Conformation of nucleosides: Circular dichroism study on the syn-anti conformational equilibrium of 2-substituted benzimidazole nucleosides

(adenosine/purine nucleosides/iterative extended Hückel theory)

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ABSTRACT  The solution conformations of 2-substituted derivatives of 1-(β-D-ribofuranosyl)benzimidazole have been determined by circular dichroism spectroscopy in aqueous solutions. It is shown that analogs with methyl, amino, or methylenamino substituents at position 2 of the benzimidazole ring (position 8 of the purine ring) have predominantly anti conformations, whereas analogs with chloro, azo, methoxy, or methymercapto substituents have predominantly syn conformations. The preferred solution conformations of the benzimidazole nucleosides and analogous purine nucleosides are compared. The results demonstrate that the replacement of nitrogen by carbon at position 3 of the purine ring of purine (β) nucleosides leads to important conformational consequences, which are strengthened or neutralized by substituents at position 8 of the purine ring.

In this paper the syn-anti glycosidic conformational equilibrium of 2-substituted derivatives of 1-(β-D-ribofuranosyl)benzimidazole is related to the circular dichroism (CD) spectra. The analysis is facilitated by studies on reference benzimidazole nucleosides that are unequivocally constrained to the syn conformation by bulky substituents and to the anti conformation by covalent bonding. Calculations of glycosic conformational energy are also included. It is found that analogs with methyl, amino, and methylenamino substituents at position 2 have predominantly anti conformations in aqueous solution, whereas analogs with chloro, azo, methoxy, or methymercapto substituents have predominantly syn populations. Because the 2-substituent of a benzimidazole nucleoside may be considered analogous to the 8-substituent of a purine nucleoside, our work may provide insights into the pivotal conformational factors that regulate the glycosic conformation of biologically important purine nucleosides. Considerable attention has been devoted to understanding these factors, because the biological activities of some purine nucleoside and nucleotide substrates are mediated by their glycosidic conformational preferences (1–9). In a recent article (8), we presented evidence that replacement of N3 of the purine ring by CH produced important conformational effects, which are now shown to be strengthened or neutralized by substituents at position 8 of the purine ring. The preference for the anti conformation engendered by 2-amino and 2-methylenamino is apparently realized in purine nucleosides as well according to a recent NMR study (7). Our CD results for the 2-methylbenzimidazole nucleoside derivative indicate that the syn conformation is produced by the methyl substituent. The chemical shift changes for 8-methyladenosine indicate that the syn conformation is produced by the analogous methyl substituent (6) on the adenine ring.

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EXPERIMENTAL

CD measurements were made with the Cary 60 spectropolarimeter at very low scan speeds when necessary. In general, the signal-to-noise ratio obtained on benzimidazole nucleosides is several times more favorable than those obtained on purine nucleosides. In regions of low signal-to-noise ratio the pen response-time was increased to a maximum. Ultraviolet absorption spectra were recorded on a Cary 14 spectrophotometer. All spectroscopic measurements were made at least three times in aqueous solution at optical densities of 1.5, 2.0, and 2.5. The preparation and characterization of the benzimidazole nucleosides included in this paper are described in the literature (10–12).

RESULTS AND DISCUSSION

Structures of the compounds discussed in the text are given in Fig. 1, along with the names and numbering scheme used in the text. The definition of the glycosyl torsional angle, XCN, that we will follow is that of Sundaralingam (13). In Fig. 1, compound 8 illustrates the syn conformation and compound 9 illustrates the anti conformation.

The CD spectra of the parent compound, 1-(β-D-ribofuranosyl)benzimidazole (1) and its 2-substituted derivatives may be placed in one of two categories according to the sign pattern and general shape of the spectra. Spectra of the first kind (see Fig. 2) show a positive, negative, positive or missing, and negative sign pattern. The 2-dimethylamino derivative 4a and compounds 4b and 4c, which contain the very bulky dialkylamino type substituents, are sterically forced into the syn conformation. These compounds exhibit the alternating sign pattern belonging to the first category. The CD spectra of the 2-chloro, 2-azo, 2-methoxy (Fig. 3), and 2-methylmercapto derivatives also fall into this category, although the third CD band is missing in the spectrum of the 2-methoxy derivative 5. It should be pointed out that at least partial cancellation of the intensity of the third CD band is expected, because it is overlapped by CD bands of the opposite sign. The cancellation effect is expected to be much stronger for the 2-methoxy derivative due to the methoxy-induced merging of the second and third absorption bands.

CD spectra of the second kind show an all-positive sign pattern (see Fig. 3). The fused ring derivative, 9, which is restricted covalently to the anti range (at about XCN = 30°) shows this pattern. The first CD band is seen to be positive throughout the 260- to 280-nm range. The second CD band appears as a low-intensity tail at the long-wavelength side of the third CD band which is, itself, positive. The merging of the second and third CD bands for this compound, which has virtually the same absorption spectra as the 2-methoxy derivative, is consistent...
Fig. 1. Structural formulas of the nucleosides discussed in the text. Compound 9 is fixed in the anti conformation as drawn. All other compounds are drawn in the syn conformation to conserve space. Numbering system used in referring to the substituents and atoms is indicated. 1, 1-(β-D-ribofuranosyl)benzimidazole; 2, 2-chloro-1-(β-D-ribofuranosyl)benzimidazole; 3, 2-methylthio-1-(β-D-ribofuranosyl)benzimidazole; 4a, 2-dimethylamino-1-(β-D-ribofuranosyl)benzimidazole; 4b, 2-(N-piperidino)-1-(β-D-ribofuranosyl)benzimidazole; 5, 2-chloro-1-(β-D-ribofuranosyl)benzimidazole; 6a, 2-amino-1-(β-D-ribofuranosyl)benzimidazole; 6b, 2-methylamino-1-(β-D-ribofuranosyl)benzimidazole; 7, 2-methyl-1-(2'-deoxy-β-D-ribofuranosyl)benzimidazole; 8, 1-(β-D-ribofuranosyl)benzotriazole; 9, 2,5'-anhydro-1-(β-D-ribofuranosyl)benzimidazole.

with the blue shift effect of 2-alkoxy substituents mentioned above. The CD spectra of the 2-methyl, the 2-amino, and the 2-methylamino derivatives also exhibit an all-positive sign pattern.

In the absence of an overriding chromophoric substituent effect on the optical properties, the observed alternating sign pattern should be diagnostic of a preferred syn conformation. Similarly, the all-positive sign pattern is diagnostic of a preferred anti conformation. The fact that the CD spectra fall neatly into two categories may indicate that the glycosyl conformation of the benzimidazole nucleosides is less variable than the glycosyl conformation of the purine nucleoside.

Theoretical considerations appear to support the above simplistic analysis of the CD data. For nucleosides, in general, the dominant mechanism operative in the generation of the CD bands is the coupled oscillator, in which there is electric dipole–dipole coupling of the transition moments of the base chromophore with those of the ribose unit (14). The sign is determined by the chirality of these oscillators. The chirality according to this mechanism may be changed by chromophoric optical effects or by vicinal optical effects as well as by conformational changes. Vicinal optical effects are ruled out by restriction of our study to nucleosides with the same ribose unit. Molecular orbital calculations of the Pariser–Parr–Pople type (15) or the all-valence electron method of Ellis et al. (16) performed on the benzimidazole unit and its 2-substituted analogs seem to rule out chromophoric optical effects. These calculations show that the individual characters of the first four π →
We wish to emphasize the multiband character of our study in that the CD spectrum is analyzed over the entire accessible range with attention focused on the sign pattern. A single band analysis must be used with discretion, because published rotational strength versus sugar-nucleobase torsion angle curves based on the coupled oscillator effect occasionally exhibit a quadrant behavior predicting that sectors of the syn and anti ranges have the same signed rotational strength (17, 18). It is very likely that the rotational strength of the first and third transitions of the benzimidazole nucleosides exhibit quadrant behavior, because these transitions generate positive CD bands in both the reference syn and reference anti compounds.

Additional support for our conformational assignments has been obtained from conformational energy calculations. The iterative extended Hückel theory used recently in our studies of nucleoside conformation (8, 19, 20) gives the conformational profiles exhibited in Fig. 4. The 2-chloro derivative, which is found in the syn conformation in the crystal state (21), is, according to our calculations, favored by 7 kcal/mol over the local minimum found in the anti region. The syn conformation is also favored for the parent compound 1 (see ref. 8). The corresponding conformational energy curve for the 2-methyl derivative has the global energy minimum in the anti range at 90° of the glycosyl torsional angle with a local minimum in the syn range. The anti conformation is calculated to be more stable by about 2 kcal/mol. Molecular model studies indicate that the conformation predicted for the 2-methyl derivative is very close to the fixed conformation of the fused-ring derivative 9, as expected from the fact that both exhibit the same signed CD spectra.

It is interesting to compare the conformational energy profiles of the benzimidazole nucleosides (see also ref. 8) with those obtained for purine nucleosides (see refs. 8, 19, 20). Overall, the benzimidazole nucleosides show narrower and deeper depressions in the syn and anti regions than is generally found for the purine nucleosides. This distinctive characteristic of the benzimidazole nucleosides at least partially explains why they give much better signal-to-noise ratios in the CD spectra than is generally found for purine nucleosides—i.e., less dispersion of the local population within the allowed conformational range. The fact that the CD spectra of the benzimidazole nucleosides fall neatly into two categories also indicates that the glycosyl conformations of these compounds are more restricted. This is a direct consequence, in all probability, of the conformational effects of substituting CH for N3 in the purine ring.

The CH group at position 7 (position 3 of the purine ring) destabilizes those regions of the syn range of overlapping electron clouds (steric repulsions) and stabilizes those regions of essentially nonoverlapping electron clouds. In this latter region, the London interaction is always present and is always an attractive force arising from fluctuation dipole-induced dipoles. Other interactions include permanent dipole–permanent dipole interactions and permanent dipole–induced dipole interactions. It is convenient to think of interactions between the nucleobase and ribose in terms of an interplay between these forces. At very short separation distances between groups, London attraction has the upper hand due to the inverse distance to the sixth power character. At intermediate distances, the static repulsion prevails. Replacement of the nitrogen by the CH group drastically reduces the potential for electrostatic and hydrogen-bonding conformational effects but enhances the potential for the London force because the CH group is more polarizable. The London force can become very specific in those regions of the syn range that provide for snug fitting of the ribose unit against the benzene ring unit. This selectivity of London attraction at close range provides for preferential stabilization of the syn conformation. The tendency for the hydrocarbon face of the ribose and the benzene ring to stick together may also be enhanced in aqueous solution by the entropy effect, which promotes the clustering of hydrocarbon groups in water.

The CD data and conformational energy profiles indicate that electronegative substituents such as chloro, azo, methoxy, or methylmercaptan at position 2 strengthen the intrinsic preference of the benzimidazole nucleosides for the syn conformation.

Contrary to the other 2-substituted benzimidazole nucleosides the methyl, amino, and methy lamino derivatives show a preference for an anti arrangement about the glycosidic bond. Substitution of a methyl group at position 2 produces strong London attractions in the anti range. Molecular model studies indicate a region in the anti conformation that provides for snug fitting of the hydrocarbon face of the ribose against the 2-methyl substituent. On the basis of our CD data and our conformational energy curve for the 2-methyl derivative, it appears that the London stabilization of the syn range produced by replacing N by CH is effectively neutralized by 2-methyl substitution. The anti conformation of the 2-amino and 2-methylamino derivatives may be explained by the same rationale recently given by Phorille et al. (22) for the anti preferences exhibited by 8-amino and 8-methylamino AMP. This conformation can be stabilized by an intramolecular hydrogen bond between the purine and the exocyclic group.

In summary, our study shows that replacement of N by CH produces important conformational effects, which are strengthened or neutralized by substituents at position 2 of the benzimidazole ring.

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