Notes

Molecular Modeling Studies of "Flap Up" Mannosyl Cation Mimics

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The importance of inhibitors of glycosidases as therapeutic agents for viral, proliferative, and metabolic diseases is being increasingly recognised. Several years ago we reported that the activities of mannosidase inhibitors may be explained in terms of their similarity to the mannosyl cation intermediate postulated to form during the enzyme-catalyzed processing of oligosaccharide substrates. Recently, the validity of this model has been called in to question by some authors. We report recent molecular modeling studies undertaken to clarify this apparent contradiction. Mannostatin can indeed bind in a fashion which bears a close similarity to the mannosyl cation. Moreover we have shown that (−)-mannostatin is not able to adopt a similar binding mode to that of the mannosyl cation. As additional proof, Farr *et al.* have synthesized a trihydroxycyclopentylamine as a direct mimic of our mannosyl cation model. Satisfyingly, this compound shows potent inhibition of Jack Bean α-mannosidase, as predicted by the model. The inactivity of aminotrihydroxyhexahydro-1*H*-azepine against mannosidases can be explained in terms of the relative energies of the axial versus equatorial conformations of the critical hexahydroazepine ring substituents.

Introduction

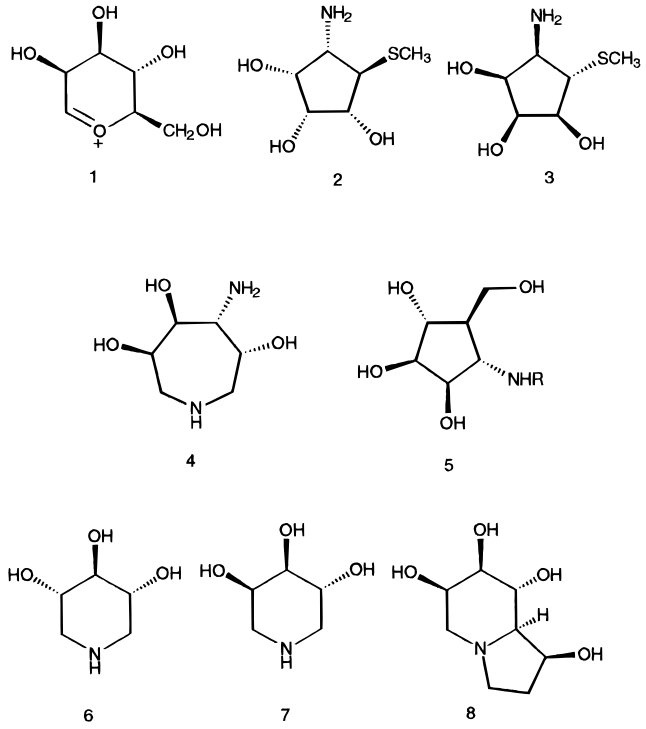
Compounds which inhibit glycosidases are becoming of increasing interest as antiviral, anticancer, immunoactive, and antihyperglycaemic therapeutic agents.[1−3] Several years ago, Winkler and Holan reported[4] a study of mannosidase inhibitors, in which they proposed, on the basis of molecular orbital calculations and molecular modeling, that the best inhibitors resembled one of the low-energy conformers of the mannosyl cation (**1**, Chart 1). The model was successful in explaining the activity of potent inhibitors and the poor activity of other compounds (such as 6-epi-castanospermine) which superficially resemble good inhibitors. It also suggested which electronegative binding groups in the inhibitor structures were essential for activity and selectivity and which were less important.

Several recent papers[5−8] have reported the mannosidase activity of apparently paradoxical inhibitors of mannosidases which do not appear to conform to the mannosyl cation structural features we proposed.

Ganem and his co-workers[5−7] have reported in several papers that the potent mannosidase inhibitor (+)-mannostatin A (**2**) bears little similarity to the mannosyl cation structure. Knapp and Murali Dhar[8] reported that, paradoxically, its inactive enantiomer (−)-mannostatin A (**3**) appeared to more closely mimic the mannosyl cation structure than the highly active (+) enantiomer. This was also reported by Ganem.[7]

Farr and his co-workers at Merrell Dow[9] synthesized a trihydroxyhexahydro-1*H*-azepine (**4**) as mimic of the "flap up" mannosyl cation transition state. It showed negligible mannosidase activity, and they concluded that either the hexahydroazepine ring conformation was not optimal for interaction with the enzyme or that a binding group topographically equivalent to the 6-OH of the mannosyl cation was essential for activity, contrary to our findings.

Chart 1



The purpose of this communication is to illustrate how the potent activity of mannostatin, the inactivity of its enantiomer, and the inactivity of hexahydro-1*H*-azepine mimics of the mannosyl cation are consistent with the mannosyl action model originally proposed.

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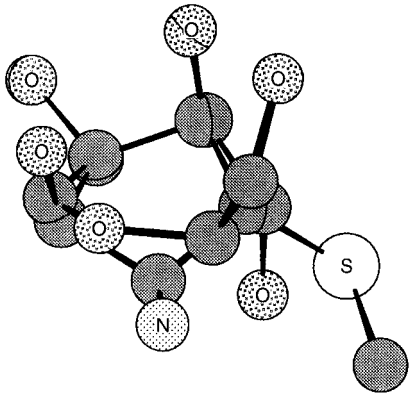

Figure 1. Superimposition of (+)-mannostatin (**2**) on the mannosyl cation structure **1**. Hydrogen atoms have been omitted for clarity.

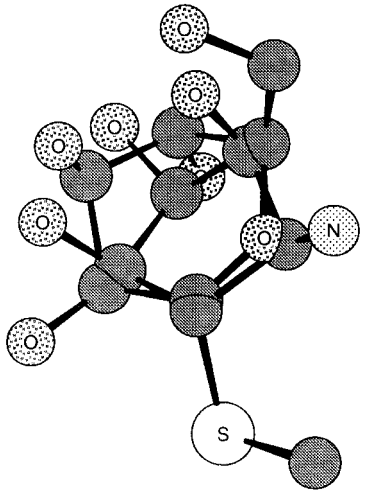

Figure 2. Superimposition of (−)-mannostatin (**3**) on the mannosyl cation structure **1** in the orientation suggested by Knapp and Murali Dhar.[8] Hydrogen atoms have been omitted for clarity.

Results

Figures 1 and 2 show the superimposition of the (+) and (−) enantiomers of mannostatin A on the flap up mannosyl cation model. The biologically active (+) enantiomer clearly superimposes well on the mannosyl cation model. There is very good overlap of two of the three OH groups of mannostatin onto the 2- and 3-OH groups of the mannosyl cation. These OH groups are those considered most important for activity and selectivity in our original study.[4] The remaining OH group on mannostatin lies in the region of the anomeric carbon of the α-mannosidase substrates. The amino nitrogen atom of mannostatin occupies a region of space near the oxonium ion, as predicted. The sulfur atom of the thioether side chain of mannostatin lies near the 4-OH of the mannosyl cation. In mannostatin B, in which this sulfur is oxidized to the sulfoxide, the sulfoxide oxygen atom lies very close to the 4-OH of the mannosyl cation. Such a conversion of to the sulfoxide is likely to occur *in vivo*. The hydroxymethyl group on the mannosyl cation does not superimpose on any structural features of mannostatin. This is consistent with our original work which proposed that this functional group may not be essential for activity. The modeling studies (Figure 2) also show that it is not possible to superimpose the biologically inactive (−) enantiomer in any effective way on the mannosyl cation model.

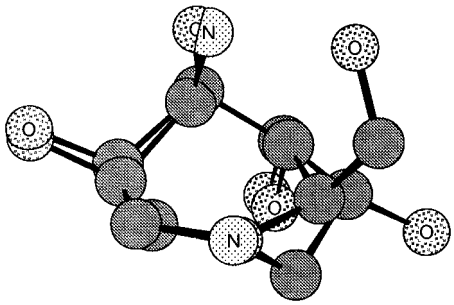

Figure 3. Superimposition of diaxial conformation of hexahydroazepine **4** on the mannosyl cation structure **1**. Hydrogen atoms have been omitted for clarity.

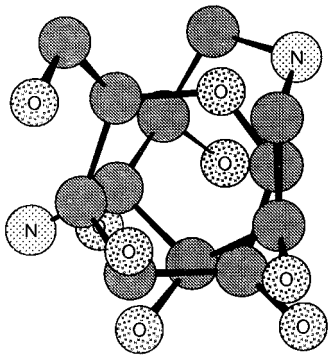

Figure 4. Superimposition of diequatorial conformation of the hexahydroazepine **4** on the mannosyl cation structure **1**. Hydrogen atoms have been omitted for clarity.

Figures 3 and 4 show the superimposition of the "diaxial" and "diequatorial" hexahydro-1*H*-azepine model compounds on the mannosyl cation structure. Clearly, as Farr *et al.*[9] reported, the diaxial conformer superimposes well on the mannosyl cation except for the 6-OH. In this case all heteroatoms in both structures, except for the hydroxymethyl of the mannosyl cation and the 6-OH of the hexahydroazepine, occupy very similar regions of space. Our molecular orbital calculations show that the diequatorial conformation is 1.82 kcal/mol lower in energy than the diaxial conformer. While caution must be exercised when interpreting small energy differences in flexible rings, such an energy difference is consistent with often observed preference of ring substituents for equatorial conformations. As Figure 4 illustrates, it is not possible to superimpose a significant number of potential binding groups on the hexahydroazepine in the diequatorial conformation onto corresponding groups in the mannosyl cation model.

Discussion

The effective superimposition of (+)-mannostatin A on our mannosyl cation structure is consistent with its potent mannosidase activity. Similarly, the inability to adequately superimpose its (−) enantiomer on the mannosyl cation suggests a rationale for its inactivity. Additional evidence for the validity of the mannosyl cation model was reported by Farr, Peet, and Kang.[14] They designed and synthesized a trihydroxycyclopentylamine (**5**) *specifically* as a mimic of the mannosyl cation structure we proposed. In their compound the α-amino group is appropriately positioned to be protonated in the enzyme active site, and the four hydroxy groups are ideally oriented to match those in the mannosyl cation. Molecular modeling studies show

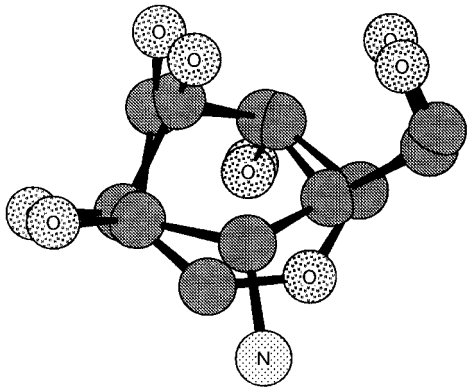

Figure 5. Superimposition of mannosyl cation mimic **5** on the mannosyl cation structure **1**. Hydrogen atoms have been omitted for clarity.

(Figure 5) that their compound superimposes well on the mannosyl cation structure. All of the polar substituents on **5** overlay with analogous groups in the mannosyl cation. The exocyclic amine lies between the ring oxygen and C1 of the mannosyl cation, which is a favorable orientation to form hydrogen bonds with the residues in the catalytic region of the enzyme active site. Significantly the compound was a potent inhibitor of Jack Bean α-mannosidase (IC_{50} = 62 nM), consistent with its similarity to our model.

Hexahydroazepines exhibit a higher degree of ring flexibility than do smaller ring heterocycles. There appear to be no theoretical studies of hexahydroazepine ring conformations in the literature. While the inactivity of **4** may indicate that a binding group equivalent to the 6-OH of the mannosyl cation model is essential for interaction with the mannosidase enzyme, a more likely reason is that the diaxial conformation is energetically unfavorable. Our molecular orbital calculations are consistent with the NMR evidence of Farr *et al.* that their compound preferentially adopts a conformation in which the adjacent 4-OH and 5-NH_2 groups are equatorial rather than axial. In this conformation there would be a poor overlap with the mannosyl cation model. It is this feature, rather than the lack of a binding group analogous to the 6-OH of the model structure, which may explain its poor mannosidase inhibition. There are a number of reports of glycosidase inhibitors in which the functional groups topographically equivalent to the 6-OH of the mannosyl cation are missing. Bernotas *et al.* reported that des(hydroxymethyl)deoxynojirimycin (**6**) inhibited sweet almond β-glucosidase with an almost identical K_i to that of deoxynojirimycin.[15] More significantly, they reported that des(hydroxymethyl)deoxymannojirimycin (**7**) competitively inhibited Jack Bean α-mannosidase in a manner comparable to deoxymannojirimycin. They concluded that the presence of a hydroxymethylene side chain on inhibitors was relatively unimportant for these two enzymes.

Our work suggests that superficial similarity of compounds to model structures can often be misleading, and it is necessary to carry out molecular modeling studies to verify whether the compounds really mimic their target structures. The apparent similarity of 6-epi-castanospermine (**8**) to the mannosyl cation structure is a useful example,[4] as are mannostatin and the hexahydroazepine examples discussed here. The mannosyl cation model thus appears to be a valid paradigm for design of mannosidase inhibitors.

Experimental Section

The crystal structure of mannostatin A tetraacetate (**2**) was used in the superimpositions[10] and the (−) enantiomer (**3**) was generated by suitable inversion using the Sybyl modeling package.[11] The hexahydro-1*H*-azepine structure (**4**) was constructed in the modeling program and the Sybyl MAXIMIN molecular mechanics method was used to optimize the two ring conformers. The conformers used were the diaxial or $^4T_5$ conformer where the 4-OH and 5-NH_2 were both axial and the diequatorial or $^5T_4$ conformer where these two groups were equatorial. The two conformers were optimized by the MOPAC semiempirical MO package using the AM1 parameterization.[12] The optimization used the PRECISE criterion and the NLLSQ optimizer in order to give more reliable geometries and energies, as suggested by Ferguson *et al.*[13] The superimpositions were performed using the Sybyl FIT routine with the topographically equivalent electronegative atoms being superimposed using a least squares fitting algorithm.

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