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Equilibrium of Formation of the 6-Carbanion of UMP, a Potential Intermediate in the Action of OMP Decarboxylase

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Orotidine 5′-monophosphate decarboxylase (ODCase) catalyzes the formation of uridine 5′-phosphate (UMP) from orotidine 5′-monophosphate (OMP) (Figure 1a). This last step in pyrimidine biosynthesis is of chemical interest because ODCase enhances the rate of this reactio in water by a factor of $\sim 10^{17}$ without the assistance of metals or other cofactors.[1]

Crystal structures of enzyme complexes with several inhibitors suggest that the altered substrate in the transition state is stabilized by a network of charged residues in the active site,[2−4] whereas the carboxylate group of OMP may be destabilized by electrostatic repulsion at the active site.[5,6] Opinion differs as to whether a discrete carbanion is generated at position 6 of the pyrimidine ring (Figure 1a) during the course of the enzyme reaction.[7]

In the reverse reaction, ODCase should be capable of catalyzing removal of a proton from the 6-CH goup of UMP (Figure 1a), and it would be desirable to have information about the intrinsic acidity of this carbon acid in water. In the vapor phase, the 6-CH group of UMP has been shown to exhibit a much lower proton affinity than the C−H groups of such apparently similar compounds as 1-methyl-2-pyridone and 1-methyl-4-pyridone.[8] In water, the carboxylic acid group of orotidine and 1-methylorotic acid have been found to be unusually acidic, with pK_a values of $\sim$0.4.[1]

To evaluate the approximate pK_a value of the 6-CH group of UMP in water, we determined the rate of deuterium exchange at elevated temperatures, and extrapolated the results to room temperature (Figure 1b). To circumvent difficulties that might arise from ionization at other positions we chose the model compound 1,3-dimethyluracil (DMU), whose methyl groups prevent ionization and furnish internal integration standards for ^{1}H NMR.

A typical reaction mixture contained DMU (25 mM), potassium acetate buffer (80 mM) and sufficient KCl to adjust the constant ionic strength to 1.0, in D_2O. Reaction mixtures were sealed in quartz tubes and incubated for various intervals at temperatures between 165 and 215 °C. For ^{1}H NMR spectroscopy, samples were diluted 1:10 with D_2O containing pyrazine, added as an integration standard. The progress of the reaction was followed by the disappearance of a doublet at 7.55 ppm corresponding to the C-6 proton of DMU. Deuterium exchange occurred at both C-5 and C-6 at comparable rates and proceeded to completion with satisfactory first-order kinetics. Interestingly, buffer catalysis was observed for proton exchange at C-5, but not for proton exchange at C-6 (Figure 1). Rate constants for proton exchange at C-6 were determined at various temperatures in a series of potassium acetate buffers at five different pH values, yielding linear Arrhenius plots (Figure 2, inset).

Adopting the approach of Rios et al.,[9] the overall rate constant k for deuterium exchange can be considered to be the sum of the rate constants for the spontaneous (k_W) and hydroxide-catalyzed

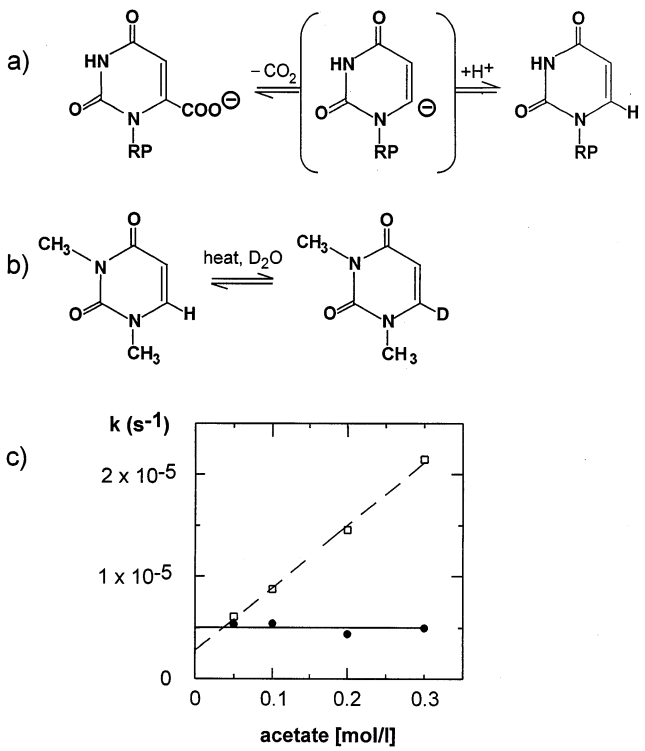

Figure 1. (a) Decarboxylation of OMP, (b) Deuterium exchange at C-6 of DMU, (c) effect of acetate on the exchange rate of the C-5 proton (□) and the C-6 proton (●) at 198 ° C

(k_{OH}) reactions in water, so that the rate of deuterium exchange is described by eq 1:

$$v = k\,[\mathrm{DMU}] = k_w\,[\mathrm{DMU}] + k_{OH}\,(\mathrm{OH}^-)\,[\mathrm{DMU}] \quad (1)$$

These investigators have shown that the observed values of log k_{OH} are linearly related to the pK_a values of a series of carbon acids.[9] Thus, the pK_a value of any member of the series can be estimated by plotting the overall rate constant k as a function of hydroxide ion concentration.

Arrhenius plots were used to obtain rate constants in different buffers at a single temperature. By determining the slope of the rate as a function of (OH$^-$) at that temperature, we obtained a k_{OH} value at that temperature (eq 1). This process was repeated at each of a series of temperatures between 175 and 217 °C. The activity of OH$^-$ was corrected to allow for the heat of ionization of acetate buffers, using data reported for 217 and 154 °C,[10] and for the variation of the ion product of D_2O with changing temperature.[11] Linear extrapolation of the resulting Arrhenius plot for k_{OH} to 25 °C (Figure 2) yields a value of $k_{OH} = 1.6 \times 10^{-9}$ M^{-1} s^{-1} for

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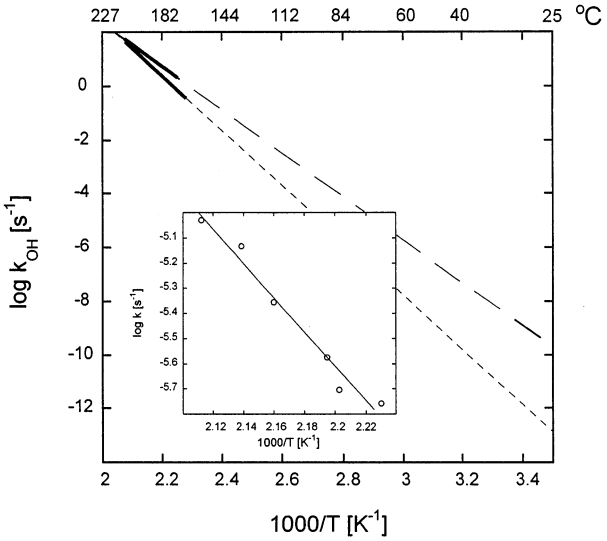

Figure 2. Arrhenius plot for the OH^- catalysis of deuterium exchange for dimethyl uracil (— —) and thiophene (- - -). (Inset) Typical Arrhenius plot for the rate of deuterium exchange into dimethyl uracil (pH 4.8).

dimethyl uracil at 25 °C. For such a strong base, the "on"-rate for combining with deuterium is limited by the rate of rotation of water into a reactive conformation, $\sim 10^{11}$ s^{-1} under these conditions.[9] Using eq 2,[9]

$$pK_a = pK_W + \log [k_{HOH}/k_{HO}] \qquad (2)$$

we estimate that the pK_a value of the 6-CH group of dimethyl uracil is 34 ± 2 at 25 °C. Applying the same experimental procedure to thiophene, we obtained a pK_a value of 37 ± 2, in reasonable agreement with a value of 35 ± 5 obtained by polarographic methods.[12]

It has been suggested that a negative charge at C-6 of UMP might be stabilized by resonance with the adjacent N−C═O group.[13] However, the results summarized in Figure 3 suggest that, in water, the 6-CH group of DMU is only slightly more acidic than the 2-CH group of indole (lit. pK_a = 36 ± 5)[12] or the 2-CH group of thiophene (37 ± 2), in which resonance stabilization of that kind is absent. Further, the absence of general acid catalysis of deuterium exchange at C-6 of DMU (as contrasted with the observed acid catalysis of deuterium exchange at C-5) appears to be consistent with the presence of a highly localized negative charge in the intermediate.

With a half-time of ~140 million years, the kinetic barrier to spontaneous cleavage of the 6-CH bond of DMU is so high ($k = 10^{-15.8}$ s^{-1} at 25 °C and pH 7) that it surpasses the barrier[1] to spontaneous cleavage of the 6-CC bond in the decarboxylation of OMP ($k = 10^{-14.5}$ s^{-1}). In avoiding formation of a discrete carbanion during decarboxylation, some features of the ODCase reaction that may be particularly significant include sequestration of the reacting substrate away from interactions with solvent water and the presence of a protonated lysine residue (Lys-93 in the yeast amino acid sequence) in a position that is optimal for interaction with the developing carbanion, to which it probably transfers its proton as CO_2 is released. Considered individually, no single enzyme−substrate interaction would seem to be capable of accounting for the remarkable high transition-state affinity ($k_{non}/(k_{cat}/K_m) \approx 10^{-23}$ M)[1] and proficiency of ODCase as a catalyst. However, enzyme−substrate interactions have been shown to exhibit exceptionally large connectivity effects in the transition state for substrate transformation by ODCase.[7] Thus, it will be of interest to learn the structural

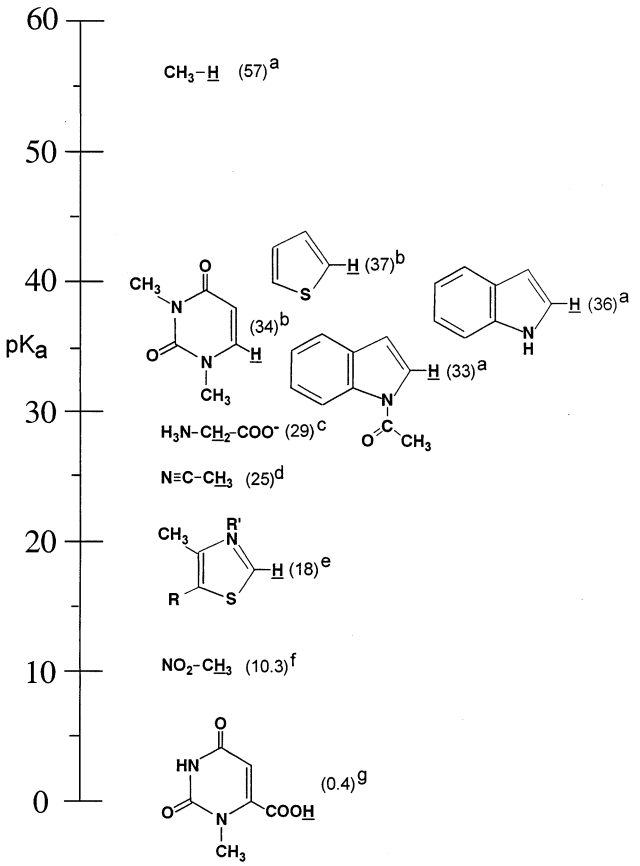

Figure 3. Acidities of some carbon acids, from (a) ref 12, (b) this work; lit.: 35 ± 5,[13] (c) ref 9, (d) ref 14, (e) ref 15, (f) ref 16, (g) ref 1.

basis of the very great difference between this enzyme's affinity for the substrate in the ground state and in the transition state, on which catalysis depends.

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