter WG, Etzler ME: Biochemistry 14:2685, 1975.
51. Galbraith W, Goldstein IJ, Biochemistry 11:3976, 1972.
52. Pereira MEA, Kabat EA, Sharon N: Carbohydr Res 37:89, 1974.
53. Poretz RD, Riss H, Timberlake JW, Chien S-M: Biochemistry 13:250, 1974.
54. Chien S-M, Lemanski T, Poretz RD: Immunochemistry 11:501, 1974.
55. Benjamini E, Shimizu M, Young JD, Leung CY: Biochemistry 7:1261, 1968.
56. Pereira MEA, Kabat EA, Lotan R, Sharon N: Carbohydr Res 51:107, 1976.
57. DeVries AL, Komatsu S, Feeney RE: J Biol Chem 245:2901, 1970.
58. Feizi T, Kabat EA: J Exp Med 135:1242, 1972.
59. Nicolson GL, Blaustein J, Etzler ME: Biochemistry 13:196, 1974.
60. Drysdale RG, Herrick PR, Franks D: Vox Sang 15:194, 1968.
61. Podder SK, Surolia A, Bachhawat BK: Eur J Biochem 44:151, 1974.
62. Yariv J, Kalb AJ, Katchalski E: Nature (London) 215:890, 1967.
63. Kalb AJ, Biochim Biophys Acta 168:532, 1968.
64. Pereira MEA, Kabat EA: Biochemistry 13:3184, 1974.
65. Rovis L, Kabat EA, Pereira MEA, Feizi T: Biochemistry 12:5355, 1973.
66. Hayes CE, Goldstein IJ: J Biol Chem 249:1904, 1974.
67. Iyer PN, Wilkinson KD, Goldstein IJ: Arch Biochem Biophys 117:330, 1976.
68. Pacák F, Kocourek J: Biochim Biophys Acta 400:374, 1975.
69. Petryniak J, Pereira MEA, Kabat EA: Arch Biophys 78:118, 1977.
70. Bretting H, Kabat EA: Biochemistry 15:3228, 1976.
71. Phillips SG, Bretting H, Kabat EA: J Immunol 117:1226, 1976.
72. Bretting H, Kabat EA, Liao J, Pereira MEA: Biochemistry 15:5029, 1976.
73. Goldstein IJ, Hammarström S, Lundblad G: Biochim Biophys Acta 405:53, 1975.
74. Cuatrecasas P, Tell GPE: Proc Natl Acad Sci USA 70:485, 1973.
75. Oikawa T, Yamanichi R, Nicolson GL: Nature (London) 241:256, 1973.
76. Robinson PJ, Roitt I: Nature (London) 250:517, 1974.