Theoretical studies of the conformational properties of ribavirin

(IMP dehydrogenase/substrate conformation/iterative extended Hückel theory/1,2,4-triazole-3-carboxamide/virazole)

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ABSTRACT One of the factors required for the antiviral activity of the synthetic nucleoside, ribavirin (1-5-D-ribofuranosyl-1,2,4-triazole-3-carboxamide), is the ability of the molecule to adopt the substrate conformation specified by the enzyme for which it is a competitive inhibitor, inosine 5'-phosphate dehydrogenase (IMP:NAD+ oxidoreductase, EC 1.2.1.14). The calculated glycosidic minimum for ribavirin is the high syn conformation, which is in agreement with experimental determinations of the molecule's solution conformation. The similarity in solution between the conformation of the active ribavirin molecule and the conformation of its inactive 5-methyl and 5-chloro derivatives indicate that some other substrate conformation is specified by the enzyme. The high anti conformation, found by these calculations to be close in energy to the high syn minimum, is postulated to be the active conformation required by the enzyme. The inactivity of the 5-methyl and 5-chloro derivatives is attributed to the much greater stability of these derivatives in the inactive high syn conformation.

Ribavirin is an important new antiviral agent (1, 2). Its solution properties have been investigated by nuclear magnetic resonance (3) and circular dichroism*. In the solid state it has been found to crystallize in two different forms (4). The ability of ribavirin to act as a strong competitive inhibitor of inosine-5'-phosphate (IMP) dehydrogenase (IMP:NAD+ oxidoreductase, EC 1.2.1.14), because of its resemblance to IMP or the feedback inhibitor guanosine-5'-phosphate (GMP), has been established (5). The broad spectrum antiviral properties (2) of this molecule and the availability of many derivatives (6-11) increase the interest in a theoretical study of its conformational properties. We have calculated the variation in the total energy of ribavirin as a function of rotation about the glycosidic bond. The calculated high syn minimum corresponds with the solution conformation (3, *). The inactivity of 5-methyl and 5-chloro derivatives of ribavirin (7) as inhibitors of IMP dehydrogenase, whose solution conformations are identical* with the unsubstituted ribavirin's solution conformation, indicate that another conformation is specified at the active site of the enzyme. Our calculations show that the high anti conformation (4) is close in energy to the high syn conformation found in solution. Conformational energy calculations on the 5-substituted “inactive” derivatives of ribavirin show that the high anti conformation, postulated here as being the conformation required by the enzyme, is ruled out for these derivatives. According to this hypothesis, any effective inhibitory analogs designed to inhibit IMP dehydrogenase should not preclude stability in the high anti region.

Insight into normal and aberrant purine metabolism (12, 13) may be obtained by an investigation of one of the key metabolic conversions involving IMP. IMP occupies an important position in purine metabolism since this nucleotide is partitioned between metabolic pathways leading to GMP and adenosine-5'-phosphate (AMP) and to the nucleoside inosine. The conformational properties required by the enzymes that operate on the substrate IMP are of interest because, if they are dissimilar, then the competing enzyme reactions can be selectively controlled through structural modifications of the substrate that favor one pathway over another. For example, the relative levels of cyclic 3':5'-AMP and of cyclic 3':5'-GMP may be modified at this point by an inhibitory analog that gives one pathway an advantage over the other.

CALCULATIONS

Potential energy calculations on an isolated substrate molecule are able to indicate the low energy conformational states of the substrate. The substrate may move into a slightly higher energy conformation during the reaction between the enzyme and substrate. The substrate's active site conformation, if different from its solution conformation, should be one of the alternate conformations predicted to be stable by a reliable theoretical conformational energy calculation. In the design of analogs of an effective antiviral or anticancer agent, it is important that the specific conformation required by the enzyme is not prohibited by the structural modifications that are made. To assist in determining the inherent active site conformation required by IMP dehydrogenase of a substrate or of a competitive inhibitor directed to the effective active site we have done conformational energy calculations on the V1 and V2 forms of ribavirin (4).

The iterative extended Hückel theory (IEHT) (14) was used to calculate the glycosidic conformational energy preferences for both the V1 and V2 forms of ribavirin, as well as for the inactive 5-chloro derivative (7). The IEHT approach offers several improvements over the extended Hückel theory of Hoffmann (15). One advantage is that a high degree of self-consistency in the net atomic charges is achieved (16). The accuracy of the IEHT method in calculating dipole and quadrupole moments (17) is a reflection of its inherent quality. We have evaluated the IEHT method by testing its ability to give the correct conformational properties of a series of small molecules and have given a brief description of its use in the calculation of conformational energy preferences of a larger molecule, fornycin (18). Other, more widely used and evaluated semi-empirical quantum mechanical approaches have been found to favor crowded structures or conformations (19, 20) that, in some cases, are clearly unstable (21). Conformational energy calculations on nucleosides and nucleotides in the past have generally neglected the stereochemical consequences (22) of the nonequivalence in both energy and orientation of lone pair interactions (23, 24). These are important in determining nucleoside and nucleotide conformations. Another advantage of the IEHT method is that it is represented as being able to take some account of lone electron pair interactions involving heterocyclic systems (14). The steric impact of the N3 lone electron

Abbreviation: IEHT, iterative extended Hückel theory.
pair in purine bases on glycosidic conformational equilibria has recently been verified through experimental circular dichroism studies that substantiated this lone electron pair effect demonstrated by IEHT conformational energy calculations (25).

For comparison, we used a Lennard-Jones 6-12 type potential function, including an electrostatic term, to calculate the glycosidic conformational energy preferences for V1 and V2 and their 5-methyl derivatives, which are known to be inactive as antiviral agents (7). The net atomic charges used for the electrostatic interaction term were those determined by the IEHT calculations; these are shown in Fig. 1. The van der Waals radii (R) and energies for interacting pairs at r = R were taken from a paper by Lakshminarayan and Sasishekar (26). A dielectric constant of 1.0 was used. Bond lengths to hydrogen atoms were corrected if they deviated significantly from standard bond lengths for both the IEHT and Lennard-Jones calculations. The bond lengths that were changed are the following: C(3')-H(3') to 1.09; C(5')-H(5,2') to 1.09; O(5')'-H(5,2') to 0.97; C(5')-H(5) to 1.09; N(8)-H(8,1) to 1.01; and N(8)-H(8,2) to 1.01. The coordinates for the 5-methyl and 5-chloro substituents were determined by placing these substituents along the C(5)-H(5) bond using standard bond lengths and bond angles (27).

RESULTS

We have followed the conformational definitions of Sundaralingam (4, 32). The dihedral angle of 0° corresponds to a cis planar arrangement of the atoms O(1')-C(1')-N(1)-C(5). When looking along the glycosidic bond, a positive rotation corresponds to a clockwise rotation of the far group. According to these definitions, the anti range is the torsion angle range from −90° to +90° and the range from +90° to +270° is referred to as the syn conformation. The intermediate syn B (32) conformation from 90° to 130° is also referred to as the high anti conformation. We will use the term high syn to refer to the intermediate range from 270° to 310°. The results of these calculations are shown in Figs. 2 and 3.

The V2 crystalline form is lower in energy than the V1 form and corresponds to the high anti conformations found in several effective chemotherapeutic agents (18, 28–31). The solution conformation determined from nuclear magnetic resonance (3) is in agreement with these calculations and with preliminary circular dichroism investigations of ribavirin's glycosidic conformational energy preferences*, all of which indicate a preference for the high syn conformation, in contrast with the high anti conformation found in the solid state for V2. However, the conformational energy calculation for the V2 form indicates that the crystalline high anti form is close in energy to the high syn minimum. The results of circular dichroism also indicate that the conformations of ribavirin and its 5-methyl inactive derivative, are identical. Thus, the solution conformation, which is high syn, is not the biologically active conformation, which we expect to be the high anti minimum found by our calculations and which corresponds to the conformation in the solid state for the V2 form of ribavirin. As can be seen in Fig. 2, the V2 form is unstable in the conventional syn and anti regions. The Lennard-Jones calculations indicate that the anti and high anti regions are unstable if a methyl group is added to ribavirin at the 5 position. Similarly, the IEHT results show that the anti

![Fig. 1. Structure and net atomic charges for ribavirin.](image1)

![Fig. 2. Potential energy calculations for the V2 form of ribavirin and its 5-substituted derivatives. IEHT calculation of ribavirin (---); Lennard-Jones calculation of ribavirin (--); IEHT calculation of 5-chloro ribavirin (- - - -); and Lennard-Jones calculation of 5-methyl ribavirin (- - - -).](image2)
and high anti regions are disfavored if a chloro substituent is added to ribavirin at the 5 position. It should be noted, however, that changes in bond lengths and bond angles induced by these substituents or modifications of sugar ring puckering would lower these barriers. A more thorough, and expensive, calculation that would allow for these variations may result in energy minima in the anti region, but these minima are expected to be higher in energy than the favored high syn minimum.

The nuclear Overhauser effects found for ribavirin-5'-phosphate (3) suggest an anti conformation corresponding to our calculated energy minimum for the V1 form of ribavirin. Ribavirin possesses its antiviral activity when converted to the monophosphate, 6-Azaauridine (31), formycin (32), and other antimetabolites that prefer a high anti conformation are expected to have similar conformational properties when converted to the monophosphate. The conformational similarity between these nucleosides and their 5'-phosphates, with respect to glycosidic orientation, may support a decision in favor of the high anti rather than the anti conformation as being the conformation required by IMP dehydrogenase of the inhibitor ribavirin-5'-phosphate or the substrate IMP. The first circular dichroism band for ribavirin and its 5-methyl derivative, which are identical, is reduced in magnitude for ribavirin-5'-phosphate, which indicates that the phosphate group modifies the conformational populations of the molecule or affects the magnitude by participating in the "aglycon, glycon" interactions which generate the circular dichroism. The change in the H(4')-H(5') and H(4')-H(5") coupling constants suggests that the predominant effect of the phosphate group is to increase the proportion of the gauche-gauche exocyclic orientation (3) in solution.

The inactivity of the 4'-thio analog of ribavirin (33), which crystallizes in the C2' endo puckering, may also support the selection of the high anti conformation since a C2' endo puckering, if significantly more stable than other puckered forms, would disallow the active high anti conformation due to steric interactions between H(5) and H(5'). That the 4'-thio derivative lacks activity (34) because of a slow rate of phosphorylation to the monophosphate may be due to an alteration of conformational preferences with respect to glycosidic or exocyclic orientation which can prevent both phosphorylation and activity.

**CONCLUSION**

The V1 and V2 forms of ribavirin, in solution, adopt one conformation. We expect that it is the high syn conformation calculated for the V2 form of ribavirin. The inactivity of 5-substituted derivatives that, for steric reasons, prohibit both the anti and more definitely the high anti ranges, indicates that it is the high anti or anti conformation that is required by the enzyme IMP dehydrogenase. An analysis of conformational and circular dichroism data to be reported later, and of theoretical and experimental investigations of others, appears to support the selection of the high anti minima calculated for the V2 form of ribavirin as the biologically important conformation specified by the enzyme IMP dehydrogenase. This is in the conformation found in the crystal structure. The utilization of IMP by competing enzymic pathways may be selectively controlled through the use of analogs designed so as to favor, through conformation, one pathway over another. This represents an important mechanism by which diseases caused by metabolic imbalances may be treated or prevented if proper regard is given to the role of feedback inhibitors and effector molecules.

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