Stepwise formation constants of folic acid, \( N-[p-[(2\text{-amino-4-hydroxy-6-pteridyl})\text{methyl}][\text{amino}]\text{benzyl}]\text{glutamic acid} \), with the carcinogenic metal ions, aluminium (III), chromium (III), beryllium (II), lead (II) and uranium (VI) have been determined at 30 °C, and at various ionic strengths, viz. 0.01 m, 0.05 m, 0.10 m, maintained by potassium nitrate solution. The formation of the chelate is evident from the shift between the (i) ligand and (ii) ligand and metal titration curves. From the values for the stepwise protonation constants and metal-ligand stability constants at various ionic strengths, thermodynamic formation constants were evaluated by extrapolation to zero ionic concentration. Logarithms of the overall thermodynamic stability constants (\( \log K^\circ \)) are: 20.40 for H; 15.15 for Al; 10.10 for Cr; 9.35 for Be; 6.50 for Pb; 9.00 for U-chelates. Corresponding free energy changes (\( \Delta F^\circ \)) are -28.47, -21.14, -14.09, -13.05, -9.07, -12.56 Kcal·mol\(^{-1} \) respectively.

Structural analogue of normal pteroyl compounds, folic acid antagonists are important in experimental cancer therapy mainly in leukemia. Folic acid itself has certain effects on tumor inhibition. The withdrawal of the vitamin from the diet or administration of folic acid antagonists may change the folic acid metabolism essential for several biosynthetic pathways. It has also been found, in many cases, that the dietary restriction of folic acid is more effective than treatment with aminopterin formed by the replacement of OH group by NH group in folic acid. Thus a deficient diet of folic acid during the aminopterin treatment might be more sensitive.

From chemical point of view, agents related to cancer are carcinogenic and anticancer organic compounds, and carcinogenic metal ions. A large amount of work has been done to prove the true carcinogenic and true anticancer compounds to be chelating agents initially or after the metabolic changes. Considering this concept of chelation various chemotherapeutic agents and their chelates with transition metal ions are being prepared. Similarly the carcinogenic metal ions are generally transition metal ions having a pronounced tendency for chelate formation, and are associated with vitamins, protein, enzymes, and nucleic acids and have altered composition in cancer cell. Also, these carcinogenic (abnormal) metal ions may replace essential metals from the normal specific enzyme with similar electronic configuration. These may change the structure and function of genetic materials by acting with nucleic acids which may be manifested in the formation of cancer. In the cancer cell the antimitotic agents may deactivate the carcinogenesis binding the carcinogenic (abnormal) metals.

Since, folic acid and its antagonists have a chelating tendency, more information is needed about the chelates themselves formed with carcinogenic metal ions under physiological conditions, for an understanding of the chelate hypothesis in cancer problem. The comparison of \( K_a \), free energy change (\( \Delta F^\circ \)) and thereby the free metals in equilibrium with the chelate may give good information. Also, since some metal salts and metal chelates are used as anticancer compounds, the metal-chelates formed under physiological pH conditions may be used for the inhibition of tumor.

In the present investigation the chelate formation of folic acid, \( N-[p-[(2\text{-amino-4-hydroxy-6-pteridyl})\text{methyl}][\text{amino}]\text{benzyl}]\text{glutamic acid} \), with carcinogenic metal ions, Al(III), Cr(III), Be(II), Pb(II) and U(VI) has been studied using Bjerrum-Calvin pH-titration technique. Chelation of folic acid during the aminopterin treatment might be more sensitive.
acid with several metal ions like copper(II), nickel (II), zinc(II), cobalt(II), cadmium(II), iron(II), manganese (II), magnesium (II) has already been done by Albert and an extension was considered desirable.

Materials and Methods

Apparatus

A Leeds and Northrup pH-meter was used with a glass-calomel electrode assembly. Solutions were maintained at 30 °C. Checks were made using standard buffer solution before each titration.

Materials

Aqueous solutions of chromic nitrate (AnalaR, B.D.H.), aluminium nitrate (AnalaR, B.D.H.), beryllium nitrate (prepared from BeC03, E. Merck), lead nitrate (AnalaR, B.D.H.) and uranyl nitrate (AnalaR, B.D.H.) were prepared and standardized. The strengths of each solution was kept 0.002 M by dilution.

Folic acid (B.D.H.) was dissolved in aqueous potassium hydroxide. A 1.5 M standard solution of KN03 (AnalaR, B.D.H.) was prepared by direct weighing and employed to maintain the ionic strength. A solution of potassium hydroxide (AnalaR, B.D.H.) was prepared and standardized against oxalic acid. A nitric acid (AnalaR, B.D.H.) solution was standardized against a standard alkali. Twice-distilled water free from carbon dioxide was used for preparing all the solutions.

Procedure

The ratio of metal to ligand was kept 1 : 5, in order to fulfil the maximum coordination number of metal. The mixtures: (A) 5 ml of 0.024 M of HN03, (B) A+20 ml of 0.0025 M of folic acid, (C) B+5 ml of 0.002 M of metal nitrate was prepared. The ionic strengths were maintained 0.01 M, 0.05 M, and 0.1 M by KN03 solution keeping the total volume 50 ml. These mixtures were titrated individually against the standard alkali (0.2032 M) by the pH-titration method. Pure nitrogen was bubbled through the titration cells. The graph between pH and the volume of alkali required to reach the corresponding pH change was plotted. The shape of the titration curves were as usual.

Calculations

Folic acid behaves as acid owing to the presence of a phenolic group in pteridine ring and two carboxylic acid groups. The stepwise dissociation of the ligand is shown as below:

\[
\begin{align*}
\text{OH} & \quad \text{NH} \quad \text{CH}_2 \quad \text{NH} \\
\text{CH}_2 \quad \text{NH} \quad \text{CH}(\text{CH}_2)_2 \quad \text{COOH} \\
\text{COOH} & \\
\end{align*}
\]

\[
\begin{align*}
\text{OH} & \quad \text{NH} \quad \text{CH}_2 \quad \text{NH} \\
\text{CH}_2 \quad \text{NH} \quad \text{CH}(\text{CH}_2)_2 \quad \text{COOH} \quad +\text{H}^+ \\
\end{align*}
\]

\[
\begin{align*}
\text{OH} & \quad \text{NH} \quad \text{CH}_2 \quad \text{NH} \\
\text{CH}_2 \quad \text{NH} \quad \text{CH}(\text{CH}_2)_2 \quad \text{COOH} \quad \cdot 2\text{H}^+ \\
\end{align*}
\]

Proton-Ligand Stability Constants

The average number of protons (\(\bar{n}_A\)) associated with ligand molecule is defined as

\[
\bar{n}_A = \frac{\text{total concentration of proton bound to ligand}}{\text{total concentration of ligand not bound to metal}}
\]

\[
= \frac{C_{LH}+2C_{LH_2}+\ldots+jC_{LH_j}}{C_L+C_{LH}+C_{LH_2}+\ldots+jC_{LH_j}}
\]

in terms of formation constants the eq. (1) can be represented as below:

\[
\bar{n}_A = K_1 \frac{C_{L}H^+}{C_L} + K_2 \frac{C_{L}H_2^+}{C_L} + \ldots + jK_j \frac{C_{L}H^+_j}{C_L}
\]

where \(j\) is the maximum number of associated protons.

To calculate the formation constant, \(\bar{n}_A\) at different pH were calculated using the acid and ligand titration curves. The formula as used by Irving and Rossotti has been used:

\[
\bar{n}_A = y \frac{T_C{L}_0 - (v''-v')(N^0+E^0)}{(v'+v''+v')-1} \frac{1}{T C{L}_0}
\]

where \(v', v''\) are the volume of alkali in acid and ligand titration to reach the same pH value. \(V^0, E^0, N^0\) are the total concentration of the titrating mixture, total concentration of acid, total concentration of alkali respectively, \(y\) is the number of dissociable protons.

The formation curves at different ionic strengths were obtained by plotting the graph between \(\bar{n}_A\) values and pH. The shape of the formation curve is as usual.

Metal-Ligand Stability Constants

For a stepwise complex formation system the formation constants are given by

\[
K_n = \frac{C_{ML^n}}{C_{ML^{n-1}}C_L} (n=1, 2, \ldots N),
\]
Kn is the nth metal-ligand stability constant. The formation constants were obtained from analysis of the formation curves drawn between \( \bar{n} \) (average number of ligands attached per metal ion), and \( pL \) (free ligand exponent). The values of \( \bar{n} \) and \( pL \) were calculated from the equations below:

\[
\bar{n} = \frac{(v''-v')\left(N^m+E^p\right)}{(v^0+v')\bar{n}_A T C_{M_0}},
\]

\[
p_L = \log_{10}\left(\frac{\beta_n^{\text{III}}\left(1-\text{antilog } B\right)^n}{\left(T C_{M_0} - \bar{n} T C_{M_0}\right)}\right),
\]

where \( T C_{M_0} \) is total concentration of metal present in solution. \( \beta_n^{\text{III}} \) is overall proton-ligand stability constant and other terms have their usual meanings.

**Results and Discussion**

**Proton-Ligand System**

There are three dissociable protons in the ligand but from the formation curve (Fig. 1) only two can be obtained as the higher values of \( \bar{n}_A \) cannot be calculated because folic acid is insoluble at lower pH. For the dissociation of the second carboxylic proton, the constant was obtained by mid-point slope method i.e. \( \log K_1 K_2 K_3 = 3pL \). The values of proton-ligand stability constants at various ionic strengths are shown in table 1.

**Metal-Ligand System**

It has been seen that the chelates at biological pH are formed only at the phenolic position, as also found by Albert in the case of other transition metal chelates of folic acid. Although the carboxylic groups might also be able to form chelates at lower pH, this could not be studied, because the ligand is not soluble in aqueous media completely in presence of more acid.

The metal-ligand stability constants are reported in table 1. The values were calculated by interpolation at half \( \bar{n} \) values and other computational methods. The values obtained by the different methods are in fair agreement.

The formation curves are shown in figs. 2 – 4 at different ionic strengths. There is no sharp change in the shape of the chelate-formation curves, suggesting that the various species coexist in solution.

Thermodynamic stability constants were obtained by extrapolating the values of \( \log K \) to zero

<table>
<thead>
<tr>
<th>Ionic strength</th>
<th>( \log K_1 )</th>
<th>( H^+ )</th>
<th>Al(III)</th>
<th>Cr(III)</th>
<th>Be(II)</th>
<th>Pb(II)</th>
<th>U(VI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01 M</td>
<td>( \log K_1 )</td>
<td>8.80</td>
<td>5.25</td>
<td>3.30</td>
<td>5.15</td>
<td>3.25</td>
<td>4.55</td>
</tr>
<tr>
<td></td>
<td>( \log K_2 )</td>
<td>6.55</td>
<td>4.55</td>
<td>3.30</td>
<td>3.90</td>
<td>3.20</td>
<td>4.20</td>
</tr>
<tr>
<td></td>
<td>( \log K_3 )</td>
<td>4.30</td>
<td>4.50</td>
<td>3.35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>( \log \beta_n )</td>
<td>19.65</td>
<td>14.30</td>
<td>9.95</td>
<td>9.05</td>
<td>6.45</td>
<td>8.75</td>
</tr>
<tr>
<td>0.05 M</td>
<td>( \log K_1 )</td>
<td>8.45</td>
<td>4.80</td>
<td>3.15</td>
<td>4.80</td>
<td>3.15</td>
<td>4.20</td>
</tr>
<tr>
<td></td>
<td>( \log K_2 )</td>
<td>5.70</td>
<td>4.28</td>
<td>3.20</td>
<td>3.70</td>
<td>3.18</td>
<td>3.95</td>
</tr>
<tr>
<td></td>
<td>( \log K_3 )</td>
<td>2.95</td>
<td>4.15</td>
<td>3.35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>( \log \beta_n )</td>
<td>17.10</td>
<td>13.23</td>
<td>9.70</td>
<td>8.50</td>
<td>6.33</td>
<td>8.15</td>
</tr>
<tr>
<td>0.10 M</td>
<td>( \log K_1 )</td>
<td>8.30</td>
<td>4.65</td>
<td>3.12</td>
<td>4.65</td>
<td>3.13</td>
<td>4.05</td>
</tr>
<tr>
<td></td>
<td>( \log K_2 )</td>
<td>5.35</td>
<td>4.20</td>
<td>3.20</td>
<td>3.70</td>
<td>3.20</td>
<td>3.85</td>
</tr>
<tr>
<td></td>
<td>( \log K_3 )</td>
<td>2.35</td>
<td>4.10</td>
<td>3.30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>( \log \beta_n )</td>
<td>16.00</td>
<td>12.95</td>
<td>9.62</td>
<td>8.35</td>
<td>6.33</td>
<td>7.90</td>
</tr>
</tbody>
</table>

Table 1. Stability Constants at 30°.

Fig. 2. Metal-ligand formation curves ($\mu=0.01 \text{ m}$). A. Pb-chelate; B. Cr-chelate; C. Be-chelate; D. U-chelate; E. Al-chelate.

Fig. 3. Metal-ligand formation curves ($\mu=0.05 \text{ m}$). A. Pb-chelate; B. Cr-chelate; C. Be-chelate; D. U-chelate; E. Al-chelate.

Fig. 4. Metal-ligand formation curves ($\mu=0.10 \text{ m}$). A. Pb-chelate; B. Cr-chelate; C. Be-chelate; D. U-chelate; E. Al-chelate.

Fig. 5. Extrapolation of log $K$ to zero ionic concentration (Proton-ligand system). A. $K_1$; B. $K_2$; C. $K_3$.

Fig. 6. Extrapolation of log $K$ to zero ionic concentration (Al-ligand system). A. $K_1$; B. $K_2$; C. $K_3$.

Fig. 7. Extrapolation of log $K$ to zero ionic concentration (Cr-ligand system). A. $K_1$; B. $K_2$; C. $K_3$. 
Table 2. Values of Thermodynamic Constants and Change in Free Energies of Metal-Complexes at 30°.

<table>
<thead>
<tr>
<th></th>
<th>H⁺</th>
<th>Al(III)</th>
<th>Cr(III)</th>
<th>Be(II)</th>
<th>Pb(II)</th>
<th>U(VI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>log $K_{i=0}$</td>
<td>9.00</td>
<td>5.80</td>
<td>3.40</td>
<td>5.30</td>
<td>3.30</td>
<td>4.70</td>
</tr>
<tr>
<td>log $K_{e=0}$</td>
<td>6.75</td>
<td>4.70</td>
<td>3.35</td>
<td>4.05</td>
<td>3.20</td>
<td>4.30</td>
</tr>
<tr>
<td>log $K_{g=0}$</td>
<td>4.65</td>
<td>4.65</td>
<td>3.35</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>log $K_{c=0}$</td>
<td>20.40</td>
<td>15.15</td>
<td>10.10</td>
<td>9.35</td>
<td>6.50</td>
<td>9.00</td>
</tr>
<tr>
<td>$\Delta F_1^0$, K.Cal/mole</td>
<td>-12.56</td>
<td>-8.09</td>
<td>-4.74</td>
<td>-7.39</td>
<td>-4.61</td>
<td>-6.56</td>
</tr>
<tr>
<td>$\Delta F_2^0$, K.Cal/mole</td>
<td>-9.42</td>
<td>-6.56</td>
<td>-4.67</td>
<td>-5.65</td>
<td>-4.46</td>
<td>-6.00</td>
</tr>
<tr>
<td>$\Delta F_3^0$, K.Cal/mole</td>
<td>-6.49</td>
<td>-6.49</td>
<td>-4.67</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

Fig. 8. Extrapolation of log $K$ to zero ionic concentration (Be-ligand system). A. $K_1$; B. $K_2$.

Fig. 9. Extrapolation of log $K$ to zero ionic concentration (Pb-ligand system). A. $K_1$; B. $K_2$.

The energy change ($\Delta F^0$) was calculated by the formula:

$$\Delta F^0 = -2.303 \frac{R T}{\mu} \log K_{i=0},$$

where $K_{i=0}$ is the formation constant at zero ionic strength.

The values of log $K_1^0$, log $K_2^0$, log $K_3^0$ and the sum log $K_{i=0}$ are given in table 2.

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