CHAPTER 5

STABILITY OF c-MYC 27 mer QUADRUPLEX DNA WITH PORPHYRIN
5.1 Introduction

The main controller of cellular propagation and cell development is human c-MYC gene. This c-MYC is associated with a variety of nasty tumors (Resar et. al. 1999). The nuclear hypersensitivity element III₁ (NHE₁), upstream of the P1 promoter of c-MYC controls, 80-90% of c-MYC transcription level (Michelotti et. al. 1995, Tomonaga et. al. 1996, Simonsson et. al. 1998, Postel et. al. 2000, Simonsson et. al. 2000). The NHE III₁, is a guanine loaded strand of DNA which contains 27 base pair sequence and can form an intramolecular G-quadruplex structure. It functions as a transcription suppressor (Siddiqui-Jain et. al. 2002). Telomerase reducers such as a telomestatin (Lemarteleur et. al. 2004), TMPyP4 (Grand et. al. 2002) and Se2SAP (Seenisamy et. al. 2005) can be used to reverse the transcription action of c-MYC.

Last few years has seen the development of such small molecules which can choose the G-quadruplex structure as a target to bind and can also stabilize them. So the G-quadruplex shape of DNA has come forward as a new and promising target for the discovery and design of new classes of anticancer drugs (Sun et. al. 1997, Han et. al. 2000, Hurley et. al. 2001).

Matthew et. al. (2007) had reported thermodynamic and structural characterization of G-quadruplex binding with Porphyrin (Fig. 5.1).

![Figure 5.1 Chemical structure of Porphyrin.](image)

Figure 5.1 Chemical structure of Porphyrin.
In their work an interaction between porphyrin and c-MYC PU 27-mer sequence 5’-TGGGGAGGGTGGGGAGGGTGGGGAAGG have been presented. The thermal melting profiles were obtained for c-MYC 27-mer quadruplex construct in 20, 50, 100, 200 mM KCl (all of these with 30 mM phosphate buffer, pH 7.0), using differential scanning calorimetry.

In the present study, we have used the theory of co-operativity for a finite system to generate the transition profiles and λ-point anomaly in heat capacity at transition point. The heat capacity measurements of Matthew et. al. (2007), had provided the input information. The alteration in nucleation parameter, which is inverse measure of binding strength, reflects the consequences of porphyrin binding.

5.2 Theoretical Approach

The amended Zimm and Bragg theory (1959) has been applied because the melting of Porphyrin bonded to DNA can be treated as a two phase problem. In brief, we write an Ising matrix for two phase system, the bonded state and unbounded state. As discussed earlier (Zimm et. al. 1959, Polkar et. al. 1996, Srivastava et. al. 1999, Srivastava et. al. 2001, Srivastava et. al. 2004), the Ising matrix $M$ can be written as:

$$
M = \begin{pmatrix}
  f_r & f_k & f_h \\
  \frac{1}{f_r} & \frac{1}{f_k} & 0 \\
  \frac{1}{f_k} & 0 & \frac{1}{f_h} \\
  0 & \frac{1}{f_h} & \frac{1}{f_r}
\end{pmatrix}
$$

Eq.(5.1).

Where $f_r$, $f_h$ and $f_k$ are the contributions of the base pair partition functions in the three states (i.e disordered, ordered and boundary or nucleation). The Eigen values for $M$ are given by:
\[ \lambda_1 = \frac{1}{2} \left[ (f_r + f_h) + \{(f_r - f_h)^2 + 4f_rf_k\}^{1/2} \right] \]

\[ \lambda_2 = \frac{1}{2} \left[ (f_r + f_h) - \{(f_r - f_h)^2 + 4f_rf_k\}^{1/2} \right] \]

\[ \lambda_3 = 0 \quad \text{Eq.(5.2).} \]

The detailed theoretical approach of above theory has been discussed in chapter 2, section 2.2.

5.3 Results

5.3.1 Transition Profiles

When Porphyrin binds to c-MYC 27-mer quadruplex, DNA still remains highly cooperative and hence two state theory of order and disorder transitions can be applied. The Zimm Bragg theory is amended so as to consider ordered and disordered transitions (bounded & unbounded states), as the two states coexist at the transition point. The transition is characterized mainly by the nucleation parameter and overall change of entropy (enthalpy), which are also the main thermodynamic forces driving the transitions. The change in enthalpy obtained from differential scanning calorimetric (DSC) measurements takes all this into account. This is evident from the melting point given in Table 5.1. The result obtained from the theoretical study suggested that the binding of Porphyrin increases the melting temperature of c-MYC 27-mer quadruplex. For c-MYC 27-mer binding to porphyrin the data is provided for several values of strand concentrations (Table 5.2).

The sharpness of the transition has been defined as a product of \(\Delta H\) and nucleation parameter which provides the ‘best fit’ to the experimental data on melting. The half widths for the transition profile and heat capacity are a measure of sharpness and related to each other. The latter are also given in Table 5.2. All the theoretical transition curves
are in a very good agreement with the experimental data of Matthew et. al. (2007). The enthalpy change and melting temperature, at which the free energy is zero, enable us to evaluate the transition profile. \( N \) which is the number of hydrogen bonds broken during the melting process is equal to 74 in our case. The transition profiles of 27-mer quadruplex revealed that greater stabilization is achieved as ionic strength is increased.

Table 5.1 Transition temperatures for Porphyrin binding to c-MYC 27-mer quadruplex at 100 mM KCl.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>c-MYC 27-mer Quadruplex DNA without Porphyrin at 100 mM KCl</th>
<th>c-MYC 27-mer Quadruplex DNA bonded with Porphyrin at 100 mM KCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transition Temperature</td>
<td>355 (Tian et. al. 2007)</td>
<td>363</td>
</tr>
</tbody>
</table>

Table 5.2 Transition Parameters for Porphyrin binding to c-MYC 27-mer quadruplex.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>G-Quadruplex DNA bonded with Porphyrin at 20 mM KCl</th>
<th>G-Quadruplex DNA bonded with Porphyrin at 50 mM KCl</th>
<th>G-Quadruplex DNA bonded with Porphyrin at 100 mM KCl</th>
<th>G-Quadruplex DNA bonded with Porphyrin at 200 mM KCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T_m ) (K)</td>
<td>353</td>
<td>358</td>
<td>363</td>
<td>370.5</td>
</tr>
<tr>
<td>( \Delta H ) (Kcal/Mbp)</td>
<td>1576.413</td>
<td>1083.03</td>
<td>1095.06</td>
<td>1432</td>
</tr>
<tr>
<td>( \sigma )</td>
<td>0.0081</td>
<td>0.004</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>( N )</td>
<td>74</td>
<td>74</td>
<td>74</td>
<td>74</td>
</tr>
<tr>
<td>Half Width (Exp)</td>
<td>20</td>
<td>20</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>Half Width (Theo)</td>
<td>22</td>
<td>24</td>
<td>28</td>
<td>29</td>
</tr>
<tr>
<td>Sensitivity parameter (( \Delta H/\sigma ))</td>
<td>194618.9</td>
<td>270757.5</td>
<td>219012</td>
<td>286400</td>
</tr>
</tbody>
</table>
5.3.2 Heat Capacity

The heat capacity with λ-point anomaly along with their transition profiles for bonded 27-mer quadruplex construct in 20 mM KCl is shown in Fig 5.2, similarly Fig 5.3, Fig 5.4 and Fig 5.5 represents the heat capacities with λ-point anomaly along with their transition profiles for bonded 27-mer quadruplex construct in 50 mM KCl, 100 mM KCl and 200 mM KCl. The results revealed that the theoretically obtained heat capacity profiles agreed with the experimentally reported one and could be brought almost into concurrence with the use of scaling factors. Minor insignificant deviations at the tail end is primarily due to the presence of various disordered states and presence of short helical segments found in random coil states. The predicted values of transitional profiles for bonded states at 20 mM KCl, 50 mM KCl, 100 mM KCl and 200 mM KCl are given in Fig 5.2, Fig 5.3, Fig 5.4 and Fig 5.5. It must also be noted that the heat capacities and the transition profiles of 27-mer quadruplex revealed that greater stabilization is achieved as ionic strength is increased.
Figure 5.2 Specific Heat versus Temperature and Absorbance Curve of Porphyrin binding with DNA at 20mM KCl.
Figure 5.3 Specific Heat versus Temperature and Absorbance Curve of Porphyrin binding with DNA at 50mM KCl.
Figure 5.4 Specific Heat versus Temperature and Absorbance Curve of Porphyrin binding with DNA at 100mM KCl.
Figure 5.5 Specific Heat versus Temperature and Absorbance Curve of Porphyrin binding with DNA at 200mM KCl.
5.4 Conclusion

The accomplishment made in this chapter is that the DNA molecule is a highly co-operative structure and when Porphyrin binds to it, the co-operativity is not altered greatly. Therefore the amended Zimm Bragg theory (phase transition theory) can be effectively applied to it. It generates the experimental transition profiles and λ-point anomaly successfully. These results will allow us to assess the thermodynamic profile of binding process. Our theoretical data also demonstrates that the binding of Porphyrin to 27-mer quadruplex is an endothermic process and that the binding increases the melting temperature of the 27-mer quadruplex. Therefore the theoretical analysis presented in this study can be implicated to understand bimolecular interactions and may also be applied in biomedical industries for drug design and development.