Published in final edited form as:
Bioorg Med Chem Lett. 2011 November 1; 21(21): 6341–6342. doi:10.1016/j.bmcl.2011.08.109.

Polarization in the Structures of Uracil and Thiouracils: Implication for Binding with Orotidine 5'-Monophosphate Decarboxylase

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Abstract

The structures of the uracil and thiouracils were examined using NMR spectroscopy and crystal structure data when available. The relationships between the extent of polarization and the C5–C6 bond length as well as the H5–H6 coupling constants were probed. It was found that the bond length and coupling constants correlate well with the proton affinities at the carbonyl or thiocarbonyl groups at C4 but not C2. The possible implication in the tighter binding of thiouracil based nucleotides to orotidine-5'-monophosphate decarboxylase was discussed.

Keywords

uracil; thiouracil; dipole moment; polarization; ODCase; coupling constant

The structure and properties of uracil and derivatives have been examined quite extensively due to its status as one of the nucleic base in RNA.[1–7] Some studies have focused on the protonation of the two carbonyl oxygens in the mechanistic studies of the reaction catalyzed by orotidine-5'-monophosphate decarboxylase (ODCase).[8–12]

The inhibition of ODCase by uridine-5'-monophosphate (UMP) and its thialated analogues 2-thioUMP and 4-thioUMP has been compared.[13] The replacement of either carbonyl oxygen by sulfur was found to enhance the ability of these nucleotides to inhibit ODCase.[13] In this Letter, we examine the structures of uracil (**1**) and thiouracils **2**–**4**, especially the extent of polarization, as an attempt to provide insight into the enhanced inhibition seen in thioUMPs.

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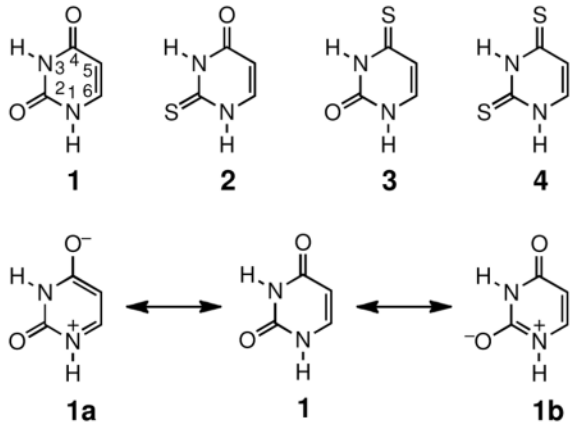

One way to estimate the extent of polarization in the structures of uracil and thiouracils is to probe the contribution of zwitterionic resonance forms to the overall structure. Structures **1a** and **1b** of uracil **1** are depicted as examples. It is evident that in these resonance forms, specifically **1a**, the C5–C6 double bond has become single bond. The more the zwitterions such as **1a** contributes to the overall structure, the less double-bond character for the C5–C6 bond. The decreased bond order will result in the lengthening of the C5–C6 bond. The bond lengths of the C5–C6 bonds in uracil **1**, 2-thiouracil **2**, and 2,4-dithiouracil **4** are available from published crystal structures as listed in Table 1.[14–16] The impact of thialation on the uracil structure is evident from the comparison of the C5–C6 bond lengths. Thialation clearly increases the bond lengths and suggests increased contribution of zwitterionic resonance forms as well as enhanced polarization of the π-electrons.

In addition to the examination of their crystal structures, the structures of uracil and thiouracils in solution were probed with NMR spectroscopy, which has been used to examine resonance effects in conjugated cyclic structures and the zwitterionic contribution to the structures of pyridone and thiopyridone.[17,18] In NMR spectroscopy, the chemical shifts of the ring hydrogens are indicative of the extent of aromaticity and the coupling constants of the ring hydrogens are dependent on the bond length.[17,18] For two hydrogens on the two ends of a C–C bond, a shorter bond length results in closer distance between the two hydrogens and thus a larger coupling constant.[17,18] In the case of uracils **1**–**4**, increased importance of zwitterions such as **1a** to the overall structure will lead to the lengthening of the C5–C6 bond and thus smaller coupling constants. Interestingly, the H5–H6 coupling constants measured for **1**–**4** come in two distinct sets of values, one value for uracil **1** and 2-thiouracil **2** and a different one for 4-thiouracil **3** and 2,4-dithiouracil **4**. Thialation of the carbonyl group at C4 results in smaller H5–H6 coupling constants, indicating decreasing bond order and thus more contribution from zwitterionic resonance forms. The greater importance of zwitterionic resonance forms with negative charge on sulfur, relative to oxygen, has been observed in thiopyridones and phosphorothioates.[17,19]

The polarity of uracil and its thio-analogues can also be assessed by their dipole moments. The polarization of uracil derivatives upon thialation is evident from their reported dipole moments listed in Table 1.[20,21] Thialation, especially at C4, results in significant increases of the dipole moments of thiouracils.

To further understand the effect of thialation, the proton affinities of the carbonyl or thiocarbonyl groups at C2 and C4 were calculated. The calculated values of proton affinity are in good agreement with those previously reported.[7,9,22] The values have further demonstrated the higher basicity of the carbonyl or thiocarbonyl groups at C4. This site benefits most from resonance form **1a**, which is enhanced by the stretch in the C5–C6

distance. Interestingly, gas-phase calculations showed little difference in the structures (such as C5–C6 bond lengths) of uracil or thiouracils. This discrepancy between the experimentally determined and calculated structural parameters may be a result of self-interactions in the crystal form or in solution through hydrogen bonds. Indeed, crystal structures of uracil and thiouracils have revealed strong hydrogen bonding-mediated self-interactions between these molecules in the crystal lattice.[14–16]

In summary, theoretical studies indicate that thialation leads to higher basicity at the sulfur site, but not the oxygen site of uracil derivatives. Structural information on uracil and thiouracil from studies of these molecules in the crystal form and in solution has demonstrated that thialation results in a greater extent of polarization. The relationship between the polarity of the uracil derivative and the binding of their corresponding nucleotides to ODCase is worth exploring. Although outright proton transfer to the carbonyl groups[11,23] appears unlikely due to the absence of adjacent basic residues at the active site of ODCase[24–27], the enhanced polarization and larger dipole moments may allow thioUMPs to interact with ODCase more favorably. It has been proposed that the unique charge distribution at the active site of ODCase forms a polar environment that interacts with substrate analogues through dipole interactions.[28–31] The more readily polarizable nature and the larger dipole moments of the thiouracil moieties may lead to stronger interactions at the active site of ODCase and thus the enhanced inhibitory ability of the thioUMPs as seen in Table 1.

These results have provided some preliminary support for the dipole interaction mechanism, which hypothesizes that ODCase selectively binds nucleotides with large and directionally matched dipole moment.[28–31] However, other factors such as the lower pK_a of the thiouracils may also play a role in the tighter binding of thioUMPs.[13] We are currently examining other substrate analogues to further test this hypothesis.

Acknowledgments

This investigation was supported by the National Institutes of Health (MBRS SCORE Program – Grant S06 GM52588), by a grant from the Center for Computing for Life Sciences at SFSU, and the National Science Foundation (CHE–1011771). The NMR facility was funded by the National Science Foundation (DUE-9451624 and DBI 0521342). Computational studies were performed at the VCU Center for High-Performance Computing. S.H. was supported by a Summer Research Fellowship from the Department of Chemistry and Biochemistry.

References and Footnotes

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Table 1

Some structural and electronic properties of uracil and thiouracils and binding affinities of corresponding nucleotides

Uracil Derivatives	1	2	3	4
Proton Affinity C2=O(S) (kcal/mol)[a]	197.8	203.7	197.3	204.1
Proton Affinity C4=O(S) (kcal/mol)[a]	205.5	205.4	209.1	208.9
C5–C6 bond length (Å)[b]	1.340	1.354	—	1.365
$J_{5,6}$ (Hz)	7.60	7.58	7.03	7.03
Dipole moment (D)[c]	4.19	4.20	4.47	4.67
Relative binding affinity[d]	1	29	61	—

[a] Calculated values at G3MP2 level.

[b] Values determined by X-ray crystallographic studies reported in references 14–16.

[c] Experimental values reported in references 20 and 21.

[d] Calculated from K_i values reported in reference 13.