Deprotonation energetics of purine and uric acid in water from emf measurements at different temperatures

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The first step and second step deprotonation constants of purine and uric acid in water have been determined from emf measurements of cells comprising H$_2$ and Ag-AgI electrodes at five equidistant temperatures ranging from 15°-35°C. The pK values are fitted in the temperature equation $pK = AT^{-1} + B + CT$ by least squares method and the related standard free energies ($\Delta G^\circ$), entropies ($\Delta S^\circ$) and enthalpies ($\Delta H^\circ$) of the deprotonation processes in water have also been evaluated using the values of the coefficients A, B and C of the respective acids.

In continuation of our earlier work on deprotonation energetics of uridine 5'-monophosphate and guanosine 5'-monophosphate we are reporting the deprotonation constants and the related energetics of purine (Pur) and its 2,6,8-trihydroxy derivative uric acid (Uri) at zero ionic strength and in the range of 15°-35°C at 5 degree intervals as determined by emf method using Harned Ehler-type galvanic cells without liquid junction comprising Pt, H$_2$ (g, 1 atm) and Ag-AgI electrodes. The Harned Ehler-type cells used for the determination of $pK_1$ and $pK_2$ and related energetics are (A)-(D)

For HA = Pur, H$_2$A$^+$ = protonated Pur and A$^-$ = conjugate base of Pur
Pt, H$_2$ (g, 1 atm)/H$_2$A$^+$I$^-$ (m$_1$), HA (m$_2$), KI (m$_3$)/AgI-Ag ... (A)
Pt, H$_2$ (g, 1 atm)/HA (m$_1$), NaA (m$_2$), KI (m$_3$)/AgI-Ag ... (B)

For H$_2$A = Uri, HA$^-$ = conjugate base of Uri and A$^{2-}$ = dianion of Uri
Pt, H$_2$ (g, 1 atm)/H$_2$A (m$_1$), NaHA (m$_2$), KI (m$_3$)/AgI-Ag ... (C)
Pt, H$_2$ (g, 1 atm)/NAHA (m$_1$), Na$_2$A (m$_2$), KI (m$_3$)/AgI-Ag ... (D)

**Experimental**

Purine (grade P-1655) and uric acid (grade U-2625) (both from Sigma Chemical) were used as such after drying in a vacuum desiccator. The purity of all the chemicals were checked by UV spectroscopy and were found to lie within 98%-99%. Sodium hydroxide (GR), potassium iodide (GR) and hydroiodic acid (GR) were obtained from E. Merck.

Cell solutions at different ionic strengths were prepared by mixing appropriate weighed amounts of biochemicals, NaOH and KI solutions of known molality and triply distilled CO$_2$ free water in well stoppered Jena bottles. Appropriate amounts of standard NaOH solutions were used for the stepwise neutralization of the acids so as to get the desired concentrations of the corresponding conjugate bases. For the determination of $pK_1$ for Pur, HI acid was used instead of alkali solution. The method of HI distillation was similar to that as described elsewhere.

General experimental procedure including the preparation of Ag-AgI and H$_2$(Pt) electrodes were similar to those described. The equilibrium was attained in 3-4 hours. The constancy of emf readings to about ±0.1 mV for one hour was taken as the criterion of equilibrium. The readings were first taken at 15°C and then successively at higher temperatures of 5°C intervals. The readings of 25°C when back checked after 35°C agreed within ±0.1 mV.

**Results and discussion**

The measured emf's of cells corrected to $pH_2$ = 1 atm gave the value of E. The emf data (E) of cells (A)-(D) at different temperatures and corresponding molalities of KI, H$_2$A$^+$I$^-$, HA for cell (A), KI, HA, NaA for cell (B), KI, H$_2$A, Na-
Table 1—EMF values at different temperatures for different cell solutions in pure water for first and second step deprotonation constants of purine and uric acid

<table>
<thead>
<tr>
<th>( \mu )</th>
<th>( m_1 \times 10^3 )</th>
<th>( m_2 \times 10^3 )</th>
<th>( m_3 \times 10^3 )</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mol kg(^{-1}))</td>
<td>( \text{emf (V)} )</td>
<td>( \text{emf (V)} )</td>
<td>( \text{emf (V)} )</td>
<td>( \text{emf (V)} )</td>
<td>( \text{emf (V)} )</td>
<td>( \text{emf (V)} )</td>
<td>( \text{emf (V)} )</td>
<td>( \text{emf (V)} )</td>
</tr>
<tr>
<td>0.0100</td>
<td>4.8</td>
<td>6.6</td>
<td>10.0</td>
<td>0.1357</td>
<td>0.13825</td>
<td>0.1403</td>
<td>0.1430</td>
<td>0.1446</td>
</tr>
<tr>
<td>0.0231</td>
<td>9.9</td>
<td>26.0</td>
<td>23.1</td>
<td>0.1237</td>
<td>0.12575</td>
<td>0.1276</td>
<td>0.12925</td>
<td>0.1308</td>
</tr>
<tr>
<td>0.0322</td>
<td>19.9</td>
<td>16.3</td>
<td>32.2</td>
<td>0.0916</td>
<td>0.0930</td>
<td>0.0946</td>
<td>0.0963</td>
<td>0.0968</td>
</tr>
<tr>
<td>0.0439</td>
<td>9.9</td>
<td>26.0</td>
<td>43.9</td>
<td>0.1087</td>
<td>0.1114</td>
<td>0.1124</td>
<td>0.11375</td>
<td>0.1151</td>
</tr>
<tr>
<td>0.0655</td>
<td>19.8</td>
<td>16.6</td>
<td>65.5</td>
<td>0.07575</td>
<td>0.07715</td>
<td>0.0783</td>
<td>0.07975</td>
<td>0.0802</td>
</tr>
<tr>
<td>0.0786</td>
<td>19.7</td>
<td>16.5</td>
<td>78.6</td>
<td>0.07175</td>
<td>0.0725</td>
<td>0.0738</td>
<td>0.0751</td>
<td>0.0758</td>
</tr>
<tr>
<td>0.1064</td>
<td>20.2</td>
<td>16.1</td>
<td>106.4</td>
<td>0.0640</td>
<td>0.0652</td>
<td>0.06625</td>
<td>0.06725</td>
<td>0.06795</td>
</tr>
</tbody>
</table>

\( \text{Pur:} \ pK_1 = (pK_i)_\text{HA}^+; m_1 = m_\text{HA}^+, m_2 = m_\text{HA}^-; m_3 = m_1. \)

\( \text{Pur:} \ pK_2 = (pK_i)_\text{HA}^+; m_1 = m_\text{HA}^-, m_2 = m_\text{A}^-, m_3 = m_1. \)

\( \text{Uri:} \ pK_1 = (pK_i)_\text{HA}^+; m_1 = m_\text{HA}^-, m_2 = m_\text{HA}^+, m_3 = m_1. \)

\( \text{Uri:} \ pK_2 = (pK_i)_\text{HA}^+; m_1 = m_\text{HA}^-, m_2 = m_\text{A}^-, m_3 = m_1. \)
HA for cell (C) and KI, NaHA, Na₂A for cell (D) for each of the cell solutions are given in Table 1. Similar to our previous paper, the pK₁ and pK₂ of Pur and Uri were evaluated by using the functions pK₁ and pK₂ defined by Eqs 1-4

\[
pK_1(Pur) = -\log(m_{H₄} + m_{H₅}) - \log(m_{H₃} + m_{H₄})/
\]

\[
pK_2(Pur) = -\log(m_{H₃} + m_{H₅})/
\]

where

\[
-\log(m_{H₅}) = (E - E₀)/k + \log m_{H₄} d₀^{1/2}
\]

In the above equations m and γ denotes respectively the molality and molal activity coefficients of the species involved. The standard state is so chosen that at infinite dilution in a given solvent γᵢ of any species i is equal to unity, k = 2.3026 RT/F, μ (ionic strength) = m₁ = m₃ and Sᵢ (Debye-Hückel constant) = 1.824 × 10⁶ (εₑ T)^{−3/2}, m₁ denotes the apparent molality of hydrogen due to deprotonation of the acid and hydrolysis of conjugate base of the acid, E₀ the standard electrode potential of Ag-AgI electrodes in pure water, d₀ = density of pure water, R = universal gas constant, T, absolute temperature, F, Faraday, εₑ, the dielectric constant of water and f(μ) stands for a function of μ which is usually linear.

\[
pK_1(Pur) = (E - E₀)/k + \log(m_{H₄} + m_{H₅}) + \log(\gamma⁺/\gammaₜ)
\]

\[
pK_2(Pur) = (E - E₀)/k + \log(m_{H₃} + m_{H₅})/
\]

where \(\log m_{OH⁻}= log K_w + p_w H \quad \ldots (2')\)

\[
p_w H = -\log a_{H⁺} \quad p_w H = -\log a_{H⁺} \quad γ⁺ = (E - E₀)/k + m_{H₄}
\]

\[
μ = m_2 + m_3; m_{OH⁻} stands for the molality of hydroxyl ion due to hydrolysis; K_w, the ionic product of water, p_w H = Bates acidity function.

\[
pK_1(Uri) = (E - E₀)/k + \log(m_{H₄} + m_{H₅}) + \log(\gamma⁺/\gammaₜ)
\]

\[
pK_1(Uri) = (E - E₀)/k + \log(m_{H₃} + m_{H₅}) + \log(\gamma⁺/\gammaₜ)
\]

\[
pK_2(Uri) = (E - E₀)/k + \log(m_{H₃} + m_{H₅})/
\]

where \(μ = m_2 + m_3\)

\[
pK_2(Uri) = (E - E₀)/k + \log(m_{H₃} + m_{H₅})/
\]

The involved activity coefficients in water are obtained by the Bronsted form of Debye-Hückel equation

\[
-\log γᵢ = Sᵢ zᵢ^2 (μ d₀)^{1/2} + bᵢ μ \quad \text{where} \quad zᵢ = \text{charge of the species involved and} \quad bᵢ = \text{a constant for the species i depending upon the nature of the solvent and temperature. As indicated in our previous paper, due corrections for m₁ to m₃ and m_{OH⁻} have been made for pK₁ and pK₂ of Pur and Uri respectively. Since in all the cases pK' values were found to be linear with respect to μ, the pK values at different temperatures were obtained from linear fit of the type}
\]

\[
pK = AT⁻¹ + B + CT \quad \ldots (5)
\]

by the method of least squares. The thermodynamic parameters \(ΔG°\), \(ΔS°\) and \(ΔH°\) accompanying the deprotonation of the acids were evaluated using the same relations as described in our previous paper. The values of \(ΔG°\), \(ΔS°\) and \(ΔH°\) so obtained and the values of the constants A, B and C are presented in Table 3. The maximum uncertainties in \(ΔG°\), \(ΔS°\) and \(ΔH°\) are ±0.01 kJ mol⁻¹, ±1 JK⁻¹ mol⁻¹ and ±0.3 kJ

<table>
<thead>
<tr>
<th>Compd</th>
<th>15°C</th>
<th>20°C</th>
<th>25°C</th>
<th>30°C</th>
<th>35°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pur</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pK₁</td>
<td>2.551 ± 0.002</td>
<td>2.528 ± 0.001</td>
<td>2.501 ± 0.001</td>
<td>2.471 ± 0.001</td>
<td>2.439 ± 0.001</td>
</tr>
<tr>
<td>pK₂</td>
<td>9.248 ± 0.001</td>
<td>9.141 ± 0.0025</td>
<td>9.040 ± 0.001</td>
<td>8.932 ± 0.002</td>
<td>8.830 ± 0.002</td>
</tr>
<tr>
<td>pK₃</td>
<td>5.863 ± 0.003</td>
<td>5.810 ± 0.002</td>
<td>5.760 ± 0.003</td>
<td>5.710 ± 0.002</td>
<td>5.660 ± 0.002</td>
</tr>
<tr>
<td>Uri</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pK₁</td>
<td>10.910 ± 0.004</td>
<td>10.804 ± 0.001</td>
<td>10.690 ± 0.001</td>
<td>10.601 ± 0.003</td>
<td>10.522 ± 0.003</td>
</tr>
</tbody>
</table>
Table 3—Values of A, B, C and relevant energetics $\Delta G^\circ$, $\Delta S^\circ$ and $\Delta H^\circ$ (molal scale) for purine and uric acid at 25°C

<table>
<thead>
<tr>
<th>Compd</th>
<th>A (K)</th>
<th>B</th>
<th>C (K$^{-1}$)</th>
<th>$\Delta G^\circ$ (kJ mol$^{-1}$)</th>
<th>$\Delta S^\circ$ (JK$^{-1}$ mol$^{-1}$)</th>
<th>$\Delta H^\circ$ (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pur</td>
<td>-1550</td>
<td>14.575</td>
<td>-0.02306</td>
<td>14.275</td>
<td>-15.8</td>
<td>9.60</td>
</tr>
<tr>
<td></td>
<td>(± 10)</td>
<td>(±.08)</td>
<td>(±.00002)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd step dissociation</td>
<td>1196</td>
<td>7.24</td>
<td>-0.00744</td>
<td>51.56</td>
<td>-53.7</td>
<td>35.60</td>
</tr>
<tr>
<td></td>
<td>(± 12)</td>
<td>(±.06)</td>
<td>(±.00006)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uri</td>
<td>0</td>
<td>8.74</td>
<td>-0.01000</td>
<td>32.87</td>
<td>-53.2</td>
<td>49.9</td>
</tr>
<tr>
<td></td>
<td>(± 0)</td>
<td>(±.05)</td>
<td>(±.00006)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd step dissociation</td>
<td>6077</td>
<td>-24.18</td>
<td>0.04872</td>
<td>61.23</td>
<td>-93.3</td>
<td>33.4</td>
</tr>
<tr>
<td></td>
<td>(± 5)</td>
<td>(±.06)</td>
<td>(±.00009)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The sites of proton ionization from Pur and Uri are discussed below and the equilibria involved in the deprotonation processes are shown in Schemes 1 and 2.

The fairly low magnitude of entropy change accompanying the first step ionization of Pur indicates that the ionization process is a charge transfer process (CTP) involving a BH$^+$ type acid and the deprotonation sites (with $pK_a = 2.50$) to be either N$_1$H$^+$ or N$_3$H$^+$ group.

On the other hand, negative magnitude of entropy of ionization of second step dissociation of Pur suggests the ionization process involved as charge separation process (CSP) involving a HA-type acid. In purine hydrogen was found to be located at N(7) and N(9)$^a$. However, from charge density distribution$^5$ it appears that deprotonation from neutral Pur occurs from N(9) and $pK_a = 9.04$ is assigned to it. Notably, $pK_a = 2.50$ for protonated Pur and $pK_a = 9.04$ for neutral Pur suggest that it is a weaker acid than pyrimidine.
(pKₐ = 1.3) and a much stronger acid than imidazole (pKₐ = 14.5). These properties are consistent with pyrimidine ring withdrawing electrons from the imidazole ring of purine.

Similar to neutral Pur, the first step dissociation of Uri with a negative magnitude of entropy change is also a CSP. By analogy with hypoxanthine (Hyp) and xanthine (Xan) and from charge density distribution it appears that in aqueous solution the lactam-lactim tautomeration exists and the deprotonation sites in Uri (with pKₐ = 5.96) are either 2 or 6 hydroxy group. Interestingly enough, on comparing pK₁ of 2,6,8-trihydroxyurine (Uri) with 6-hydroxyurine (Hyp) and 2,6-dihydroxyurine (Xan) it is found that acidity increases in the order Hyp < Xan < Uri. This might be due to the electron withdrawing inductive effect of the hydroxyl groups which tend to destabilize the negative charge on the oxygen of the 2 or 6 hydroxyl group which deprotonates.

The ΔS° values for the second step ionization of Uri, Hyp and Xan are similar indicating that deprotonation occurs from HA⁻ type acid and −N₉H on the ring is the favourable ionization site. Moreover, the magnitude of pK₂ of Uri, Xan and Hyp indicates an increase in pKₐ in the order Uri < Xan < Hyp which is again a result of graded increase in +I effect of hydroxy groups in the order Hyp < Xan < Uri. However, in unsubstituted purine N₉H site is more acidic than the same in its mono-, di- and tri-hydroxy derivatives. This is due to the fact that deprotonation from N₉H site of hydroxy derivatives of purine occurs from their conjugate bases and negative charge on oxygen at 2 or 6 position in nibits to some extent the proton ionization from N₉H group and thereby results in a decrease in acidity.

The highly negative magnitude of entropy change accompanying the second step deprotonation of uric acid and that ΔS°₂, Uri is more negative than ΔS°₁, Uri conform to what is expected from the nature of the equilibria involved in the dissociation processes involving

\[ \begin{align*}
K₁ & = \frac{[H⁺][HA⁻]}{[H₂A]} \\
K₂ & = \frac{[HA⁻][A⁻]}{[HA²⁻]} \\
\end{align*} \]

\[ \Delta S°₁(\text{Uri}) = (S°_{\text{H₂O}} - S°_{\text{H₂O}}) + (S°_{\text{HA⁻}} - S°_{\text{H₂O}}) \quad (6) \]

\[ \Delta S°₂(\text{Uri}) = (S°_{\text{H₂O}} - S°_{\text{H₂O}}) + (S°_{\text{HA²⁻}} - S°_{\text{HA⁻}}) \quad (7) \]

\( S°_{\text{H₂O}} - S°_{\text{H₂O}} \) is negative as H₂O⁺ carrying a charge must be solvated resulting in a more or-derliness and there is a decrease of entropy of H₃O⁺ relative to water i.e. \( S°_{\text{HA⁻}} < S°_{\text{H₂O}} \). Similarly, \( S°_{\text{HA²⁻}} < S°_{\text{HA⁻}} \) and \( S°_{\text{HA²⁻}} < S°_{\text{HA⁻}} \). Since entropy of hydration of any ion is directly proportional to the square of ionic charge, \( (S°_{\text{HA²⁻}} - S°_{\text{HA⁻}}) < (S°_{\text{HA⁻}} - S°_{\text{HA⁺}}) \) i.e. \( \Delta S°_{\text{HA²⁻}} < \Delta S°_{\text{HA⁻}} - S°_{\text{HA⁺}} \). In other words \( \Delta S°₂,\text{Uri} < \Delta S°₁,\text{Uri} \).

References