The Journal of Immunology
RESEARCH ARTICLE | JANUARY 01 1960

The Upper Limit for the Size of the Human Antidextran Combining Site[1]

Elvin A. Kabat

J Immunol (1960) 84 (1): 82–85.
https://doi.org/10.4049/jimmunol.84.1.82

THE UPPER LIMIT FOR THE SIZE OF THE HUMAN ANTIDEXTRAN COMBINING SITE[1]

ELVIN A. KABAT

From the Departments of Microbiology and Neurology, College of Physicians and Surgeons, Columbia University and the Neurological Institute, Presbyterian Hospital, New York, New York

Received for publication May 15, 1959

Earlier studies from this laboratory (1–3) have established that in the homologous series of α 1 $\rightarrow$ 6 glucose oligosaccharides up to isomaltohexaose, the relative capacity of the oligosaccharides on a molar basis to inhibit the precipitin reaction of several human antidextran sera of 1 $\rightarrow$ 6 specificity by clinical or native NRRL B512 dextran increased with increasing chain length up to the hexasaccharide. The relative increment in inhibiting power of oligosaccharides containing more than three hexose residues decreased markedly as if a limit was being reached. With all of the antisera studied, however, the hexasaccharide was significantly better than the pentasaccharide, so that it was possible that isomaltoheptaose might be even more effective than the isomaltohexaose. Moreover, differences in the relative inhibiting power of the lower oligosaccharides to the hexasaccharide with different antisera indicated that each individual produced a population of antibody molecules which varied in the complementary area of their combining sites, some being specific for longer sequences of α 1 $\rightarrow$ 6 linked oligosaccharides of glucose. It was, therefore, also possible that the antibody combining sites in those individuals who produced a higher proportion of antibody directed against the longer chain oligosaccharides might contain some antibody directed against a chain more than six units in length. The availability of a sample of isomaltoheptaose made it possible to compare it with isomaltohexaose in inhibiting power. With the human antidextran sera from the four individuals previously studied (2), the hexa- and heptasaccharides were about equal in inhibiting power; but with a fifth serum (3), the heptasaccharide was slightly but definitely better than the hexasaccharide. These findings indicate that in most sera only a relatively small number of antibody combining sites are directed against units larger than a hexasaccharide, but in at least one serum the antibody combining sites complementary to a heptasaccharide were present in quantities sufficient to be detectable.

[1] These studies were supported by the William J. Matheson Commission, the National Science Foundation (G-5208 and G-1586) and the Office of Naval Research, Navy Department, Washington, D. C. Reproduction in whole or in part is permitted for any purpose of the United States Government.

EXPERIMENTAL

Isomaltoheptaose was prepared by Doctor S. Haq in Doctor W. J. Whelan's laboratory at the Lister Institute and generously made available by Doctors Haq and Whelan. Isomaltohexaose, pentaose and tetraose, the preparations previously used, had been provided by Doctors J. R. Turvey and W. J. Whelan (4). Isomaltotriose was obtained from Dr. Allene Jeanes of the Northern Regional Utilization Laboratory (5).

The four antidextran sera from individuals 1, 20, 30 and 36, as well as clinical dextran N150 N and native dextran NRRL B512 preparations and the oligosaccharide inhibition technique, have all been described previously (1–3). Quantitative precipitin determinations were carried out by the Heidelberger and MacPherson procedure (6). A sample of Russian dextran polyglukin (*cf.* (7)) was obtained through the National Research Council.

RESULTS

The essential findings for the four antisera previously studied (2) are given in Figure 1. The actual value for the percentage inhibition for each point is given. An estimate for the experimental error in each determination is also indicated for each point on the assumption that the antibody N determined might be in error by ± 1.5 μg N and the spread in percentage inhibition resulting from such an error is indicated.

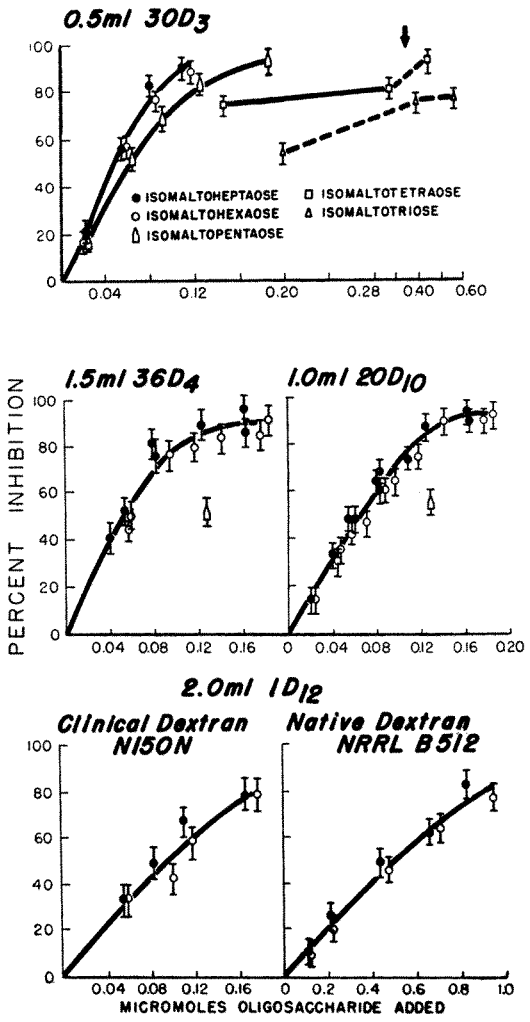

Figure 1. Inhibition by oligosaccharides of the precipitation of four human antidextran sera by dextran.

It is evident that for all four antisera—$30D_3$, $36D_4$, $20D_{10}$, and $1D_{12}$—isomaltohexaose and isomaltoheptaose are about equally effective as inhibitors on a molar basis. With antiserum $30D_3$, data for the penta-, tetra- and trisaccharides of the isomaltose series show them all to be less effective per mol as the chain length decreases, substantiating earlier findings (2, 3). The significantly lower capacity of the isomaltopentaose in inhibiting is also evident from the single points shown with antisera $36D_4$ and $20D_8$. With antiserum $1D_{12}$ isomaltohexaose and isomaltoheptaose also proved to be identical in inhibiting potency on a molar basis when either native or clinical dextran was used to precipitate the antibody. The curves in Figure 1 are drawn on a much larger scale than those previously published, and the interpolated values for the quantities giving 50% inhibition previously reported (2, 3) for the hexasaccharide are slightly different because of the different size scale.

Figure 2 shows the results with antiserum $176D_2$ which had been obtained by the injection of a fraction from partially hydrolyzed B512 dextran with an average molecular weight of 91,700; the heptasaccharide was slightly but significantly better as an inhibitor than the hexasaccharide, but the increase in inhibiting power was less in going from the hexa- to the heptasaccharide than it was in going from the penta- to the hexasaccharide (Fig. 2). The results with the antiserum in Figure 2 were confirmed on another sample of serum from the same subject.

Table I shows a comparison of quantitative precipitin data with antidextrans $30D_3$ and $20D_{10}$

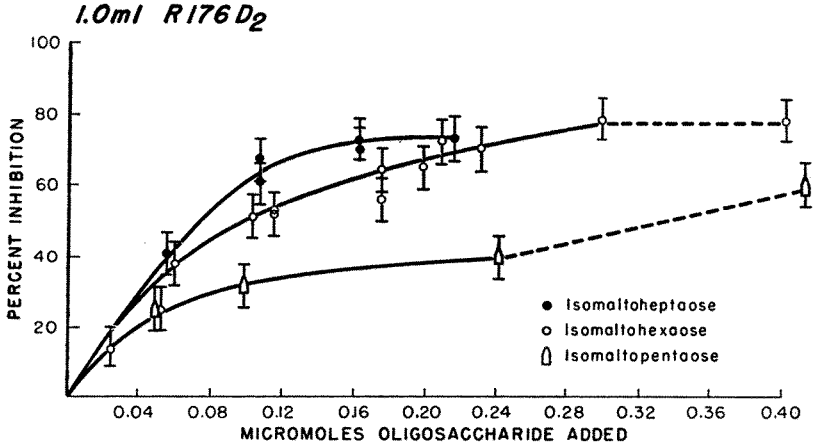

Figure 2. Inhibition by oligosaccharides of precipitation of human antidextran by dextran.

TABLE I

Quantitative precipitin studies of N150 N and Russian dextran polyglukin

Dextran Added	Antibody N Precipitated			
	0.5 ml Antiserum $30D_3$		1.0 ml Antiserum $20D_{10}$	
	N150 N	Polyglukin	N150 N	Polyglukin
μg	μg	μg	μg	μg
2	18	18	17	17
4	28	29	26	26
6	31	31	27	26
10	34	31	28	28
20	27	25	23	24

using equal amounts of N150 N and the Russian dextran polyglukin (cf. (7)). It is evident that both dextrans react identically.

DISCUSSION

Although individuals have been shown to produce heterogeneous populations of antibodies which vary in the size of their combining sites, it is not known what proportions of combining sites complementary to these various sized units are formed. Under such circumstances determination of the upper limit in size of the combining site presents certain difficulties. If only a small proportion of the antibody combining sites were complementary to a heptasaccharide with the remainder being complementary to the hexa-, penta- and tetrasaccharides, it would be difficult to detect the increased inhibiting power contributed by this small number of combining sites complementary to the heptasaccharide. With the four sera in Figure 1 this may actually be the case, since, although the inhibition data show the two to be equal in inhibiting power within experimental error, there was a tendency for the heptasaccharide points to be slightly above the hexasaccharide points on the curves. The data in Figure 2, however, show that in the serum of at least one individual enough antibody complementary to the heptasaccharide may be formed to be detectable by this technique. It is of interest that the original inhibition curves in this serum (3) showed the tetrasaccharide to be only slightly better as an inhibitor than the trisaccharide, with the penta- and hexasaccharides being considerably better than either. These inhibition curves tended to differ in this respect from those of the other individuals studied (2, 3) and would be consistent with a larger proportion of antibodies having combining sites larger than usual. The possibility that a small proportion of antibody molecules could have antibody combining sites complementary to an octasaccharide cannot be excluded, but it is evident that in most instances as determinable by the present technique the largest proportion of antidextran molecules are complementary to a hexasaccharide or to smaller units, although detectable amounts of antibody complementary to a heptasaccharide can sometimes be formed.

It is important to be able to estimate the proportions of antibodies with the various sized combining sites and also to ascertain the lower limit for the size of an antibody combining site. Antibody with smaller combining sites would be expected to have higher discriminating power in elucidating structures by oligosaccharide inhibition techniques for the first few residues from the terminal nonreducing ends, whereas antibody with larger combining sites would be most useful in determining structural relationships further from the nonreducing end. Such differences were readily apparent from the data with antiserum from individuals 30 and 20 given in (2, 3); those antisera with higher proportions of antibody with the larger combining sites require larger amounts of the smaller oligosaccharides to obtain a given degree of inhibition. In the case of the blood group B substance, galactinol, an α-galactoside of galactose and inositol, the best inhibitor thus far tested gave 50% inhibition with one antiserum at a concentration of about 0.4 μM, whereas with another antiserum, 20 μM was required (8). Thus, since only limited quantities of the various oligosaccharides are usually available, the ability to explore unknown structures at any stage of development may depend upon the availability of suitable antiserum. Similar problems have also been encountered in inhibition studies with the polysaccharides of salmonella and horse and rabbit antisera (9).

The question naturally arises whether a hexasaccharide or heptasaccharide constitutes the largest unit to which an antibody combining site can be formed, or whether for structural reasons the dextran molecules only present chains of this size to the antibody-forming mechanism. The latter possibility might be envisaged if the maximum length of all terminal nonreducing chains in the dextran were six or seven units long because

of branching on the seventh or eighth residue. Knowledge of dextran structure is at present based on statistical analysis and does not provide an explicit answer. From studies on the oligosaccharides formed at 1–2% hydrolysis, Dimler *et al.* (10 *cf.* 11) have inferred that 70% of the branches of B512 dextran are one unit long, 15% 2 units, 8% 3 units, 4% 4 units and 3% are longer. The degree of uncertainty is greater for the higher oligosaccharides and involves several assumptions, so that there may well be significant amounts of long branches even in this calculation. Moreover, Rozenfeld (7), using a dextran 1,6 glucosidase which splits dextran from the nonreducing ends of chains to the branch points, found four clinical dextrans to have from 14–18% up to 30–35% of their weight hydrolyzed. From the values for the proportion of non-1,6 linkages by periodate oxidation for the Russian clinical dextran, polyglukin, 97% 1,6 and 4% non-1,6 linkages, which very closely resembles the American clinical dextran prepared from the B512 strain and containing 96% 1,6 and 4% 1,3 like linkages, the 30–35% liberated would indicate average chains of 7 or 8 units. It is of interest that the clinical dextrans gave identical quantitative precipitin curves (Table I). With the British intradex prepared from the B512 strain with 30–35% splitting and 6% non-1,6 linkages, the average chain length would be 5 to 6 units. Even with the other dextrans which showed lower proportions of splitting and more non-1,6 linkages, long terminal chains of 1,6 linked units may exist. Finally, methylation studies on four clinical dextrans from the B512 strain have shown them to have 9.5–11 α 1 $\rightarrow$ 6 glucopyranose units/branch (12). There is thus little reason to think that the antibody forming mechanism may not be presented with some terminal chains of α 1,6 linked glucoses larger than the maximum size of the combining site thus far encountered. In studies of the cross reactions of Type II antipneumococcal horse and rabbit antisera with dextrans, it has been found (13) that units as large as isomaltopentaose can fit into these antibody combining sites.

SUMMARY

With four human antidextran sera of 1,6 specificity isomaltoheptaose was not significantly better as an inhibitor than isomaltohexaose, but with a fifth antiserum the heptasaccharide was slightly better. The data indicate that in most instances the upper limit in size for most of the antibody combining sites is complementary to a hexasaccharide but that in one instance a detectable amount of antibody complementary to a heptasaccharide was found. A Russian and an American clinical dextran gave identical quantitative precipitin curves with two human antidextran sera of 1,6 specificity.

REFERENCES

1. Kabat, E. A., J. Am. Chem. Soc., **76:** 3709, 1954.
2. Kabat, E. A., J. Immunol., **77:** 377, 1956; J. Cell & Comp. Physiol., **50** (Supplement 1): 79, 1957.
3. Kabat, E. A. and Bezer, A. E., Arch. Biochem. **78:** 306, 1958.
4. Turvey, J. R. and Whelan, W. J., Biochem. J., **67:** 49, 1957.
5. Jeanes, A., Wilham, C. A., Jones, W., Tsuchiya, H. M. and Rist, C. E., J. Am. Chem. Soc., **75:** 5911, 1953.
6. Heidelberger, M. and MacPherson, C. F. C., Science, **97:** 405, 1943; correction, **98:** 63, 1943.
7. Rozenfeld, E. L., Biokhimiya, **23:** 597, 1958 (English translation).
8. Kabat, E. A., In *Chemistry and Biology of Mucopolysaccharides*, Ciba Foundation Symposium, J. & A. Churchill, Ltd., London, 1958; Behringwerke Mitteilungen, **34:** 39, 1958.
9. Staub, A. M., Tinelli, R., Lüderitz, O. and Westphal, O., Ann. Inst. Pasteur, **96:** 303, 1959.
10. Dimler, R. J., Jones, R. W., Schaefer, W. C. and Van Cleve, J. W., Am. Chem. Soc. Abstracts, **129:** 2D, 1956.
11. Jeanes, A., In *Transactions of Third Conference on Polysaccharides in Biology*, Edited by G. F. Springer, p. 138, Josiah Macy, Jr., Foundation, New York, 1958.
12. Jones, J. K. N. and Wilkie, K. C. B., Canad. J. Biochem. Physiol., **37:** 377, 1959.
13. Goodman, J. W. and Kabat, E. A., J. Immunol., In press.