Activity of Reduced Oligosaccharides Isolated from Blood Group H, Le^b and Le^a Substances by Alkaline Borohydride Degradation†

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ABSTRACT: The oligosaccharides isolated by alkaline borohydride degradation of an Le^a-active (N-1) and an H, Le^b-active (JS) glycoprotein have been tested as inhibitors of precipitation in five different antibody– or lectin–blood group substance systems. The biological activities found agree with the structures deduced from data on analytical composition, periodate oxidation, and methylation and permit a structure to be proposed for some oligosaccharides for which the chemical data did not allow a unique solution. All of the fucose-containing oligosaccharides isolated from the N-1 glycoprotein are good inhibitors in the goat $Lewis^a$ system. The goat anti-$Lewis^a$ serum used has been shown to be specific for the $\beta(1\rightarrow3)$ linkage joining the βDGal(1→3)-[αLFuc(1→4)]βDGlcNAc sequence to the next residue. None of the oligosaccharides isolated from the JS glycoprotein showed $Lewis^b$ activity. All but one of the mono- and difucosyl JS oligosaccharides tested had fucoses on type 2 chains, as indicated by their reactivity with the purified lectin from *Lotus tetragonolobus*. This lectin is highly specific for type 2 chains and has proven especially important in establishing some of the structures of the compounds obtained from JS. The oligosaccharides containing terminal unsubstituted DGal cross-reacted with type XIV horse antipneumococcal serum to different extents depending on the sequence of sugars, the linkage of the DGal residue to the DGlcNAc, and the subsequent linkage. An oligosaccharide isolated from N-1, Lewis R_{IMs} 1.95, had two unsubstituted type 2 chains linked $\beta(1\rightarrow6)$ and was the best inhibitor of both the type XIV horse antipneumococcal and anti-I Ma systems.

Although each has its own characteristic blood group specificity, human blood group A, B, H, Le^a, Le^b, and precursor I active materials cross-react to different extents with type XIV horse antipneumococcal serum (Kabat *et al.*, 1948; Kabat, 1956; Morgan, 1960) and may show more than a single glood group activity (Watkins, 1972). Thus A or B substances may show H and Le^b activity (Watkins, 1958, 1959) and Le^a substances may show blood group I activity (Feizi *et al.*, 1971). These multiple specificities appear to be a consequence of structural heterogeneity in the carbohydrate portion of these macromolecules and reflect both the existence of a population of presumably incomplete chains and the action of the products of the various blood group genes. The preceding paper (Rovis *et al.*, 1973b) reported the isolation and characterization of a number of individual undegraded reduced oligosaccharide chains from two immunochemically distinct glycoproteins, N-1 ($Lewis^a$ active) and JS (H and Le^b active). The isolated oligosaccharides are shorter than the majority of the carbohydrate chains and originate from a heterogeneous population of chains directly attached to the polypeptide backbone. Many of them contain L-fucose and carry the specific determinants expected from the original activity of the glycoprotein from which they were derived.

Quantitative assays of inhibition by haptens of precipitation of antibody by antigen are standard methods for elucidating structures of antigenic determinants (*cf.* Kabat, 1961, 1968). As applied to blood group glycoproteins they have yielded important structural information on the sequences of sugars involved in the various blood group specificities. Additional structural information has been gained by inhibition by various oligosaccharides of hemagglutination or of enzymic degradation of blood group substances (Watkins, 1972). Inhibition of precipitation or of hemagglutination has also been used with various lectins (Morgan and Watkins, 1953; Hammarström and Kabat, 1969; Etzler and Kabat, 1970).

In the present study the ability of the reduced oligosaccharides described in the preceding paper to inhibit precipitation in five different blood group substance–antibody and blood group substance–lectin systems has been studied. These assays have made it possible to identify structural relationships at the nonreducing ends of the various reduced oligosaccharides and to confirm the structures deduced from the chemical data or to choose between alternative structures compatible with the chemical findings (Rovis *et al.*, 1973b).

Materials and Methods

Inhibitors. The structures of the oligosaccharides isolated from N-1 and from JS are in the preceding paper (Rovis *et al.*, 1973b); refer to that paper for symbols used. Values obtained with the few oligosaccharides containing inert nonsugar material have been corrected to sugar weight. Lacto-*N*-fucopentaose II, lacto-*N*-tetraose, lacto-*N*-neotetraose, lactodifucotetraose, lacto-*N*-fucopentaose II, and lacto-*N*-difucohexaose I were gifts of the late Professor R. Kuhn (Figure 1). OG R_L 0.44 and N-1 R_L 0.71b are described in Vicari and Kabat (1970) and Lloyd *et al.* (1968), respectively. βDGal-

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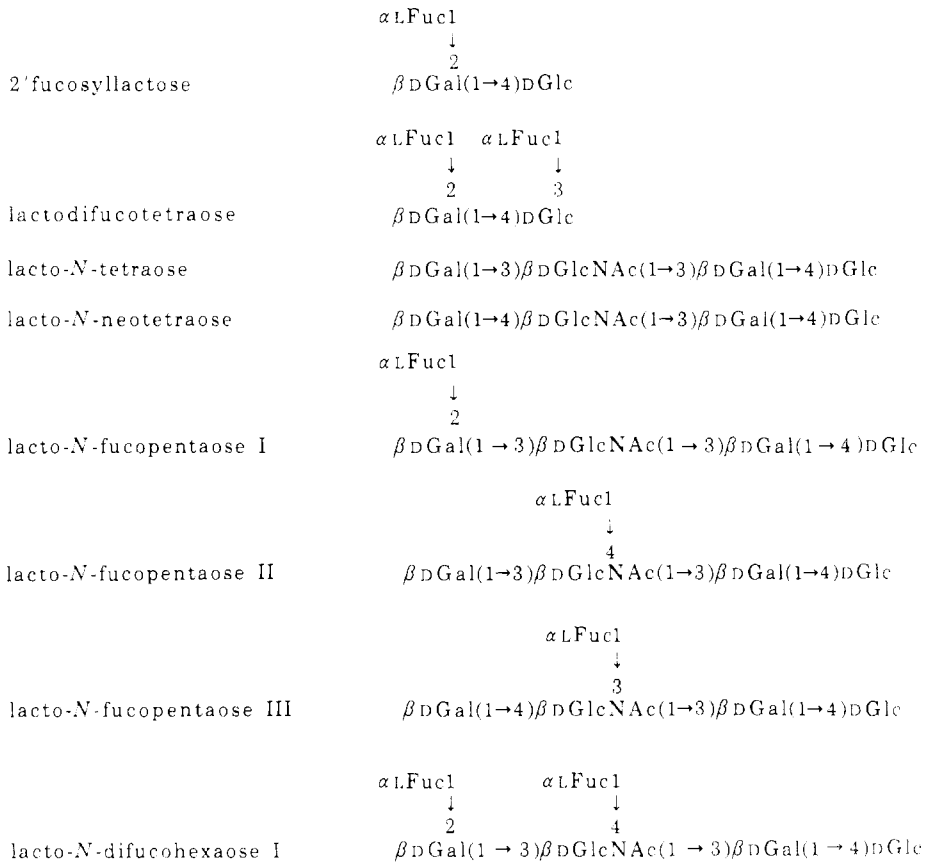

FIGURE 1: Oligosaccharides from milk (from Ginsburg, 1972).

(1→4)DGlcNAc and βDGal(1→3)DGlcNAc[1] were a gift from Dr. F. Zilliken. These are representative of the type 2 and type 1 blood group chains, respectively (Painter *et al.*, 1963).

Antisera. Type XIV horse antipneumococcal serum (H 635, '39 bleeding) has been described (Kabat, 1962). Goat anti-Lewis[a] and anti-Lewis[b] sera (Marcus and Grollman, 1966) were provided by Dr. Marcus. *Lotus tetragonolobus* lectin was prepared from seeds purchased from Thompson and Morgan Ltd., England; a highly purified protein preparation was used (Pereira and Kabat, 1973). Anti-I Ma serum has been previously described (Feizi *et al.*, 1971).

Immunochemical Methods. Quantitative precipitin inhibition assays were carried out on a microscale (Schiffman *et al.*, 1964). Tests for inhibition of I anti-I precipitation (Feizi *et al.*, 1971; Feizi and Kabat, 1972) and of the cross-reaction of blood group substance OG with type XIV antipneumococcal horse serum (Kabat, 1961) were performed at 0°. The oligosaccharides and reduced oligosaccharides were assayed for their ability to inhibit the precipitation of about 3–6 μg of nitrogen in the following systems: (a) type XIV antipneumococcal horse serum, 60 μl, and OG 20% from 10%, 11.2 μg (Vicari and Kabat, 1970), total volume 185 μl; (b) goat anti-Le[a] serum, 75 μl, and N-1 20% of second 10% 8.5 μg (Rovis *et al.*, 1973a), total volume 175 μl; (c) goat anti-Le[b] serum, 60 μl, and JS phenol insoluble, 4 μg (Schiffman *et al.*, 1964), total volume 220 μl; (d) *Lotus tetragonolobus* purified lectin, 6.1 μg of N, and JS phenol insoluble, 19.6 μg, total volume 225 μl; (e) anti-I Ma serum, 15 μl, and OG 20% from 10%, 13.6 μg, total volume 725 μl.

[1] Abbreviations used are: Glc, glucose; Gal, galactose; Fuc, fucose; GlcNAc, 2-acetamido-2-deoxy-D-glucose; GalNAc, 2-acetamido-2-deoxy-D-galactose; *N*-acetylgalactosaminitol, 2-acetamido-2-deoxy-D-galactitol.

Experimental Section and Results

Figure 2A illustrates the ability of the oligosaccharides to inhibit the cross-reaction between type XIV antipneumococcal horse serum and OG substance. The importance of the three terminal nonreducing residues as a determinant in this cross-reaction has been established, and lacto-*N*-neotetraose was found to be the best inhibitor of the system (Watkins and Morgan, 1959; Kabat, 1962). However, oligosaccharides containing the sequence βDGal(1→4)βDGlcNAc followed by a β(1→6) linkage were also good inhibitors and better on a molar basis than the disaccharide βDGal(1→4)DGlcNAc. This is shown by the activity as inhibitors of Lewis R_L 0.41 (Lloyd *et al.*, 1968), OG R_L 0.44 (Vicari and Kabat, 1970), and Lewis R_L 0.44 (Figure 2A), all of which have the same structure βDGal(1→3)[βDGal(1→4)βDGlcNAc(1→6)]-*N*-acetyl-D-galactosaminitol and about the same activity. The same oligosaccharide has also been isolated in a small amount from JS glycoprotein (JS R_{IM_8} 2.35b) but it contains some impurities (Rovis *et al.*, 1973b) and it is slightly less active than Lewis R_L 0.44. Lewis R_{IM_8} 1.95 is more than twice as active as Lewis R_L 0.44 and is the best inhibitor among the structures studied. Its high activity is due mostly to the presence in this oligosaccharide of two type 2 chains linked β(1→6) to the next sugar. In addition it contains also one type 1 chain linked β(1→3) to the branched Gal which should also be slightly active as inferred from the inhibiting power of βDGal(1→3)DGlcNAc and lacto-*N*-tetraose (Figure 2A). Lewis R_{IM_8} 1.28 has the same structure as Lewis R_{IM_8} 1.95,

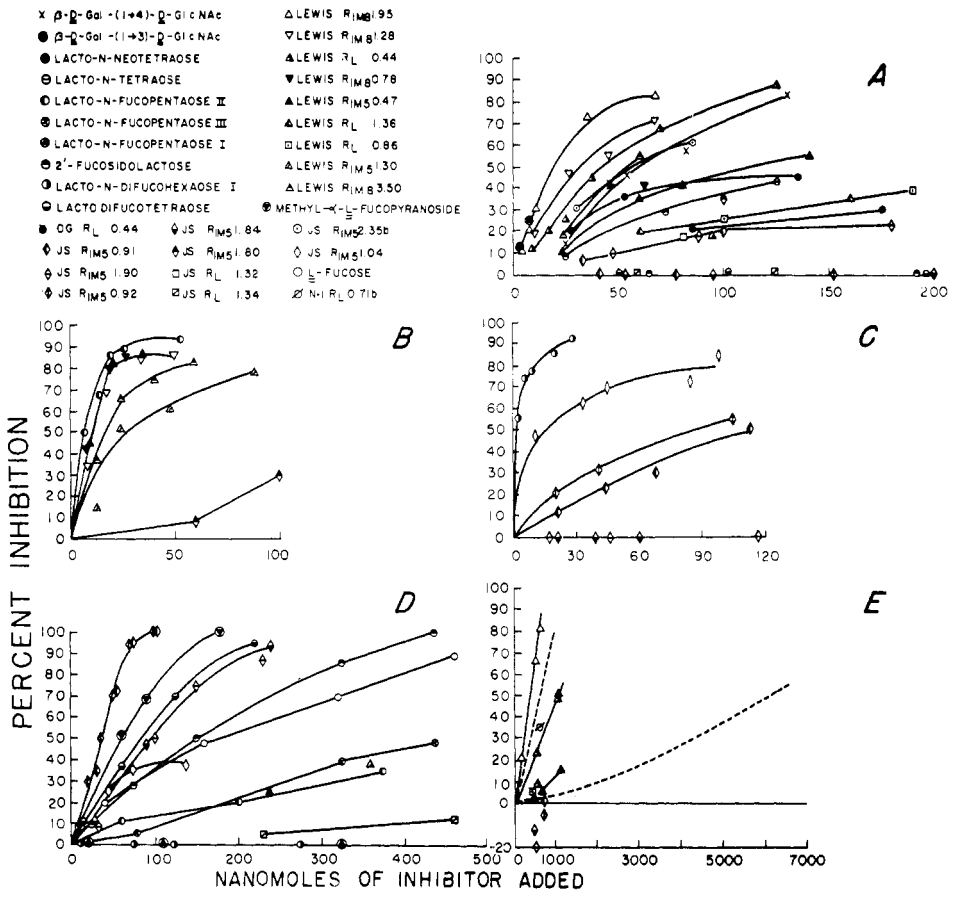

FIGURE 2: Inhibition by oligosaccharides of precipitation of: (A) type XIV antipneumococcal horse serum and OG 20% from 10%; (B) goat anti-Le^a and N-1 20% of second 10%; (C) goat anti-Le^b serum and JS phenol insoluble; (D) *Lotus tetragonolobus* purified lectin and JS phenol insoluble; (E) anti-I Ma serum and OG 20% from 10%.

the sole difference being an additional fucosyl residue on C-4 of the GlcNAc of its type 1 chain. This, together with the presence of a contaminant (Rovis *et al.*, 1973b), may explain its slightly lower inhibiting power. Lewis R_{IM8} 3.5 has a monofucosyl type 1 and an unsubstituted type 2 chain both linked to a branched Gal; as expected it is about as active as Lewis R_L 0.44. Lewis R_{IM5} 0.47 has intermediate activity as does Lewis R_{IM8} 0.78; however, the latter compound is contaminated by Lewis R_{IM8} 1.28 and since only very small amounts were isolated, a complete inhibition curve could not be done. Lewis R_L 1.36, R_L 0.86, R_{IM5} 1.30, JS R_{IM5} 0.91, R_{IM5} 1.90, R_L 1.32, and R_{IM5} 1.80 are all poor inhibitors in this system, and their poor and equivalent reactivity is probably due to the terminal DGal linkage to *N*-acetylgalactosaminitol.

Compounds without a terminal DGal, such as JS R_{IM5} 0.92, R_{IM5} 1.84, R_L 1.34, lactodifucotetraose, and 2′-fucosidolactose, showed no inhibiting power at the concentrations studied. Moreover, Lewis R_{IM5} 1.30, JS R_{IM5} 0.91, R_{IM5} 1.90, and R_{IM5} 1.80 have a structure which would be expected to react well except for the fucosyl substitutions on the DGal or DGlcNAc residues (Kabat, 1962). Figure 2B shows inhibition experiments in which various oligosaccharides are tested for their ability to inhibit the reaction between Lewis^a substance (N-1 20% of second 10%) and a goat anti-Lewis^a antiserum. Lacto-*N*-fucopentaose II, the best low molecular inhibitor of this system thus far reported, has been used as a standard. Lewis R_{IM8} 0.78, R_{IM8} 1.28, and R_{IM5} 0.47 are about as active as the standard. This finding confirms the presence, in all, of an LFuc linked to C-4 of the GlcNAc of the type 1 chain. Lewis R_{IM8} 3.5 is slightly less active than the others, as expected, since it was known from the structural studies reported in the previous paper (Rovis *et al.*, 1973b) to contain about 20% of a contaminant lacking fucose. Lewis R_{IM5} 1.30 has the sequence βDGal(1→3)-[αLFuc(1→4)]βDGlcNAc; however it is significantly less active on a molar basis than the other N-1 oligosaccharides tested. This suggests that the specificity of the anti-Le^a serum may also involve the linkage next to the DGlcNAc carrying the LFuc on C-4; in all the more active structures as well as in lacto-*N*-fucopentaose II, this linkage is β(1→3), while in Lewis R_{IM5} 1.30 the GlcNAc is linked β(1→6) to the terminal *N*-acetylgalactosaminitol. The poorest inhibitor is JS R_{IM5} 1.80. This oligosaccharide was not distinguishable from Lewis R_{IM5} 1.30 by the structural studies, since the methylation analysis does not give information on the nature of the sugars linked to both C-3 and C-4 of the DGlcNAc. However, its low inhibiting power relative to lacto-*N*-fucopentaose II and to Lewis R_{IM5} 1.30 indicates the presence of βDGal on C-4 and of αLFuc on C-3 of the DGlcNAc of a type 2 chain.

Figure 2C shows inhibition by various oligosaccharides of precipitation of JS phenol insoluble and goat anti-Lewis^b serum. None of the oligosaccharides tested showed inhibition comparable to lacto-*N*-difucohexaose I, the milk oligosaccharide used as a standard (Marcus and Grollman, 1966), indicating that none contains a difucosyl type 1 determinant. JS R_{IM5} 1.84 and JS R_{IM5} 1.90 showed no inhibition at the concentrations tested. JS R_{IM5} 0.91 and JS R_{IM5} 0.92 inhibit the precipitation to the same extent and are equally active to structures containing a fucosyl type 2 determinant (Lundblad and Kabat, 1970). The best inhibitor among the compounds

tested is JS R_{IM5} 1.04 which is also believed to contain a difucosyl type 2 determinant. However, in JS R_{IM5} 1.04 the sequence αLFuc(1→2)βDGal(1→4 or 3)[αLFuc(1→3 or 4)]-βDGlcNAc is linked β(1→3) to Gal instead of β(1→6) to the terminal *N*-acetylgalactosaminitol as in JS R_{IM5} 0.91 and JS R_{IM5} 0.92. Thus its resemblance to lacto-*N*-difucohexaose I could explain the greater cross-reactivity of this oligosaccharide and suggests that it is the type 2 chain.

Figure 2D illustrates the ability of N-1 and JS oligosaccharides to inhibit the precipitation of purified *Lotus tetragonolobus* lectin (Pereira and Kabat, 1973) by human H and Le^b active substance (JS phenol insoluble). Inhibition curves obtained with known haptens and milk oligosaccharides are also presented for comparison. JS R_{IM5} 0.92 and JS R_{IM5} 0.91 are the best inhibitors of the system. They both have a difucosyl type 2 chain and show the striking specificity of the lectin for this determinant. The two oligosaccharides are equally active on a molar basis, indicating that the third fucose present on C-2 of the DGal linked to the terminal *N*-acetylgalactosaminitol of JS R_{IM5} 0.92 probably does not contribute to the activity. This is confirmed by the inability to inhibit shown by lacto-*N*-fucopentaose I and JS R_L 1.34. The much greater inhibiting power of the two JS oligosaccharides if compared with lactodifucotetraose may be due either to the presence of a DGlcNAc instead of DGlc in their determinants, or could imply that the specificity of the lectin involves also the next linkage of the difucosyl type 2 chain. In the two oligosaccharides this linkage is β(1→6) to the terminal *N*-acetylgalactosaminitol. In accord with this, JS R_{IM5} 1.04, a compound with a difucosyl type 2 determinant linked β(1→3) to a DGal, is a much poorer inhibitor. The same implications regarding the specificity of the lectin are suggested by the behavior of JS R_{IM5} 1.90 and 1.84, which are also good inhibitors. Both have a monofucosyl type 2 chain, and are therefore less active than JS R_{IM5} 0.91 and 0.92. However, they are almost as good as lactodifucotetraose and about twice as active as 2′-fucosidolactose. The two oligosaccharides differ from each other only by the presence in JSR_{IM5} 1.84, of a second L-fucose linked α(1→2) to the DGal attached to C-3 of the *N*-acetylgalactosaminitol; since they are equally active, they confirm the failure of the lectin to react with the αLFuc(1→2)βDGal(1→3) linkage and in general with type 1 chains substituted by LFuc on the DGal with or without a second LFuc on the DGlcNAc (*cf.* lacto-*N*-difucohexaose I and lacto-*N*-fucopentoase I). Lewis R_{IM5} 0.47 and Lewis R_{IM5} 1.30, both of which have a $Lewis^a$ determinant, are poor inhibitors and as active as lacto-*N*-fucopentaose II and lacto-*N*-fucopentaose III which contain a single fucosyl residue linked only to the DGlcNAc of the type 1 and type 2 chains.

Figure 2E shows inhibition assays with anti-I serum Ma and OG 20% from 10%. For reference, inhibition data previously obtained with βDGal(1→4)DGlcNAc and with oligosaccharides containing βDGal(1→4)βDGlcNAc(1→6) structures (Feizi *et al.*, 1971) are shown by dashed lines, the latter being the more active. Lewis R_{IM5} 1.95 appears to be a rather better inhibitor than any of the oligosaccharides thus far tested; N-1 R_L 0.44 is slightly less active than the previously isolated identical compound Lewis R_L 0.71b and Lewis R_L 0.41. However, the number of tests is small and it is uncertain whether these differences are significant. Lewis R_{IM5} 0.47 has intermediate activity. Lewis R_L 0.86 and JS R_{IM5} 0.91, 0.92, 1.90, and 1.84, in which a free βDGal(1→4)βDGlcNAc(1→6) group was lacking, had no inhibitory activity in the range tested. Other oligosaccharides isolated from JS and N-1 glycoproteins were not assayed as they were available in very limited amounts.

Discussion

The hapten inhibition technique using five different antibodies or lectins as reagents has proven especially useful in confirming the structures of many of the oligosaccharides which were isolated from blood group Le^a and H, Le^b substances (Rovis *et al.*, 1973b) and in permitting a choice when alternative structures were possible from the analytical and chemical data.

All the fucose-containing oligosaccharides isolated from N-1, although different in location and number of residues, are $Lewis^a$ active and the great majority of the JS oligosaccharides have the H determinant. Lewis R_{IM8} 0.78 and Lewis R_{IM5} 1.28 have the βDGal(1→3)[αLFuc(1→4)]βDGlcNAc sequence joined β(1→3) to a branched DGal, while in Lewis R_{IM5} 0.47 the same determinant is substituted on a DGal at C-3. These three oligosaccharides are all about as active as lacto-*N*-fucopentaose II suggesting that the branched DGal is not involved in the specificity of the goat antiserum, and that the structure, apart from the βDGal(1→3)[αLFuc(1→4)]-βDGlcNAc(1→3), does not contribute significantly to the determinant (Figure 2B). Moreover, they are all better inhibitors than Lewis R_{IM5} 1.30 which has a monofucosyl type 1 chain linked β(1→6) to the terminal *N*-acetylgalactosaminitol, indicating that the β(1→3) linkage next to the determinant makes the haptens more complementary to the antibody combining site. This contributes additional important evidence to show that the type 1 chain is linked β(1→3) and the type 2 chain β(1→6) to the branched DGal.

JS R_{IM5} 1.80 is a poor inhibitor in the $Lewis^a$ system; its activity is comparable to that of Lewis R_L 0.71a isolated by Lloyd *et al.* (1968), which also has an LFuc linked to C-3 of a DGlcNAc in a type 2 chain. This confirms the great difference in activity shown by the type 1 and type 2 determinants in the $Lewis^a$ system (Lloyd *et al.*, 1968); in the A-anti-A or B-anti-B systems the differences between type 1 and type 2 monofucosyl chains are not very great (Lloyd *et al.*, 1966). The type 2 determinant with LFuc on C-3 of the DGlcNAc is the product of a specific fucosyltransferase different from the one responsible for Le^a activity (Kobata and Ginsburg, 1969). It is expressed in Lewis R_{IM5} 0.78 and JS R_{IM5} 1.80, but we have no way of detecting it immunologically. Yang and Hakomori (1971) obtained a specific antiserum by immunizing rabbits with a glycolipid isolated from adenocarcinomas and containing a carbohydrate moiety identical with lacto-*N*-fucopentaose III (Figure 1).

JS R_{IM5} 1.04, R_{IM5} 0.91, and R_{IM5} 0.92 have a difucosyl type 2 chain which results from the action of the fucosyltransferase products of the H gene and the fucosyltransferase discussed above (Lloyd and Kabat, 1968; Kobata and Ginsburg, 1969). Accordingly they do not show $Lewis^b$ activity comparable to that of lacto-*N*-difucohexaose I (Figure 2C). Experiments with the goat anti-Le^b serum confirmed the importance of the type 1 difucosyl determinant for high $Lewis^b$ activity (Marcus and Grollman, 1966), and an oligosaccharide (JS R_{IM5} 1.04) also showed considerable Le^b cross-reactivity leading to the conclusion that it contained a type 2 difucosyl determinant linked β(1→3) to the next sugar. No oligosaccharide containing a $Lewis^b$ determinant was isolated from JS, although the original glycoprotein has very strong Le^b activity (Figure 2C). This suggests that type 1 chains are mostly present in larger oligosaccharides (A fraction, Rovis

et al., 1973a) and that the finding of large amounts of type 2 determinants is due to the presence of the structure βDGal-(1→4)βDGlcNAc(1→6)[βDGal(1→3)]-*N*-acetylgalactosaminitol as an intermediate which is constantly present in blood group substances and is substituted by one or more LFuc residues in JS (Rovis *et al.*, 1973b). This structure (Lewis R_L 0.44), together with all the compounds containing one or more unsubstituted type 2 chains (as in Lewis R_{IM8} 1.95), seems to be responsible for most of the type XIV antipneumococcal cross-reactivity found in the original N-1 glycoprotein (Feizi *et al.*, 1971). The low cross-reactivity with type XIV antipneumococcal serum found in H substances and the increase following mild acid hydrolysis or enzymatic action are presumably due to the presence of the type 2 chain with LFuc residues, substituted as in JS R_{IM5} 1.80, 1.90, 0.91, 0.92, and 1.04, which are removed by acid or enzymatic action (Kabat *et al.*, 1948; Morgan, 1960; Howe and Kabat, 1953; Watkins, 1953, 1962; Watkins and Morgan, 1962). In general the different quantitative distribution of the LFuc substitutions is probably responsible for the different amounts of type XIV cross-reactivity found in all blood group substances. Only determinants with type 2 chain seem to be present in glycolipids isolated from human erythrocytes (Koscielak *et al.*, 1972, 1973; Hakomori *et al.*, 1972; Stellner *et al.*, 1973). However, the carbohydrates of the glycolipids show heterogeneity in chain length comparable to the oligosaccharides isolated from soluble blood group substances (Rovis *et al.*, 1973b), suggesting a similar degree of complexity in the biosynthetic pathway.

Antisera specific for H and for the difucosyl type 2 determinant were not available. The striking specificity of the *Lotus tetragonolobus* lectin for type 2 chains linked β(1→6) and containing LFuc on the C-2 of the DGal, with or without a second LFuc on C-3 of the DGlcNAc, and its failure to react with type 1 chains substituted by LFuc on C-2 of the DGal (Figure 2D) make this lectin an extremely valuable reagent for elucidating the structures of the oligosaccharides studied. The results confirmed the proposed structures and the biological data obtained in the Lewis[b] and type XIV systems.

The partial structure of the I determinant reacting with anti-I Ma serum was already established (Feizi *et al.*, 1971). The importance of the sequence βDGal(1→4)DGlcNAc plus a β(1→6) linkage is again confirmed by the activity of Lewis R_L 0.44. The higher inhibiting power of Lewis R_{IM8} 1.95 (Figure 2E) could be due either to the presence of two such sequences, or that one of the type 2 chains is joined β(1→6) to the branched DGal. A compound in which the β(1→6) substitutes an intact DGal has not been available, all active compounds studied (Feizi *et al.*, 1971) having a β(1→6) linkage to hexenetetrol(s), hexanepentol(s), galactitol, or *N*-acetylgalactosaminitol resulting from the alkaline borohydride degradation (Lloyd *et al.*, 1966; Anderson *et al.*, 1972).

The finding that H, Le[a] and Le[b] specificities are associated with the fucosyl substitutions on the βDGal(1→3)[βDGal(1→4)βDGlcNAc(1→6)]-*N*-acetylgalactosaminitol raises the question that perhaps in A and B substances αDGalNAc and αDGal residues may be added to these chains. This would provide an additional kind of type 2 chain which conceivably could be involved in A_1 and A_2 specificity (Moreno *et al.*, 1971; Schachter *et al.*, 1973).

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Lateral Diffusion, Protein Mobility, and Phase Transitions in *Escherichia coli* Membranes. A Spin Label Study†

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ABSTRACT: The coefficient of lateral diffusion in *Escherichia coli* membranes is determined as $D_{diff} = 3.25 \times 10^{-8}$ cm²/sec at 40° using a spin label technique developed previously. This value compares well with the rate of lateral diffusion in dipalmitoyllecithin (DPL) membranes at 50°. The rate of rotational and translational diffusion of membrane proteins may be estimated from the hopping frequency ($\nu \approx 10^7$ Hz) of the lipid molecules. For a protein with a radius $R = 25$ Å we obtain a rotational relaxation time τ_r of about $\tau_r = 75$ μsec and a coefficient of lateral diffusion $D_{diff}^{P} \sim 3 \times 10^{-10}$ cm²/sec. The electron spin resonance (esr) spectra of spin labels incorporated in DPL model membranes undergo characteristic changes in the temperature range of the lipid phase transition. The transition temperature, T_t, determined from the changes in spectral intensity and the order parameter S depends on the distance between the paramagnetic center and the membrane surface. If the NO group is deeply buried within the hydrocarbon phase the obtained values of T_t agree well with dilatometric and spectroscopic measurements (90° light scattering, 8-anilino-1-naphthalenesulfonate (ANS) fluorescence). Lower values of T_t are, however, observed if the NO group is near the semipolar region of the membrane. The same spectral changes have been observed in intact membranes of an *E. coli* fatty acid auxotroph (containing predominantly *trans*-Δ⁹-octadecenoic acid, *cis*-Δ⁹-octadecenoic acid, and *trans*-Δ⁹-hexadecenoic acid), indicating a lipid phase transition in these membranes. The transition temperatures T_t obtained with stearic acid labels carrying the NO group near the methyl end of the chain agree well with the previously reported breaks in the temperature dependence of some transport systems of the respective *E. coli* mutant (P. Overath *et al.*, *Nature (London), New Biol., 234*, 264 (1971)). This shows that these breaks are caused by phase transitions of the membrane lipids. The occurrence of a lipid phase transition in *E. coli* membranes and the approximate equality of the coefficient of lateral diffusion in these membranes with the value of D_{diff} in DPL model membranes strongly support the presence of (continuous) lipid layers in the *E. coli* membranes. "Polar" spin labels (Tempo, digitoxigenin) and stearic acid labels with the NO groups near the carboxyl end indicate a "pretransition" some 6–8° below the main transition. This shows that the result of spin label studies with membranes may depend critically on the position of the paramagnetic center within the membrane.

In recent years spin label probes have been used extensively to study the structure and dynamics of lipids in biological membranes (see reviews by Jost *et al.*, 1971 and Mehlhorn and Keith, 1972). Several groups have applied the spin label technique to the crystalline–liquid crystalline phase transition in dispersions of synthetic phospholipids (Barratt *et al.*, 1969; Hubbell and McConnell, 1971; Jost *et al.*, 1971). Discontinuities in the temperature dependence of the rotational correlation time τ (Barratt *et al.*, 1969), the line width ΔH (Sackmann and Träuble, 1972), and the so-called order parameter S (Hubbell and McConnell, 1971) have been taken as evidence for the occurrence of phase transitions. Similar discontinuities in the electron spin resonance (esr) spectra of spin labeled membranes of *Mycoplasma laidlawii* (Tourtelotte *et al.*, 1970), of plant and rat liver mitochondria (Raison *et al.*, 1971), and yeast cells (Eletr and Keith, 1972) suggest that similar phase transitions occur in biological membranes. This interpretation is supported by calorimetric studies (Steim *et al.*, 1969) showing peaks in the specific heat at about the same temperatures. Historically, calorimetry was the first method used to demonstrate lipid phase transitions in model membranes (Chapman *et al.*, 1967) and biological membranes (Steim *et al.*, 1969).

A large body of evidence is now available demonstrating that the crystalline–liquid crystalline phase transition involves a transition from an ordered to a fluid lipid structure. The importance of the membrane fluidity is now gaining full recognition, for example, in the processes of membrane assembly (*cf.* Rothfield and Romeo, 1971; Sumper and Träuble, 1973), membrane fusion (Frye and Edidin, 1970), rotational and translational motion of membrane components in immune response (Taylor *et al.*, 1971), and sensory transduction (Kaissling and Priesner, 1970; Adam and Delbrück, 1968; Brown, 1972; Cone, 1972).

For some purposes it may suffice to characterize the membrane fluidity by the macroscopic viscosity (η). A more direct approach to the problem of membrane fluidity is the study of the molecular motions within the lipid hydrocarbon chains and the translational motion of the lipid molecules. An attempt is made in the present paper to correlate the translational and rotational motion of membrane macromolecules with the mobility of the lipid molecules.

The rate of lateral diffusion in lipid model membranes was measured recently by an analysis of the effect of spin label

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