Molecular recognition. III. The binding of the H-type 2 human blood group determinant by the lectin I of *Ulex europaeus*

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Using a radioimmunoassay to measure the relative potencies of a wide range of chemically modified structures related to the H-type 2 human blood group determinant, evidence was accumulated that the binding of αLFuc(1→2)βDGal(1→4)-βDGlcNAc-OMe by the lectin I of *Ulex europaeus* involves a wedge-shaped amphiphilic surface which extends on one side of the molecule from the methoxy aglycon to OH-3 of the βDGal unit. A cluster which involves OH-3, OH-4, and OH-2 of the αLFuc unit along with OH-3 of the βDGal unit provides the polar interactions with the lectin. However, only OH-3 and OH-4 of the αLFuc are indispensable to complex formation and are regarded as providing the key polar interaction. The binding reaction involves both a decrease in enthalpy of 29 kcal/mol and a decrease of 68 cal/mol/K in entropy. It is submitted that the main source of the decrease in enthalpy is the establishment of nonpolar interactions that extend from the aglycon over the nonpolar portion of the β-side of the βDGlcNAc unit and on to include a major portion of the α-side of the αLFuc unit. The binding of the βDGlcNAc unit includes OH-6 intramolecularly hydrogen bonded to O-5 in order to extend the nonpolar interactions to the α-side of this unit and perhaps beyond. The decreases in enthalpy (6.0 kcal/mol) and entropy (2.7 cal/mol/K) which occur on the binding of methyl α-L-fucopyranoside are much smaller than for the H-type 2 trisaccharide and are compatible with the much smaller surface that interacts to form the complex. The inhibition data obtained using a range of structures related to methyl α-L-fucopyranoside are in general accord with expectations based on the results obtained with the more complex structures.

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Utilisant un essai radioimmunologique pour mesurer la puissance relative d'un grand nombre de composés chimiquement modifiés et apparentés au déterminant du groupe sanguin humain de type H-2, on a accumulé des données suggérant que la liaison du αLFuc(1→2)βDGal(1→4)βDGlcNAc-OMe avec la lectine I de l'*Ulex europaeus* implique une surface amphiphile en forme de coin qui s'étend sur un côté de la molécule du groupement méthoxyle de l'aglycone jusqu'au groupement OH-3 de l'unité βDGal. Un aggrégat impliquant les groupements OH-3, OH-4 et OH-2 de l'unité αLFuc ainsi que le groupement OH-3 de l'unité βDGal est responsable des interactions polaires avec la lectine. Toutefois, seuls les groupements OH-3 et OH-4 de l'unité αLFuc sont indispensables à la formation d'un complexe et on les considère comme les éléments clés des interactions polaires. La réaction de liaison implique à la fois une diminution d'enthalpie de 29 kcal/mol et une diminution d'entropie de 68 cal/mol/K. On pense que la diminution d'enthalpie est due principalement à l'existence d'interactions non polaires qui vont de l'aglycone qui se trouve au-dessus de la portion non polaire de la face β de l'unité βDGlcNAc et qui incluent aussi une partie importante de la face α de l'unité αLFuc. La liaison de l'unité βDGlcNAc comprend le groupement OH-6 qui est lié par des liaisons hydrogènes intramoléculaires au O-5 de façon à étendre les interactions non polaires à la face α de cette unité et peut être même au-delà. Les diminutions d'enthalpie (6 kcal/mol) et d'entropie (2,7 cal/mol/K) qui sont observées lorsqu'il se produit une liaison de l'α-L-fucopyrannoside de méthyle sont plus faibles que celles qui sont observées avec le trisaccharide de type H-2; ces valeurs sont compatibles avec le fait que la surface impliquée pour former des complexes est beaucoup plus petite. Les données concernant le pouvoir inhibiteur qui ont été obtenues en utilisant une grande variété de structures apparentées au α-L-fucopyrannoside de méthyle sont en général en accord avec les hypothèses fondées sur les résultats obtenus avec des structures plus complexes.

[Traduit par le journal]

Introduction

The lectin I of *Ulex europaeus* (common gorse) was found (1) to agglutinate O human red cells and proved to be a useful reagent for the classification of people of the O blood group as secretors or nonsecretors (2). This proved to be a fortunate circumstance, which is now known to arise because the lectin has a strong affinity for the H-type 2 determinant (αLFuc(1→2)βDGal(1→4)βDGlcNAc--) but does not recognize the H-type 1 determinant (αLFuc(1→2)βDGal(1→3)βDGlcNAc--) (3). The lectin is available commercially and its binding characteristics have received major previous attention (4). Our recent studies (5–7) of the binding of oligosaccharides by lectins and antibodies have shown that complex formation may require a polar interaction involving hydroxyl groups that are present in different sugar units. In such cases, the protein binds neither the simple sugars that make up the oligosaccharides nor their methyl glycosides. Thus, binding of the H-type 2 determinant by the lectin I of *Ulex europaeus* was of special interest since it was known (4) that both fucose and methyl α-L-fucopyranoside are effective as inhibitors of the agglutination of O red cells by the lectin.

Recently, we reported on the ability of a number of modified H-type 2 structures to inhibit the agglutination of O red cells (8). This study confirmed that the acetamido group is not involved in the binding reaction (4). Also, it became apparent that neither OH-3 of the βDGlcNAc unit nor OH-4 of the βDGal unit interact with the protein. Structural changes involving the αLFuc were not extended beyond replacement of its CH_3-6 group by hydrogen. It was concluded that the binding involves the recognition by the lectin of a wedge-shaped lipophilic surface which includes OH-6 of the βDGlcNAc unit intramolecularly hydrogen bonded to O-5. We now wish to report

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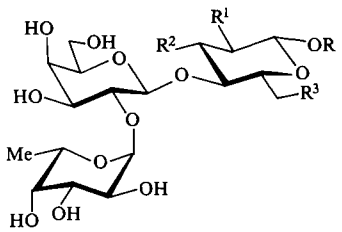
TABLE 1. Inhibition of the binding of an H-type 2 artificial antigen by the Lectin I of *Ulex europaeus*. Involvement of the βDGlcNAc-OR portion of **1**

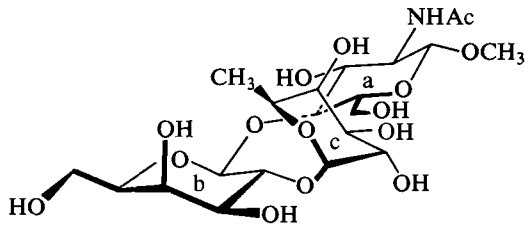


Compound	Substituent R	R¹	R²	R³	50% Inhibition μmol/L	Relative potency	$\Delta\Delta G^0$ kcal/mol at 23°C
1	CH_3	NHAc	OH	OH	9	67	0.24
2	$(CH_2)_8COOCH_3$	NHAc	OH	OH	6	100	0
3	$(CH_2)_8COOH$	NH_2	OH	OH	6*	—	—
4	$(CH_2)_8COOCH_3$	NHAc	H	OH	13	46	0.46
5	$(CH_2)_8COOCH_3$	NHAc	αLFucO	OH	23	26	0.79
6	$(CH_2)_8COOCH_3$	NHAc	OH	H	1.7	352	−0.75
7	αLFuc(1→2)βDGal-O$(CH_2)_8COOCH_3$				290	2	2.3
8	αLFuc(1→2)α,βDGalOH				730	0.8	2.8

* Based on the previous observation (8).

a continuation of these studies but using a solid-phase radioimmunoassay to determine relative inhibition potencies. This paper includes the examination of the eight possible monodeoxy derivatives of the H-type 2 determinant αLFuc(1→2)-βDGal(1→4)βDGlcNAc (9). Also, a wide number of derivatives of methyl α-L-fucopyranoside were examined.

In our previous study (8), the inhibitors were synthesized with $(CH_2)_8COOCH_3$ as aglycon. A number of these compounds were used in this investigation but most of the structures are new (9) and contain the simple methyl group as aglycon. In Tables 2 and 3, the reference inhibitor for the expression of relative potencies is the methyl glycoside (**1**) of the H-type 2 trisaccharide and the sugar units are labelled a, b, and c, as shown. The formula for **1** is drawn as a projection that



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imitates the conformational preference suggested by HSEA calculation and supported by ^1H nmr (10, 11). It is noteworthy in this regard that the lectin IV of *Griffonia simplicifolia* binds the Lewis b and Y determinants nearly equally well and that this finding is in agreement with the conformational analyses, based on ^1H nmr, for these two structures (5). We therefore have confidence that the conformer for **1** with $\phi^b/\psi^b = 55°/0°$ and $\phi^c/\psi^c = 50°/15°$ (11) well represents the compound in aqueous solution. Furthermore, the ^1H and ^{13}C nmr parameters found (9) for the various modifications of **1** used in this study indicate that this conformational preference is maintained. The specific deshielding of H-5 of the αLFuc unit by O-4 of the βDGlcNAc unit (8, 11) supports our contention that these are rigidly held conformers.

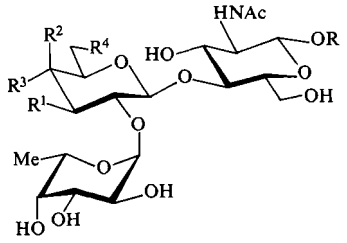
Results and discussion

The radioimmunoassay used to determine the inhibition data presented in Tables 1–4 was described in detail in paper II of this series (5) and the 50% inhibitions proved reproducible to within ±5% of the values listed.

The results presented in Table 1 confirm the conclusions reached in our previous publication (8). Previously, the activity of the Y determinant (**5**) was taken as evidence that OH-3[a] is not involved in the binding reaction. On the basis of the criteria set for noninvolvement of a hydroxyl group (5), this conclusion is now confirmed by the high potency displayed by the 3[a]-deoxy derivative **4**. The fact that **2** is only slightly more active than **1** suggests that binding of the aglycon does not extend importantly beyond the aglyconic carbon grouping, as was found, for example, for the anti-I Ma antibody (12). In contrast, in the case of a monoclonal anti-Lewis b antibody (6), the change in aglycon from CH_3 to $(CH_2)_8COOCH_3$ increased the potency by a factor of 6.5. As previously reported (8), deoxygenation of the CH_2OH^a group importantly increased the potency. On the basis of the presently used radioimmunoassay, elimination of the requirement of OH-6[a] to become intramolecularly hydrogen bonded to O-5[a] increases the potency by a factor of 3.5 and, therefore, appears to increase the driving force for reaction by about 0.75 kcal/mol. Replacement of the βDGlcNAc-O$(CH_2)_8COOCH_3$ portion of **2** by the O$(CH_2)_8$-$COOCH_3$ group led to the much less potent inhibitor **7**. However, replacement by an hydroxyl group provided an even weaker inhibitor (**8**). These results were not surprising since it had become evident that binding about the aglycon of the βDGal unit of **1** is extensive and nonpolar in nature (8). Evidently, the $(CH_2)_8COOCH_3$ group of **7** does not well provide a conformation that is complementary to the hydrophobic cleft of the lectin which binds the βDGlcNAc unit of **1**.

As seen from Table 2 and as previously noted (8), the 4[b]-epimer of **2**, namely, **10**, is only about twice less active than **2**. This observation was interpreted as noninvolvement of OH-4[b] and, therefore, probably also OH-6[b]. This latter prediction is now confirmed by the high potency displayed by the 6[b]-deoxy

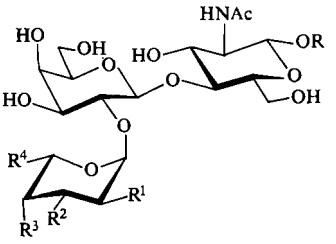
TABLE 2. Inhibition of the binding of an H-type 2 artificial antigen by the lectin I of *Ulex europaeus*. Involvement of the βDGal unit of **1**



Compound	Substituent R	R¹	R²	R³	R⁴	50% Inhibition μmol/L	Relative potency	$\Delta\Delta G^0$ kcal/mol at 23°C
1	CH_3	OH	OH	H	OH	9	100	0
9	CH_3	H	OH	H	OH	360	2.5	2.2
10	$(CH_2)_8COOCH_3$	OH	H	OH	OH	15	40*	0.5
11	CH_3	OH	OH	H	H	15	60	0.3

* Relative to **2** (Table 1).

TABLE 3. Inhibition of the binding of an H-type 2 artificial antigen by the lectin I of *Ulex europaeus*. Involvement of the αLFuc unit of **1**



Compound	Substituent R	R¹	R²	R³	R⁴	50% Inhibition μmol/L	Relative potency	$\Delta\Delta G^0$ kcal/mol at 23°C
1	CH_3	OH	OH	OH	CH_3	9	100	0
12	CH_3	H	OH	OH	CH_3	1420	0.63	3.0
13	CH_3	OH	H	OH	CH_3	16000*	0.06	4.4
14	CH_3	OH	OH	H	CH_3	Inactive†	—	—
15	$(CH_2)_8COOCH_3$	OH	OH	OH	H	160	3.8‡	1.9
16	αLFuc-OCH_3					950	0.95	2.7
17	βDAra-OCH_3					42000	0.02	5.0

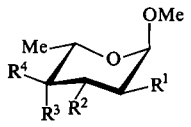
* Extrapolated from 20% inhibition at 4400 and classified as inactive.
† 8% Inhibition at 4400.
‡ Relative to **2** (Table 1).

compound (**11**). On the other hand, deoxygenation of the OH-3[b] position of **1** to form **9** decreased the potency by a factor of 40. Thus, OH-3[b] is importantly involved in a polar interaction with the combining site. For reasons previously discussed (5), a polar interaction that is not essential to complex formation is not included in the so-called key polar interaction. As will be seen below, OH-2[c], which projects in space close to OH-3[b], is also involved in an important but not essential interaction with the lectin.

The effects on binding that arose from modifications of the αLFuc unit are reported in Table 3. It is seen at once that all three hydroxyl groups become involved in polar interactions with the lectin. However, only deoxygenation at positions 3[c] and 4[c] yielded essentially inactive compounds, namely, **13** and **14**. In contrast, replacement of OH-2[c] of **1** by hydrogen to produce **12** caused a marked decrease in potency but the compound retained an activity near that of methyl α-L-fucopyranoside (**16**). Judging from the $\Delta\Delta G^0$ values, this complex is nearly 3 kcal/mol less stable than that formed from **1**. As will be discussed in more detail below, OH-2[c] projects close to OH-3[b] and these two hydroxyl groups appear to form a polar grouping adjacent to the key polar grouping provided by OH-3[c] and OH-4[c]. Important involvement of the CH_3-6[c] group is indicated by the large decrease in potency that results from the replacement of this group in the inhibitor **2** (Table 1) by hydrogen to form **15**. This observation is confirmed by the large difference in the inhibitions provided by methyl α-L-fucopyranoside (**16**) and methyl β-D-arabinopyranoside (**17**), the latter compound being essentially inactive.

The results obtained using a variety of derivatives of methyl α-L-fucopyranoside (**16**) are reported in Table 4. As was to be expected from the results obtained using the 3[c]- and 4[c]-deoxy

TABLE 4. Inhibition by L-fucose and derivatives of the binding of an H-type 2 artificial antigen by the lectin I of *Ulex europaeus*



Compound	Substituent R¹	R²	R³	R⁴	50% Inhibition μmol/L	Relative potency	ΔΔG° kcal/mol at 23°C
16	OH	OH	OH	H	950	100	0
18	H	OH	OH	H	7300	13	1.2
19	OH	H	OH	H	Inactive*	—	—
20	OH	F	OH	H	31000	3	2.1
21	OH	OH	H	H	Inactive*	—	—
22	OH	OH	F	H	Inactive*	—	—
23	OH	OH	OH	CH_3	Inactive†	—	—
24	βLFuc-OCH_3				11500	8.3	1.5
25	α,βLFuc-OH				4300	22	0.9

* Inactive at 55 000.
† Inactive at 35 000.

TABLE 5. Thermodynamic parameters for binding by the lectin I of *Ulex europaeus*

	$K_{Assoc.}$*	ΔH kcal/mol	ΔS cal/mol/K	ΔG°* kcal/mol
αLFuc-OCH_3 (**16**)	$6.4 \pm 0.2 \times 10^3\ M^{-1}$	−6.0	−2.7	−5.2
H-type 2-OCH_3 (**1**)	$1.8 \pm 0.8 \times 10^6\ M^{-1}$	−29	−68	−8.5

* At 298 K.

derivatives of **1**, namely, **13** and **14** (Table 3), the 3-deoxy (**19**) and 4-deoxy (**21**) derivatives of **16** proved inactive. In the case of the 2-deoxy derivative (**18**) of **16**, substantial residual activity (13% of **16**) was found. This decrease in activity is much less than that observed on deoxygenation of **1** to form the 2^c-deoxy derivative **12** where the decrease in potency was to less than 1% that of **1**. It appears, therefore, that the combining site formed to accept **1** with the establishment of a polar interaction with the OH-3^b–OH-2^c grouping is not completed for the acceptance of the simple methyl α-L-fucopyranoside (**16**). In fact, the thermodynamic data presented in Table 5 strongly support this indication that the extent of the organization of the lectin to accept **1** is much greater than that for the acceptance of **16**. It is seen that the decrease in entropy to form the complex with the trisaccharide **1** involves 68 entropy units in contrast to only 2.7 entropy units for the simple methyl α-L-fucopyranoside. These thermodynamic parameters were determined by measuring the change in ultraviolet absorption (A_{286}–A_{290} nm) under the same experimental conditions described in our study of the lectin IV of *Griffonia simplicifolia* (5). In contrast to the experience with this lectin, the difference ultraviolet spectra for the binding of **1** and **16** by the *Ulex* lectin were very similar. Experimental details and the discussion of the results obtained using **1**, **16**, and a number of other structures that are bound by *Ulex* are reserved for a separate communication (13).

It is considered interesting to note that the thermodynamic parameters for the binding of oligosaccharides by a variety of lectins have recently appeared in the literature. Some of the findings are summarized in Fig. 1 where it is seen that a fairly linear relationship exists between the ΔH and $T\Delta S$ terms. It appears, therefore, that any gain in complex stability resulting from a decrease in enthalpy is always accompanied by some cancelling from a decrease in entropy. Thus, it can be expected that the larger the combining site, the greater the effort may be for organization as reflected by a greater decrease in entropy. The thermodynamic parameters reported herein for the *Ulex* lectin and those previously reported (5) for the lectin IV of *Griffonia simplicifolia* appear in accord with this observation. The findings for **1** (Table 5) are the highest values reported to date ($\Delta H = -29$ and $T\Delta S = -20$ kcal/mol at 25°C).

Returning to Table 4, it is seen that replacement of either OH-3 or OH-4 of **16** by fluorine, to form **20** and **22**, led to essentially inactive compounds. It is likely, therefore, that these key hydroxyl groups act as proton donors in the complex formation.

As previously mentioned (8), the α-side of an α-L-fucopyranosyl group presents substantial nonpolar surface to its environment. As noted above, the CH_3-6 group of both **1** and **16** appears involved in the binding reaction and this result would be in keeping with the *Ulex* lectin recognizing the nonpolar α-side of the αLFuc unit. That this is the case is supported by the fact (Table 4) that αLFuc-OCH_3 (**16**) is much more strongly bound than its β-anomer (**24**). In fact, even free L-fucose (**25**) is more strongly bound than is **24**. Evidently O-1 of L-fucose becomes involved in the binding reaction but this must surely be by way of a nonpolar interaction since **16** is about 5 times more potent than **25** as an inhibitor. It is apparent, therefore, that the binding of **16** involves a polar interaction between OH-3 and OH-4 with a polar grouping provided by the lectin and which allows the establishment of nonpolar interactions between a complementary surface provided by the protein and the surface of **16** defined by H-3, H-4, H-5, CH_3-6, O-1, and the methyl aglycon. In order to test this hypothesis,

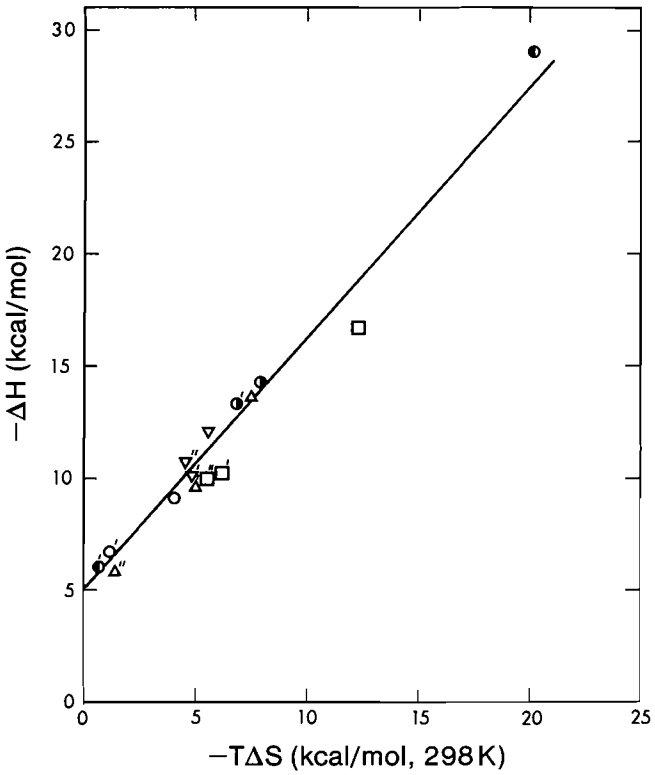


FIG. 1. A plot of selected literature values for the changes in enthalpy and entropy at 25°C which occur on the binding of a variety of carbohydrates by a variety of plant lectins. Lectin I of *Ulex europaeus:* ◐ αLFuc(1→2)βDGal(1→4)βDGlcNAc-OMe (this work); ◐′ αLFuc-OMe (this work). Lectin IV of *Griffonia simplicifolia:* ◑ αLFuc-(1→2)βDGal(1→3)[αLFuc(1→4)]βDGlcNAc-OMe (5); ◑′ Methoxymethyl(1→2)βDGal(1→3)[αLFuc(1→4)]βDGlcNAc-OMe (5). Peanut agglutinin: □ βDGal(1→4)βDGlc-OMe (14); □′ βDGal-OMe (15, 16); □″ αDGal-OMe (15, 16). *Erythrina cristagalli* agglutinin: △ βDGal(1→4)βDGlcNAc(1→2)DMan (17); △′ βDGal(1→4)DGlc (17); △″ α,βDGalNAc (17). Soybean agglutinin: ▽ βDGalNAc-OPh-*p*-NO_2 (18); ▽′ αDGal-OMe (18); ▽″ αDGalNAc-OMe (18). Concanavalin A: ○ αDMan-OMe (19); ○′ αDMan-OPh-*p*-NO_2 (20).

the 4-*C*-methyl derivative of **16**, namely, **23**, was synthesized.[4] It is seen that the simple substitution of H-4 by CH_3 led to an inactive compound. It is possible that the substitution interferes with the polar interaction and that this is the main reason why **23** is inactive. On the other hand, this inactivity could result from the methyl group preventing the establishment of a proper interfacing of complementary nonpolar surfaces. We submit that the latter is the case since the small decrease in entropy (2.7 entropy units) that accompanies the binding of **16** suggests that the combining site is virtually already present in the lectin. Thus, the formation of the complex must be highly dependent on the complementarity of the interacting surfaces. Only a slight change in the topography of one of these surfaces may so weaken a complex, which is formed through a decrease in enthalpy of only 6 kcal/mol, that complex formation is not observed.

The results of this study are summarized in Fig. 2 where the H-type 2-OCH_3 compound (**1**) is presented in the conformer indicated by ^{1}H nmr (11) and produced by HSEA calculation. It is seen that the polar interactions involve OH-4^c, OH-3^c, OH-2^c, and OH-3^b and that these groups form a polar cluster at

[4] G. Deslongchamps, unpublished.

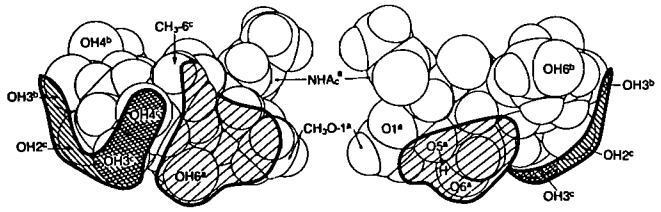


FIG. 2. The HSEA molecular model for αLFuc(1→2)βDGal(1→4)βDGlcNAc-OMe (**1**) (H-type 2). Opposite sides of the model are shown and the hatched areas display the surface which comes into interaction for complex formation with the lectin I of *Ulex europaeus*. The cross-hatched key polar interaction involving OH-4^c and OH-3^c of the fucose unit is seen to occur adjacent to the lipophilic region (lightly hatched). The OH-3^b and OH-2^c groups are seen to be adjacent to the key polar group. These hydroxyl groups also become involved in important polar interactions with the lectin but this involvement appears to arise from a conformative response that is not essential for the formation of a complex.

one end of the molecule. The OH-4^c and OH-3^c groups provide the key polar interaction which is indispensible to complex formation. In contrast, the OH-2^c and OH-3^b region, while providing important stability to the complex, are not essential. The lightly shaded area, part of which is expected to extend behind the CH_3-6^c group, represents the lipophilic surface which appears to make the major contribution to the stability of the complex. This surface may well extend to include more of the β-side of the βDGlcNAc unit and the α-side of the βDGal unit than is indicated. In order to extend the nonpolar interactions to this side of the structure, OH-6^a forms an intramolecular hydrogen bond with O-5^a. Although not reexamined in this present study, it was previously established (8) that the H-type 1 determinant (αLFuc(1→2)βDGal(1→3)βDGlcNAc-O$(CH_2)_8$COOH) is not bound but that the free amine form (αLFuc(1→2)βDGal(1→3)βDGlcNH_2-O$(CH_2)_8$COOH) interacts quite strongly with the lectin. This observation was taken as evidence that the de-*N*-acetylation exposed the α-side of the αLFuc unit for binding by the lectin while providing a lipophilic surface very similar in topography to that provided by **2**. Thus, the evidence appears convincing that the surface of **1** that is recognized by the lectin is amphiphilic in character, as represented in Fig. 2. There can be no doubt that the NHAc-2^a, OH-3^a, OH-4^b, and OH-6^b groups remain in contact with the aqueous phase. The nonpolar interactions may involve a greater surface than that shown but no evidence in this regard was obtained in this present study.

Experimental

Inhibitors

The various compounds used as inhibitors were synthesized following well-established procedures (8, 9). The structures and their purity were confirmed by appropriate ^{1}H nmr (400 MHz) and ^{13}C nmr (100 MHz) studies.

Lectin I of Ulex europaeus was purchased from Sigma Chemical Company.

Radioimmunoassay

The procedure followed was that previously reported (5) using an ^{125}I-labelled (αLFuc(1→2)βDGal(1→4)βDGlcNAc-O$(CH_2)_8$CO-NH)$_{11}$BSA antigen purchased from Chembiomed Ltd. (University of Alberta, Edmonton, Alberta, T6G 2G2). The solution provided nearly 200 000 cpm/100 μL and the tubes were incubated at room temperature (23°C). In the absence of the inhibition, about 65% of the radioactivity was bound.

Acknowledgements

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