Imidazole-Phosphate Interaction in Dimethyl Sulphoxide with Reference to Ribonuclease Inhibitor Binding

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Received September 30, 1968

The active site of bovine pancreatic ribonuclease (RNase) is known to contain the histidine residues at positions 12 and 119 in the amino acid sequence.[1-3] The precise function of their imidazole groups has been the subject of much speculation.[4,5] Nuclear magnetic resonance studies have shown that the C-2 proton absorptions of only these two residues are affected on inhibitor binding to RNase.[6,7] More recent investigations seek to fully elucidate the mechanism of interaction.[8]

Selective hydrogen-bonded interactions between purines and pyrimidines have been reported to occur in dimethyl sulphoxide (DMSO).[9,10] Downfield shifts are observed for the proton resonances in this solvent indicating magnetic deshielding. By contrast, in earlier experiments carried out in water only upfield shifts were observed, indicating hydrophobic (stacking) interactions.[11,12] I now report evidence for strong phosphate-imidazole complexing in d_6-DMSO, not observed in D_2O, providing a possible model for their interaction on inhibitor binding to RNase.

The chemical shift changes resulting on titration of imidazole with H_3PO_4 in DMSO and D_2O are shown in Fig. 1. The C-2 imidazole proton absorbs to higher field in DMSO than in D_2O due to greater bulk diamagnetic shielding. Chloroform in solutions at the extremes of the range used (zero and 20 M ratio H_3PO_4)

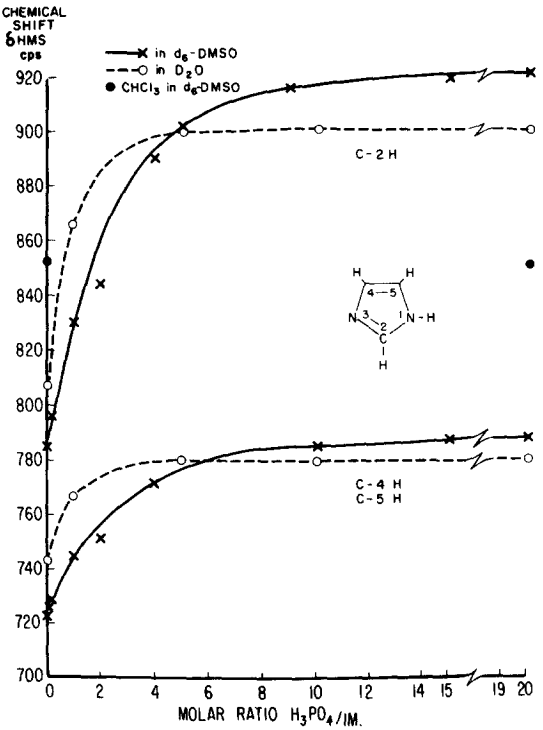
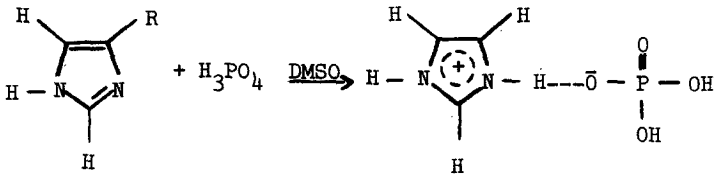


Fig. 1. Titration of imidazole with phosphoric acid in dimethyl sulphoxide and D_2O.

retained the same chemical shift (852 cps) indicating no bulk susceptibility change. The residual DMSO methyl peak shifted 7 cps downfield indicating solvent hydrogen-bond formation with phosphate. The overall chemical shift difference for the C 2 proton in DMSO is 138 cps, 46 cps greater than the difference in D_2O. 94 cps is a normal value for the acid titration of the imidazole group in histidine.[6,13] In water the shift results from intramolecular deshielding due to aromatization of the ring on protonation, and should be independent of solvent. Thus, imidazole and phosphate associate in DMSO.



It has recently been observed that in the presence of excess 3'-CMP and 2'-CMP, but not 5'-CMP or inorganic phosphate, only the C 2-H peak of histidine-119 is shifted downfield (ca. 30 cps) beyond its normal maximum displacement.[8] Thus, histidine-119 appears to be the binding site for the phosphate moiety of 2'- and 3'-CMP. Furthermore, the solvent DMSO apparently provides a model environment for the relatively hydrophobic cleft containing the active site of the enzyme.[2,3,14] These studies are being extended to include other solvents and other amino acid derivatives as possible model systems which may duplicate enzyme-inhibitor interactions.

In 50% DMSO/D_2O the shift difference for imidazole was the same as in D_2O itself. Very small shifts (1-2 cps) resulted for the C-5, 6 and 1' proton absorptions of cytidine and those of imidazole in admixture in DMSO. Cytidine-3' phosphate (3'-CMP) was almost insoluble in DMSO, but a saturated solution gave a 6 cps downfield shift of the imidazole C-2 peak. Histidine itself was insoluble in DMSO. Addition of solutions of imidazole and H_3PO_4 in d_8-dioxan and tetrahydrofuran resulted in precipitation, presumably of imidazolium phosphate. All imidazole solutions were 0.1 M or less, and there were no concentration effects upon the resonances in this range. No chemical shift changes were observed for isopropyl alcohol on addition of imidazole in a ratio of 1:10 to 10:1 in DMSO. This indicates, as expected, no strong hydroxyl H-bond with imidazole in such an environment.

NMR spectra were recorded with a Varian HA 100 spectrometer operating in the frequency sweep mode at a rate of 1 cps/sec. Hexamethyldisiloxane was used as the lock signal, and was contained in a Wilmad coaxial precision bore insert. Chemical shift values were measured directly as the difference between the sweep and reference (lock) oscillators using a Varian counter, and are quoted to the nearest cps.

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