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THE CONFORMATION OF *Salmonella* O-ANTIGENIC POLYSACCHARIDE CHAINS OF SEROGROUPS A, B, AND D_1 PREDICTED BY SEMI-EMPIRICAL, HARD-SPHERE (HSEA) CALCULATIONS*

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ABSTRACT

The preferred conformation of the tetrasaccharide repeating units of *Salmonella* Serogroups A, B, and D_1 have been calculated. The semiempirical calculations used the Hard-Sphere, Exo-Anomeric (HSEA) approach to derive a conformational model, which could be used to assist the interpretation of conformations significantly populated in aqueous solution. The calculated model was extended to include a pentasaccharide repeating unit bearing an α-D-glucopyranose-branch point. The 3,6-dideoxyhexose to D-mannose linkage was shown to possess a steep energy surface with a minimum, which results in good exposure of the dideoxyhexose O-2 and O-4 atoms. Stereochemical changes involving the equatorial or axial disposition of these atoms are the distinguishing structural features of the A, B, and D_1 serogroups. A lipophilic surface involving the 6-deoxy groups of the dideoxy-D-hexose and L-rhamnose residues was identified, and the possible implications of these features in antigenic determinants is discussed. The preferred conformation predicted by the HSEA method correlates with the known antigenic specificities of polysaccharides belonging to these *Salmonella* serogroups.

*Dedicated to Professor Raymond U. Lemieux.

INTRODUCTION

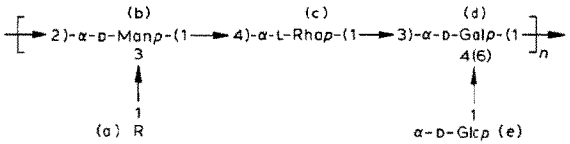
Lipopolysaccharides (LPS) of *Salmonella* have been the subject of extensive structural[1], biochemical, and genetic analysis[2–4], and these studies have facilitated the partial rationalisation of *Salmonella* serology with O-chain structure[3,4]. The Kauffman–White scheme[5], a deliberately simplified serological classification of *Salmonella* strains is based upon rabbit antibodies that detect O-factors. This scheme, devised before structural data were available, is now understood to define the gross immunological aspects of the O-chain structures, *i.e.*, O-antigens. The complexity of O-chain structure, some details of which are yet to be elucidated, and the large number of serogroups have prevented the complete rationalisation of O-factors with chemical structure. Even for those examples where precise structural data are available, specific degradation of O-chains or chemical synthesis rarely provide a sufficiently comprehensive panel of inhibitors to unambiguously map the antibody specificity of a given O-factor. Significant progress has been made in the case of O-factors 4 and 9 by use of phage-degraded polysaccharides and synthetic mono- and di-saccharides[6,7]. Nevertheless, it is not known for which oligosaccharide in the range between tetra- and octa-saccharide the antibody-combining sites are complimentary. Since antigen topography will play a crucial role in this recognition, an appreciation of O-antigen conformation is necessary for an understanding of protein-receptor and carbohydrate-antigen interaction.

Lipopolysaccharides are located at the outer membrane of the Gram-negative cell wall. The presence of O-chains confers enhanced resistance to phagocytosis of the bacterium and, at the same time, the unique structures of different O-chains serve as a finger-print, which is recognized by antibodies. Whilst the role of primary structure in O-chain-antibody interactions is well appreciated, only a few conformational interpretations have appeared[8,9]. One study[10] has focused on the complex problem of lipid A, the hydrophobic tails of the LPS molecule. In other work, we have used synthetic oligosaccharides to correlate the n.m.r. data, chemical shift, coupling constants, relaxation and n.O.e. parameters for the O-antigen of *Shigella flexneri* Y variant[11]. These data were then linked to antigen conformation *via* a semiempirical modelling using the HSEA approach of Lemieux and assoc.[12,13]. The corroboration of the calculations by n.m.r. measurements provides a crucial test of an otherwise hypothetical model. Since the genus *Salmonella* has been extensively studied and because these O-antigens possess many interesting and subtle structural variations, we have extended our work with *Shigella* O-antigens[11] to the *Salmonella* O-antigens.

In this paper, we describe the molecular-modeling of the repeating units of the *Salmonella* Serogroups A, B, and D_1, which are differentiated by variations of the 3,6-dideoxyhexose residues of otherwise identical O-chains. In the following paper, these conclusions are correlated with n.m.r. data from synthetic oligosaccharides and partially degraded O-polysaccharides obtained by the action of phage-associated *endo*-glycanases.

RESULTS

The simplest structures of the biological repeating units of *Salmonella* serogroups A, B, and D_1 are tetrasaccharides. Serogroup A LPSs possess O-factor 2, serogroup B LPSs O-factors 4 and 5, and serogroup D_1 LPSs O-factor 9 (Scheme 1). These O-factors correlate with so-called immunodominant sugars, paratose for



Scheme 1 Serogroup A , R = 3,6-dideoxy-α-D-*ribo*-hexopyranosyl (paratose);

Serogroup B , R = 3,6-dideoxy-α-D-*xylo*-hexopyranosyl (abequose); and

Serogroup D_1 , R = 3,6-dideoxy-α-D-*arabino*-hexopyranosyl (tyvelose)

serogroup A, abequose for serogroup B, and tyvelose for serogroup D_1, which occur as side chains of an identical, linear main-branch composed of trisaccharide repeating units. In serogroup B, acetylation at O-2 of the abequose residue results in the expression of O-factor 5 instead of O-factor 4. Other O-factors are expressed simultaneously on the same polysaccharide molecule, but not always on the same repeating unit. Thus, as with *O*-acetylation that converts Factor 4 to Factor 5, the introduction of an α-D-glucopyranosyl group (unit e) at O-4 (Factor 12) or O-6 (Factor 1) of the α-D-galactopyranosyl residue (unit d) occurs after polymerization of the O-chain repeating units. Hence, Factors 4 and 5 can occur simultaneously owing to incomplete acetylation[14], in the same way that incomplete glucosylation at O-4 of the α-D-galactopyranosyl residues is known[15]. Effects of such microheterogeneity are often to be seen in modified O-factor specificity.

The molecular modeling of the basic tetrasaccharide sequence a–d (Scheme 1) has been conducted for all three 3,6-dideoxy-D-hexoses, but in the presentation here reference will be made largely to serogroup B structures (R = 3,6-dideoxy-α-D-*xylo*-hexopyranosyl). The method employed to calculate oligosaccharide conformations is the HSEA method developed by Lemieux and assoc.[12,13]. The glycosidic linkage is defined in terms of the two torsion angles ϕ_H and ψ_H. The signs of these angles are defined according to IUPAC convention[16], and refer to the atom fragments H-1–C-1–O-1–C-x: ϕ_H, and C-1–O-1–C-x–H-x: ψ_H, where x refers to the linkage position on the adjacent sugar residue. The atomic coordinates employed in these calculations were taken from X-ray and neutron diffraction data, and, for abequose, bond modification was employed. Thus α-D-mannopyranose[17] (unit b), α-L-rhamnopyranose[18] (unit c), α-D-galactopyranose[19] (unit d), and α-D-gluco-

TABLE I

TORSION ANGLES (ϕ_H ψ_H) VALUES[a] FOR GLYCOSIDIC LINKAGES IN *Salmonella* O-ANTIGEN STRUCTURES[b] IN THEIR MINIMUM ENERGY CONFORMATION

Bond	*Disaccharides*	*Pentasaccharides* [d→(a→)b→c→d]			*Hexasaccharide* [c→(e→)d→(a→)b→c]
		Serogroup A	*Serogroup D_1*	*Serogroup B*	
d→b	−46/−19	−46/−23	−44/−19	−46/−22	−45/−18
b→c	−39/−13	−36/1	−36/−1	−36/−1	−36/−1
c→d	49/↩	50/6	50/6	50/6	28/21
a→b[c]	−48/−8	−51/−14			
a→b[d]	−49/−8			−51/−13	−51/−14
a→b[e]	−48/−8		−51/−12		
e→d	−40/−10				−36/−22

[a]In degrees. Defined as ϕ_H: H-1–C-1–O-1–C-x, and ψ_H: C-1–O-1–C-x–H-x. Minimum energy conformations expressed as ϕ,ψ values were calculated for ϕ,ψ steps of 2°, and the minimum energy conformation is considered significant within ±10°. [b]Structures refer to →[α-D-Glc*p*-(1→4)]-α-D-Gal*p*-(1→2)-[α-D-3,6-dideoxyhexosyl-(1→3)]-α-D-Man*p*-(1→4)-α-L-Rha*p*-(1→3)-, or simpler components such as the branched pentasaccharide d→(a→)b→c→d. [c]a = Paratose. [d]a = Abequose. [e]a = Tyvelose.

pyranose[17] (unit e) coordinates were taken from neutron-diffraction data. Bond modification of α-D-mannopyranose, α-D-galactopyranose, and α-D-glucopyranose units, by converting C-1–O-3 and C-6–O-6 to C-3–H-3*e* and C-6–H-6 bonds, respectively, of 1.1-Å length, afforded coordinates for α-tyvelose, α-abequose, and α-paratose. Subsequently, X-ray coordinates for β-paratose[20] and α-tyvelose[21] became available, and these data yielded ϕ,ψ values that differed insignificantly from those of the minimum energy conformations calculated with bond-modified coordinates, in agreement with recent results[22]. The procedures for generating hydrogen-atom coordinates from X-ray structures and coordinates of bond-modified structure have been documented[12,13]. In calculations employing hexose-sugars parameters, the hydroxymethyl group was treated as a symmetrical methyl group[23]. Hydrogen atoms of hydroxyl groups were not included in the calculations. Minimum energy conformations were first computed in five-degree steps across ϕ and ψ ranges, and then refined in two-degree steps.

In order to build a model of the basic tetrasaccharide structure (Scheme 1), successive overlapping disaccharide units a→b, b→c, c→d, and d→b were constructed. The ϕ,ψ values corresponding to the minimum energy conformation of each of these disaccharides are presented in Table I. A fifth disaccharide, α-D-Glc*p*-(1→4)-α-D-Gal*p* (e–d), was also included in order to model the more complex O-chain structures that carry this structural element. Trisaccharide sequences were next constructed in which values for one ϕ,ψ pair were set at the minimum energy values obtained for the corresponding disaccharide. The second ϕ,ψ pair values were then calculated. The operation was then repeated in the reverse direction. Thus, for the sequence Abe→Man→Rha (a→b→c), the value for a→b was

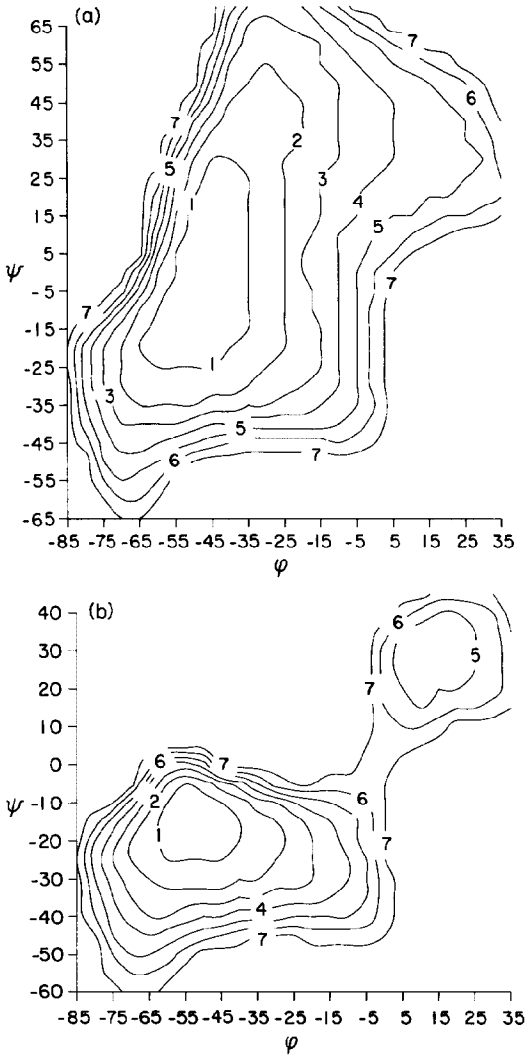


Fig. 1. (a) Isoenergy contours in kcal steps calculated for variations of ϕ,ψ values in 5-degree steps for the disaccharide α-D-Abe*p*-(1→3)-α-D-Man*p* (a→b) as a component of the linear tetrasaccharide sequence a→b→c→d. (b) Isoenergy contours calculated under the same conditions but for the disaccharide linkage a→b when it forms the branch point of the pentasaccharide sequence d→(a→)b→c→d.

held at $-49°/-18°$ and the value for the b→c glycosidic conformation was minimized. Then the value for b→c was held at $-39°/-13°$, and the value for the a→b glycoside bond was minimized. When the ϕ,ψ values coincided (within $\pm 2°$), the trisaccharide conformation was considered to be the minimum-energy conformer. Absolute energies were simultaneously computed for each conformer as a control of this procedure. The molecules of the linear trisaccharides Gal→Man→Rha and Man→Rha→Gal were constructed in analogous fashion.

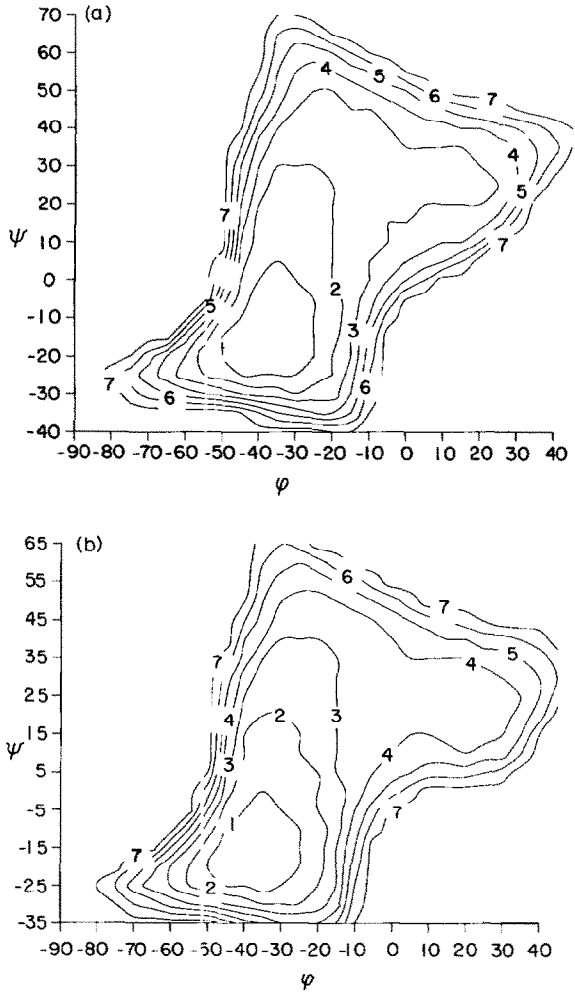


Fig. 2 (a) Isoenergy contours calculated for α-D-Man*p*-(1→4)-α-L-Rha*p* (b→c), as part of the linear trisaccharide a→b→c. (b) Isoenergy contours for the b→c linkage as a component of the main-chain trisaccharide d→b→c.

Finally, the crucial, branched trisaccharide d→(a→)b was constructed in order to examine the effects of vicinal branching at O-3 and -2 of the D-mannose unit b. Examination of the ϕ,ψ values presented for the branched tetrasaccharide d→(a→)b→c showed that the ϕ,ψ values of the trisaccharide d→(a→)b differ little from those of the component disaccharides d→b and a→b. However, the effect of this vicinal branching is to be seen in the isoenergy-contour plots for the a→b glycosidic linkage when the α-D-mannopyranose residue b either lacks or possesses a 2-*O*-galactopyranosyl group (Figs. 1a and 1b). Although no significant hard interactions occur to cause either the α-D-galactopyranosyl or α-D-abequopyranosyl

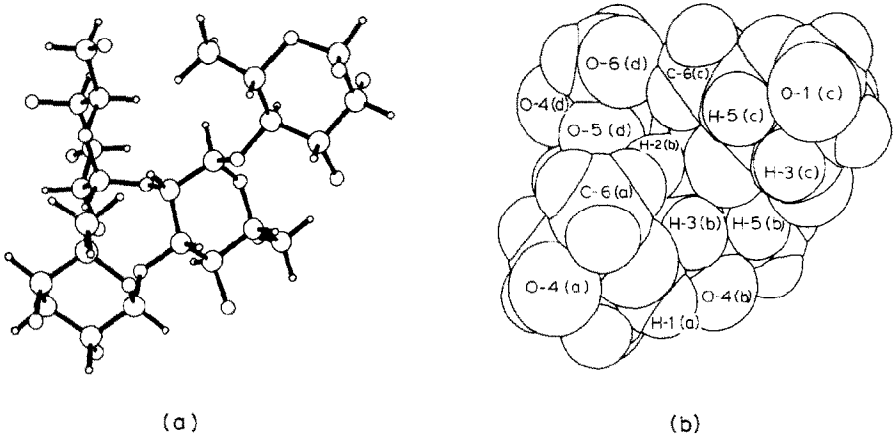


Fig. 3. The minimum energy conformation of an internal, branched tetrasaccharide repeating-unit d→(a→)b→c as a ball and stick (a), and as a space-filling model (b). Hydroxyl groups are omitted.

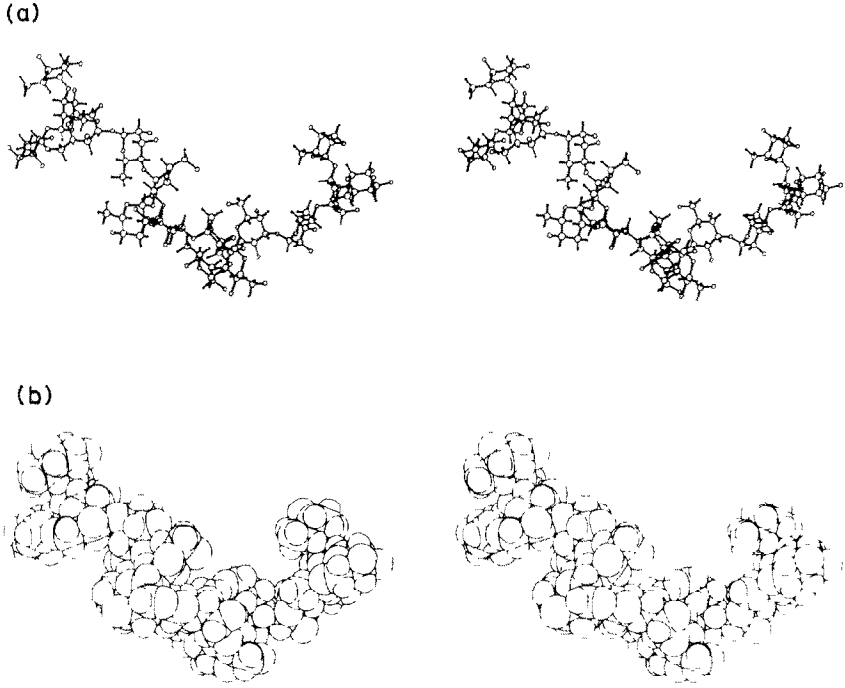


Fig. 4. Stereo projections of the idealized conformation of a hexadecasaccharide generated from four repeating units $[d\rightarrow(a\rightarrow)b\rightarrow c]_4$ with the idealized conformation of Fig. 3a, depicted as a ball and stick (a), and as a space-filling model (b).

groups substituent or α-D-mannopyranosyl residue to adopt significantly altered ϕ,ψ values, the energy well of each linkage becomes sharper and more pronounced. This indicates that previously flexible structures (ϕ,ψ ±25°) become more rigid or conformationally well-defined. The other bonds are not significantly changed by this branching (see Figs. 2a and 2b). The conformation of the linkage c→d (Rha→Gal) is largely unaffected in the transition from disaccharide through to pentasaccharide d→(a→)b→c→d, although the introduction of the α-D-glucopyranosyl group (unit e) at O-4 of the α-D-galactopyranosyl residue does cause significant interactions, and the c→d conformation changes appreciably. Thus, whereas c→d possesses ϕ,ψ values of 50°/6°, the addition of the e→d branching unit causes the ϕ,ψ values for the c→d bond to change to 28°/21° in its minimum-energy conformation. As expected, the energy surface of both the c→d and e→d linkages indicates a steeper and more pronounced minimum-energy conformation. The minimum-energy conformation of the tetrasaccharide d→(a→)b→c is represented as a ball and stick (Fig. 3a) and space-filling model (Fig. 3b), and Fig. 4a and 4b present the extension of this conformation to include four repeating-units.

DISCUSSION

Salmonella Serogroups A, B, and D_1 are structurally closely related O-antigens (Scheme 1), and since the semiempirical calculations presented here indicate that this similarity extends to conformation, the comments that follow apply in general to each serogroup. The computed conformations presented are substantiated to within a ϕ,ψ range of ±10° by n.m.r. measurements reported in the following paper[24]. These data are of particular importance since our predicted conformation differs in some respects from that reported by Lipkind and Kochetkov[8].

The generalized picture of O-antigen conformation that emerges from this work is as follows: (a) The 3,6-dideoxyhexoses protrude from the backbone of the polymer chain (see Figs. 4 and 5). (b) Although no severe atomic interactions occur between sugar residues, vicinal branching at the α-D-mannopyranosyl residue in conjunction with an α-(1→2) linkage serve to sharpen the energy surface of the glycosidic linkage a→b. This observation is in accord with principles first enunciated by Rees and Scott[23]. The result is a conformationally well-defined and quite rigid structure, especially the sequence d→(a→)b→c. (c) The α-L-Rha*p* to α-D-Gal*p* junction of the tetrasaccharide repeating sequences may be visualized as a connector between the more rigid d→(a→)b trisaccharide segments (Fig. 4). (d) A strongly hydrophobic surface exists at the α-face of the 3,6-dideoxy sugar (particularly so for abequose and tyvelose), and extends across to the 6-deoxy group of the L-rhamnose (c) unit (Figs. 3 and 5). (e) In their most ordered arrangement corresponding to the ϕ,ψ values of minimum energy, O-chains possess a helical character having ~3 repeating-units per turn (Fig. 4). An end-on view (looking at the reducing end of the O-chain) illustrates the ~120° disposition of abequose units to each other (Fig. 5 and 6). (f) Introduction of the α-D-glucopyranosyl side-group branch

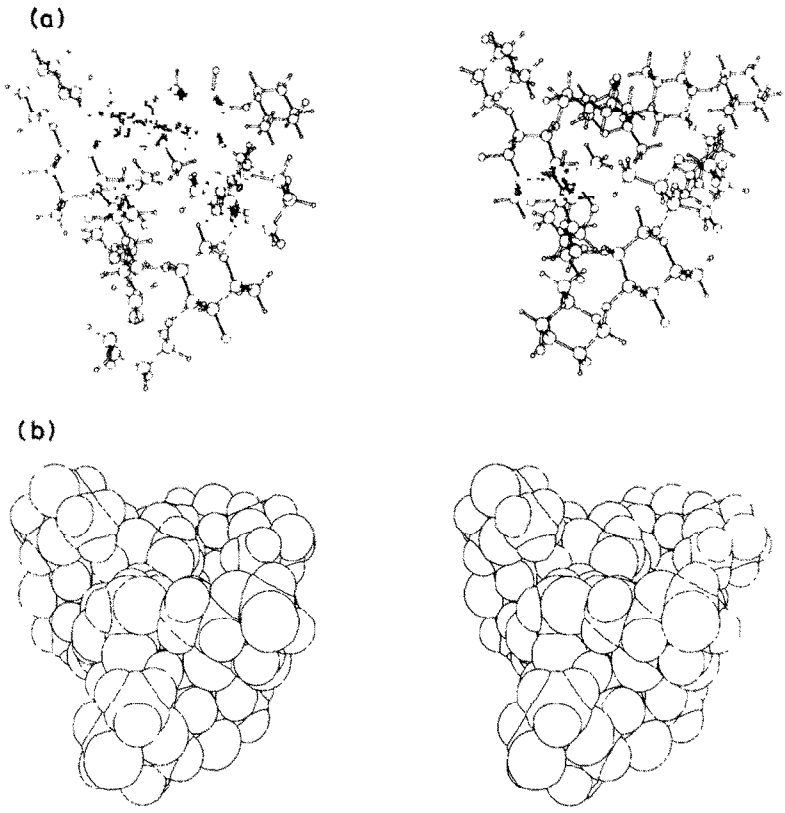


Fig. 5. Stereo projections as ball and stick (a), and space-filling model (b) of three repeating-units viewed from the reducing end of the polysaccharide O-chain.

at O-4 of α-D-galactopyranosyl units causes the antigen to become more rigid, but comparison of repeating units with and without the α-D-glucopyranosyl group show little overall-change in gross geometry. Whilst the effect of branching is to decrease ϕ from 50 to 28°, ψ opens up from 6 to 21°. (g) The α-D-glucopyranosyl side-groups are disposed between 3,6-dideoxyhexose residues of contiguous repeating units when viewed from an end-on position, and the hydroxymethyl group of this sugar disrupts the topography of the hydrophobic area associated with the dideoxyhexose and L-rhamnose residues [*cf.* (d)]. In the context of these conclusions, the known serological properties of the A, B, and D_1 antigens may now be considered.

Although there is a great deal of circumstantial evidence pointing to the structural elements that constitute the determinants of a given O-antigen[2–4], relatively few of these may be clearly defined. This results from a shortage of oligosaccharides necessary for mapping the combining sites of the respective antibodies. Consequently, speculation as to the elements that constitute the determinant of a given *O*-antigen has been rampant[3,4]. It has been recognized that *O*-antigen specifi-

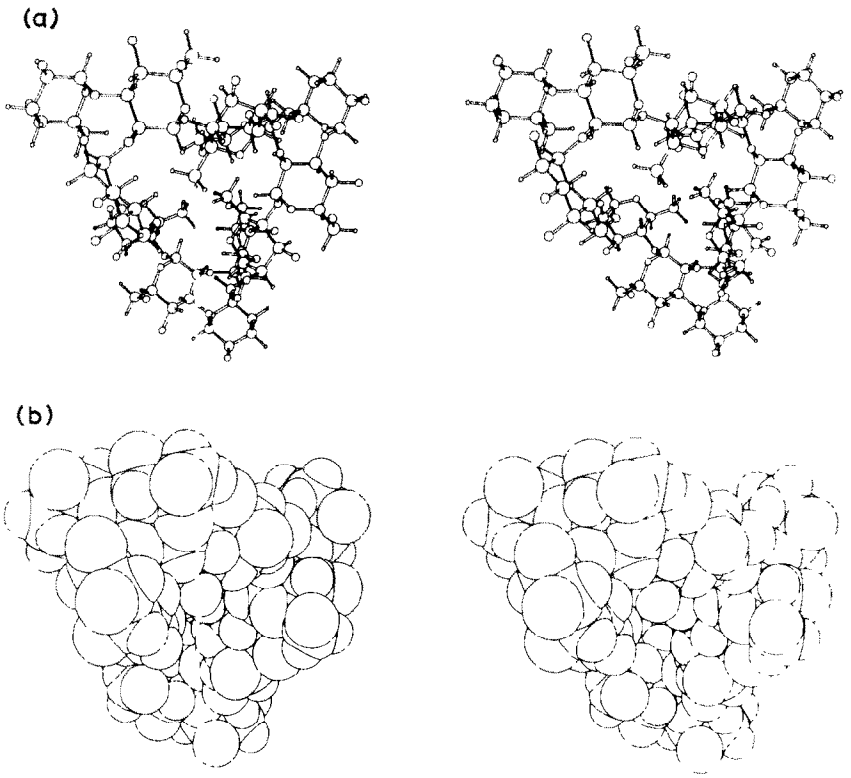


Fig. 6. Stereo projections of the model as described in Fig. 5, but viewed from the opposite direction, *i.e.*, the nonreducing end.

city extends well beyond the individual sugar that is referred to as the immunodominant sugar[3,4]. It should also be noted, as was pointed out for *S. flexneri* determinants[11], that contiguous sugar units are not always accessible to an antibody which approaches the antigen from a given direction. Similarly, with *Salmonella* the "contact" sugars of the octasaccharide sequence a→b→c→d→(a→)b→c→d could be principally abequose and L-rhamnose if the role of unit d is to hold the two Abe→Man→Rha sequences in the appropriate conformation. In the following rationalisation of conformation with O-determinant specificity, two assumptions will be made. The first is that antibodies will most likely bind oligosaccharides in their preferred conformations. Secondly, hydrophobic surfaces or surfaces that may assume less-hydrophilic character can provide an important driving force for antibody–antigen binding. These principles were recently proposed by Lemieux and associates[25–28], and experimental support for this interpretation has been reported[27,28].

It is established that determinants O2, O4, O5, and O9 are the major specificities of Serogroups A, B, and D_1. Although paratose is the principal component

of factor 2 (serogroup A), this is the least well-defined of the three serogroups[29]. Both serogroup B (determinants O4 and O5) involving respectively abequose and 2-*O*-acetylabequose, and serogroup D_1 (determinant O9; tyvelose) have sharply defined specificities. The accessibility of the 3,6-dideoxy sugar undoubtedly contributes to the dominant role that this sugar plays in the specificity of the respective O-antigens. Furthermore, the difficulty that paratose units would have in assuming an hydrophobic character (because of the difficulty of intramolecular hydrogen-bonding[30]), when compared to either abequose or tyvelose, could explain the relatively lower definition of determinant O2. Examination of the model conformation (Fig. 3) shows also that the 6-deoxy group of the 3,6-dideoxy sugar and L-rhamnose residues are in close spacial proximity, thus providing an extended hydrophobic surface.

Infection of *Salmonella* Serogroup A, B, or D_1 bacteria with phage $\phi 27$ causes a change from α-(1→2) to α-(1→6) in the Gal→Man linkage. Since it now lacks a vicinal substituent at O-2 of the D-mannose residue, the Abe→Man linkage is less restricted in terms of the permissible ϕ,ψ amplitudes. The additional degree of freedom introduced by the third torsional angle, ω, also renders the polysaccharide backbone more flexible and extends the repeating-unit length along the longitudinal axis of the polymer. As expected, this significant structural and conformational change modifies[3] the respective O-Factors O2, O4, and O9. Another phage that induces the formation of an α-(1→6) bond, albeit not in the chain but as a branch point, is phage P22. Thus, O-antigens that formerly carried a 4-*O*-α-D-glucopyranosyl group on the D-galactopyranosyl residue (d) now carry this group at O-6. The (1→4) branching caused significant constraints to be placed on the ϕ,ψ values of the Rha→Gal (c→d) linkage [*cf.*, ϕ,ψ values for the disaccharide c→d and the same bond present in the hexasaccharide c→(e→)d→(a→)b→c, Table I]. Acquisition of the third degree of freedom concomitant with the (1→6)-linked α-D-glucopyranosyl side-group allows the Glc–Gal (e→d) linkage to be more flexible[31,32], which in turn permits the main chain Rha→Gal (c→d) bonds to assume less-restricted values. The combination of these conformational effects, and the altered topography that accompanies the change of linkage of the α-D-glucopyranosyl group (d) from O-4 to O-6 is correlated with the well-known change in antigenic specificity, the O-Factor 12_2 changing to O-factor 1_{12}.

Thus, it is possible to use the model developed for O-chain conformation to rationalize these gross serological-changes with altered chain-geometry. Elaboration of this model and more extensive interpretation of structure-serology relationships are now possible.

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