Study of Spectral and Thermodynamical Interaction of Calf Thymus Deoxyribonucleic Acid (Ct-DNA) and an Anticancer Analogue Drug 10-Molybdo 2-Vanado Phosphoric Acid

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Abstract
In this study, at first the physicochemical properties of 10-molybdo 2-vanado phosphoric acid are investigated under various environmental conditions, such as ionic strength and concentration. The results represent no aggregation behavior over the concentration range of 2.86×10⁻⁵ to 5.05×10⁻⁵ M. Second, the interaction of [H₅PMo₁₀V₂O₄₀] with calf thymus deoxyribonucleic acid (ct-DNA) is considered using UV/Vis spectroscopy. The spectral data were analyzed using Origin 6.1 software. In order to determine the thermodynamic parameters, the titration of [H₅PMo₁₀V₂O₄₀] with DNA was made at several temperatures. The binding constant and the other thermodynamic parameters for this interaction are calculated. The heterogeneous binding mode for the [H₅PMo₁₀V₂O₄₀] complex to DNA was concluded from such analysis. The spectral investigations do not represent any isobestic point, which is consistent with there being more than one binding mode between [H₅PMo₁₀V₂O₄₀] and DNA. Also the results are consistent with outside and groove binding modes. The results show that the driving force for binding is entropy. This result is in agreement with outside binding mode. Third, the melting curves of DNA was studied. The results of the thermal denaturation curves of DNA represent outside binding mode.

Keywords: ct-(DNA)-Thermal denaturation- UV/Vis spectroscopy-10-molybdo 2-vanado phosphoric acid- Ligand Binding

INTRODUCTION
Deoxyribonucleic acid (DNA) contains all of genetic information required for cellular function hence it plays a role in life process since [1]. During cell division the DNA molecule replicates, so that each new cell receives an exact copy of genetic information. Replication is a process for cell pollution. Under various condition, DNA to be damaged. Some of damage may lead to various pathological changes in living organism. Therefore, explorer in case of the binding of drug with DNA is growing in recent years. Polyoxometalates are negatively charged metal oxygen cluster. These compounds have attracted much attention during last decades because of their extensive application to many fields, such as catalysis, analytical chemistry, medicine and materials science [2-5]. Several polyoxometalates had been reported to inhibit the replication of virus and cancer DNA. In situ, a significant antiviral effect of polyoxometalates against replication of retrovirus, toga virus, paramyxo virus [6], herpes simplex [7], rauscher leukemia, polio virus, Epstein-barr, rabies [8] and anti tumor effect against various cancer such as AsPC-1 human pancreatic cancer cells [9], Meth A sarcoma, OAT lung cancer, Co-4 [10], MX-1 murine mammary cancer cell line and MM46 adenocacinoma [11,12] are investigated. In this paper we studied the interaction of [H₅PMo₁₀V₂O₄₀] with DNA by UV/Vis spectroscopy method.

METHODS
Chemicals and Preparations
Preparation of 10-molybdo 2-vanado phosphoric acid , [H₅PMo₁₀V₂O₄₀], was based on a literature procedure [13], potassium dihydrogen phosphate, potassium monohydrogen phosphate were obtained from Merck Chemical Co. and were in analytical grades and used as received. Calf thymus DNA (ct-DNA) was purchased from Sigma Chemical Co. Stock solution of ct-DNA was prepared by dissolution in 5 mM phosphate buffer and 24h stirred overnight, was stored below 4°C in the dark for short periods only. The base-pairs concentration of ct-DNA was determined by its known absorbance measurements using ε =1.32×10⁴ L.mol⁻¹.Cm⁻¹ (i.e. reported in molar base pair) at the absorption maximum of 260 nm [14, 15]. Phosphate buffer solution was used to control the pH of the media (pH 7.0) and measurements were performed on a Metrohm-691 pH-meter. All other reagents were analytical reagent grade and used without further purification. Double distilled water was used throughout the experiments.

Optical Absorption
The absorbance monitoring was performed on a GBC UV/Vis Cintra 101 Spectrophotometer (Victoria, Australia) equipped with thermostat cell compartment and UV-Lite software. The UV/Vis titration experiments were made by addition of the polyoxomolybdate solution into a 1.4 mL cuvette containing the DNA solution of appropriate concentration. The titration experiments were performed at various temperatures with precision of ±0.1 °C.
Thermal Denaturation of ct-DNA
The melting curves of both free ct-DNA and POMo–DNA complex in phosphate buffer were obtained by measuring the of ct-DNA absorbance at 260 nm as a function of temperature. Melting temperatures were measured in phosphate buffer solutions pH 7.0 containing 200 µM ct-DNA. The temperature was scanned from 24 to 86ºC.

RESULTS AND DISCUSSION
Solution properties of [H₅PMo₁₀V₂O₄₀]
In order to identify the solution properties of [H₅PMo₁₀V₂O₄₀], we employed UV/Vis spectroscopy. Figure 1 shows the structure of [H₅PMo₁₀V₂O₄₀]. The optical absorption spectrum of [H₅PMo₁₀V₂O₄₀] shows three bands in 209, 223 and 306 nm. We choose 306 nm for our study. Table 1 summarizes the molar absorptivity of these bands in various temperatures. In 25°C, the band maximum of [H₅PMo₁₀V₂O₄₀] obeys Beer’s law over concentration range between 2.86×10⁻⁵ to 5.05×10⁻⁵ M in 5 mM phosphate buffer, pH 7.0. From this observation we can conclude that [H₅PMo₁₀V₂O₄₀] does not show concentration dependent aggregation.

Effect of ionic strength
The effect of NaCl on the UV/Vis spectrum of [H₅PMo₁₀V₂O₄₀] (1×10⁻⁴ M) in water is shown in Fig. 2 and the data concerning these spectral changes are presented in Table 2 that obtained by Origin 6.1 software. As the concentration of NaCl increases, the band width at half height, W₁/₂, increases and the wavelength of maximum absorption, λ_max don't show considerable changes. Also, the absorption spectrum of [H₅PMo₁₀V₂O₄₀] shows no significance electrolyte effect, no new band appears even in high concentration of salt. This result means that [H₅PMo₁₀V₂O₄₀] does not form well defined aggregates (i.e. H or J type) even at high concentrations of salt.

Binding of [H₅PMo₁₀V₂O₄₀] to ct-DNA
Optical absorption
The interaction of [H₅PMo₁₀V₂O₄₀] with calf thymus DNA was studied by UV/Vis technique. The experiments carried out at six temperatures as 25, 30, 35, 40, 45 and 50 °C. Because both DNA and POMo have same absorbance wavelength, we have to use differential absorbance titration method. The titration was performed in a fixed concentration of DNA and varying concentration of POMo in 5 mM phosphate buffer, pH 7 at 25 °C.

Table 2. UV-vis spectral characteristics of [H₅PMo₁₀V₂O₄₀] solution (1×10⁻⁴) upon increasing the NaCl concentration

<table>
<thead>
<tr>
<th>[NaCl] / M</th>
<th>A_max</th>
<th>λ_max(nm)</th>
<th>W₁/₂(nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.45</td>
<td>0.20</td>
<td>304.22</td>
<td>94.62</td>
</tr>
<tr>
<td>0.83</td>
<td>0.17</td>
<td>303.46</td>
<td>95.50</td>
</tr>
<tr>
<td>1.15</td>
<td>0.16</td>
<td>299.94</td>
<td>98.48</td>
</tr>
<tr>
<td>1.42</td>
<td>0.16</td>
<td>298.47</td>
<td>98.80</td>
</tr>
<tr>
<td>1.66</td>
<td>0.14</td>
<td>294.25</td>
<td>102.86</td>
</tr>
<tr>
<td>1.87</td>
<td>0.12</td>
<td>293.64</td>
<td>105.20</td>
</tr>
<tr>
<td>2.34</td>
<td>0.11</td>
<td>292.72</td>
<td>108.35</td>
</tr>
</tbody>
</table>

Fig. 2. Absorption spectra of [H₅PMo₁₀V₂O₄₀] solution (1×10⁻⁴) upon addition of NaCl solution (0.45, 0.83, 1.15, 1.42, 1.66, 1.87 and 2.34 M) in 5 mM phosphate buffer, pH 7 at 25 °C.

Table 1. UV-vis spectral characteristics of [H₅PMo₁₀V₂O₄₀] in aqueous solution

<table>
<thead>
<tr>
<th>C °/</th>
<th>Temperature</th>
<th>M³ cm⁻¹ / 295°C</th>
<th>M³ cm⁻¹ / 223°C</th>
<th>M³ cm⁻¹ / 260°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>16652</td>
<td>54453</td>
<td>65684</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>16712</td>
<td>51219</td>
<td>73384</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>17200</td>
<td>62310</td>
<td>88749</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>18645</td>
<td>65532</td>
<td>75324</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>22253</td>
<td>63811</td>
<td>67511</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>23431</td>
<td>71883</td>
<td>82139</td>
<td></td>
</tr>
</tbody>
</table>
at 25 °C. We analyzed the UV/Vis data by Origin 6.0 software and found the binding of POMo to DNA produces hyperchroism, to increase the band width at half height, W/1/2, without change in wavelength of maximum absorption. These effects are particularly pronounced for outside binders.

**Thermodynamic investigation of binding**

The binding constant at any specified temperature was determined by following equation [16]:

\[
\frac{[DNA]}{[DNA]} = \frac{1}{K_b} + \frac{1}{(\varepsilon_a - \varepsilon_f) K_b (\varepsilon_b - \varepsilon_f)}
\]  

(eq 1)

Where \( \varepsilon_a \) is the appearance molar absorption coefficient, \( \varepsilon_f \) is molar absorption of free POMo and \( K_b \) is binding constant. The ratio of slope to intercept of linear plot of \( [DNA] \) vs. \( [DNA] \) is \( K_b \). We calculated \( K_b \) for binding at various temperatures. The thermodynamic parameters such as standard Gibbs free energy change, \( \Delta G^\circ \), and standard molar enthalpy change, \( \Delta H^\circ \), and standard molar entropy change, \( \Delta S^\circ \), can determine by the alues at various temperatures. The standard Gibbs free energy change is usually calculated from the equilibrium constant (K) of the reaction, by the following relationship:

\[
\Delta G^\circ = -RT \ln K
\]  

(2)

Since the activity coefficients of the reactions are not known, the usual procedure is to assume them unity and to use the equilibrium concentrations instead of the activity. Therefore, it would be appropriate to adjust the terminology of apparent equilibrium constant \( K' \), and Gibbs free energy \( \Delta G'^\circ \). Apparent standard enthalpies per mole of cooperative unit can be obtained from the dependence on temperature of the apparent binding constant \( K' \), by van’t Hoff equation:

\[
\frac{\partial \ln K'}{\partial (1/T)} = -\frac{\Delta H'^\circ}{R}
\]  

(eq 3)

This is the so-called van’t Hoff enthalpy. The apparent standard entropy change, \( \Delta S'^\circ \), can be derived from the Eq. (4)

\[
\Delta S'^\circ = \frac{\Delta H'^\circ - \Delta G'^\circ}{T}
\]  

(4)

\[
\text{Fig. 4. The van’t Hoff plot [H$_5$PMo$_{10}$V$_2$O$_{40}$] binding to DNA}
\]
Table 5. Melting points of DNA temperature changes upon increasing the mole ratios of \( \frac{[H_5PMo_{10}V_2O_{40}]}{[DNA]} \)

<table>
<thead>
<tr>
<th>Mole Ratio</th>
<th>( T_m (K) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>338.72</td>
</tr>
<tr>
<td>0.024</td>
<td>338.2</td>
</tr>
<tr>
<td>0.047</td>
<td>342.8</td>
</tr>
<tr>
<td>0.088</td>
<td>338.2</td>
</tr>
</tbody>
</table>

Fig. 6. Melting profiles (\( \lambda_{max} = 260 \text{ nm} \)) for the free ct-DNA in the absence of ligand and for the different mole ratios of ct-DNA and \([H_5PMo_{10}V_2O_{40}]\)

Determination of thermodynamic parameter

Values of \( \Delta H_m \) were obtained from melting curves data and following equation:

\[
-\Delta\varepsilon_{260}(T) = (a_N + b_N T) + (a_D + b_D T) \exp \left[ \frac{\Delta H_m}{R} \left( \frac{1}{T} - \frac{1}{T_m} \right) \right] + 1 + \exp \left[ \frac{\Delta H_m}{R} \left( \frac{1}{T} - \frac{1}{T_m} \right) \right] 
\]

Where \(-\Delta\varepsilon_{260}(T)\) is negative changes of molar absorption coefficient between any temperature and 25 \(^\circ\)C, \( (a_N + b_N T)\) is the thermal dependence of \(-\Delta\varepsilon_{N,260}\) (for native DNA in 260 nm), \( (a_D + b_D T)\) is thermal dependence of \(-\Delta\varepsilon_{D,260}\) (for denatured DNA in 260 nm), \( T_m \) is melting point of DNA and \( \Delta H_m \) is change in molar enthalpy of denaturation. Table 6 show these data for different mol ratio of \( \frac{[POMo]}{[DNA]} \). we obtained these values by fitting data in equation (11) by Sigma Plot software.

\( \Delta C_p \) (the change in excess heat capacity upon DNA denaturation) for DNA was estimated from \( \Delta H_m \) versus \( T_m \). The value of \( \Delta C_p \) is 24.433 kJ mol\(^{-1}\) K\(^{-1}\).
Magnitude of this value represent that the unnatural process is cooperative and hydrophobic. With $T_m$, $\Delta H_m$ and $\Delta C_p$ by using equation (12) we estimated $\Delta G_{m,D}$ for denaturation:

$$\Delta G = \Delta H_m (1 - \frac{T}{T_m}) - \Delta C_p [(T_m - T) + T \ln \frac{T}{T_m}]$$  \hspace{1cm} (12)

Figure 7 show $\Delta G_{m,D}$ versus $T(K)$. According to this Figure, all of curves are coincide and have a maximum that represent maximum of stability.

Table 6. the curve fitting parameter for deaturation of ct-DNA

<table>
<thead>
<tr>
<th>$[H_3PMo_{10}V_2O_{40}]$</th>
<th>$[ct - DNA]$</th>
<th>0</th>
<th>0.024</th>
<th>0.047</th>
<th>0.088</th>
</tr>
</thead>
<tbody>
<tr>
<td>$(a_0 \times 10^2)$ M$^{-1}$ cm$^{-1}$</td>
<td>1.39±1.02</td>
<td>3.87±1.12</td>
<td>4.04±1.31</td>
<td>1.28±1.31</td>
<td></td>
</tr>
<tr>
<td>$(b_0 \times 10^2)$ M$^{-1}$ cm$^{-1}$</td>
<td>-4.62±3.30</td>
<td>-12.95±3.62</td>
<td>-13.48±4.19</td>
<td>-4.32±4.24</td>
<td></td>
</tr>
<tr>
<td>$(a_0' \times 10^2)$ M$^{-1}$ cm$^{-1}$</td>
<td>1.74±3.84</td>
<td>3.83±2.82</td>
<td>3.84±6.61</td>
<td>1.40±3.16</td>
<td></td>
</tr>
<tr>
<td>$(b_0' \times 10^2)$ cm$^{-2}$ K$^{-1}$</td>
<td>-7.36±1.09</td>
<td>-14.35±7.95</td>
<td>-14.53±1.87</td>
<td>-6.59±1.07</td>
<td></td>
</tr>
<tr>
<td>$T_m$ (K)</td>
<td>338.72±0.01</td>
<td>338.19±0.01</td>
<td>342.79±0.01</td>
<td>338.19±0.01</td>
<td></td>
</tr>
<tr>
<td>$\Delta H_m$ (kJ/mol)</td>
<td>314.90±1.69</td>
<td>378.00±2.79</td>
<td>402.20±3.68</td>
<td>277.50±1.53</td>
<td></td>
</tr>
</tbody>
</table>

Table 7. $T_s$ value for DNA in addition of POMo

<table>
<thead>
<tr>
<th>$[H_3PMo_{10}V_2O_{40}]$</th>
<th>$[DNA]$</th>
<th>0</th>
<th>0.024</th>
<th>0.047</th>
<th>0.088</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_s$(K)</td>
<td>323.15</td>
<td>325.15</td>
<td>327.15</td>
<td>327.15</td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSION

$[H_3PMo_{10}V_2O_{40}]$ does not show concentration dependent aggregation over an extended concentration range $2.86\times10^{-5}$ to $5.05\times10^{-5}$ M in 5 mM phosphate buffer, pH 7.0. Addition of NaCl shows no significance electrolyte effect and no new band appears even in high concentration of salt. This result suggests that $[H_3PMo_{10}V_2O_{40}]$ does not form well defined aggregates (i.e. H or J type) even in high concentrations of salt. $[H_3PMo_{10}V_2O_{40}]$ binds to external region of ct-DNA. The ct-DNA-binding process was endothermic for $[H_3PMo_{10}V_2O_{40}]$ and has a large positive entropy value. The small change of melting temperature (Tm) of ct-DNA upon addition of POMo represents the existence of outside binding mode.

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REFERENCES: