

DETERMINATION OF BINDING ENTHALPY VALUE
AT COMPLEX-FORMATION OF LIGAND WITH DNA

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The value of binding enthalpy change (ΔH) due to DNA–ligand complex-formation has been determined by Van't-Hoff mode having dependence of binding constant (K) on the reverse value of absolute temperature. Calculations that were carried out for DNA–ethidium bromide and DNA–mitoxantrone complexes showed, that if ΔH is determined from the values of K at two different temperatures, the calculated value of ΔH in the error frame coincides with its experimental value.

Keywords: DNA, ethidium bromide, mitoxantrone, complex-formation, thermodynamic parameters.

Introduction. Enthalpy change of the system emerging from ligand binding to nucleic acids (NA) (binding enthalpy, ΔH) is usually determined by micro-calorimetric method [1–3]. During such measurements relatively big amount of NA and ligands are used. The binding enthalpy is an important thermodynamic parameter characterizing complex stability. It is possible to judge about organization of complexes and their strength via the value and sign of this parameter. Simultaneously, having ΔH value of the complex-formation of a range of new synthesized pharmacologically active compounds with NA, it becomes possible to choose some of them for further clinic investigations.

ΔH may also be calculated from the binding constant values of NA–ligands at several temperatures [3–6]. Usually from titration curves of NA–ligand complexes, concentrations of both bound and free ligands in the solution are determined, through which the binding isotherm is constructed [5, 6]. Binding isotherms are usually described by linear [7] and non-linear [1, 4–6] formula, from which the binding constant value is determined. Determination of the binding constant by this mode is described in [1, 4–6] in details.

The goal of this work is to determine the binding enthalpy value from possibly easy and less experimental studies.

Materials and Methods. In experiments DNA from *M. lysodeikticus* and ethidium bromide (“Sigma”, USA) were used. Preparations were used without

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further purification. Concentrations of double-stranded DNA and ethidium bromide (EtBr) were determined spectrophotometrically, using the following values of extinction coefficients: $\varepsilon_{260}(P)=6800 M^{-1}cm^{-1}$ for DNA and $\varepsilon_{480}=5800 M^{-1}cm^{-1}$ for EtBr. The interaction of EtBr with DNA was studied in buffer containing 0.1 M NaCl and 0.01 M Tris, pH 7.5, at 308.15, 318.15 and 323.15 K temperatures. Binding isotherms were constructed from the absorption spectra. Absorption spectra were obtained via Unicam SP-8-100 spectrophotometer.

The temperature dependent values of the binding constants of DNA–mitoxantrone (MTX) complex-formation obtained by prof. Babayan and presented in [8] were used in this work.

Results and Discussion. The binding isotherms were obtained through absorption spectra. The binding isotherms were constructed by Scatchard's coordinates (r/C_f dependence on r , where $r=C_b/C_p$, C_f and C_b are the concentrations of free and bound ligands in the solutions, and C_p is DNA concentration according to nucleotides), which were circumstantially described in [5, 6, 8]. It is known that the interaction of double helical DNA with EtBr in the mentioned conditions mainly has an intercalation character [1, 5], but with MTX – a semi-intercalation [8, 9]. The binding isotherms of DNA–EtBr and DNA–MTX complexes were described by formula (1) [10].

$$\frac{r}{C_f} = K(1 - nr) \left[\frac{1 - nr}{1 - (n-1)r} \right]^{n-1}, \quad (1)$$

where K is the ligand binding constant to DNA; n is the number of DNA bases becoming unavailable, when one ligand binds.

The binding constant values determined by formula (1) from the binding isotherms of DNA–MTX and DNA–EtBr complexes were presented in Tab. 1.

Table 1

Values of several thermodynamic parameters of DNA–ligand complex-formation

Ligand type	T, K	$K \cdot 10^{-5} M^{-1}$	$-\Delta G \text{ kcal/mol}$	$-\Delta H \text{ kcal/mol}$ determined by (3)
Ethidium bromide	295.15	35±2	8.8±0.1	7.5
	305.15	23±2	8.85±0.25	
	315.15	15.5±1.5	8.9±0.2	
Mitoxantrone	308.15	6.7±0.5 [8]	8.2±0.1	10.3
	318.15	3.9±0.3 [8]	8.0±0.2	
	323.15	3.0±0.4 [8]	8.1±0.1	
	333.15	1.9±0.2 [8]	8.1±0.2	

It is followed from Tab. 1 that the binding constant values of DNA–EtBr and DNA–MTX complexes depend on temperature and differ from each other by almost one order. In the case of semi-intercalation interaction, DNA–MTX complex is more unstable compared to intercalation interaction of DNA–EtBr complex.

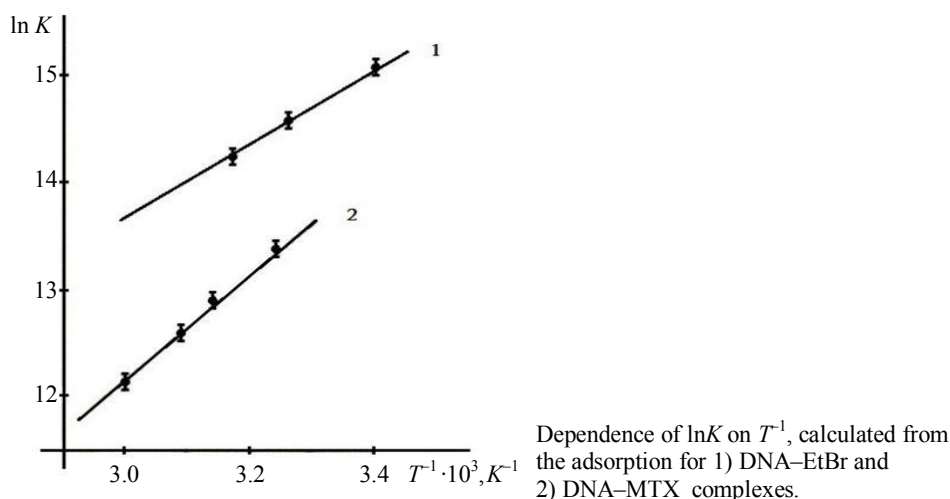
Having the value of K Gibbs free energy change due to complex-formation may be calculated:

$$\Delta G = -RT \ln K. \quad (2)$$

Taking into account that $\Delta G = \Delta H - T\Delta S$, where ΔS is system entropy change due to complex-formation, and formula (2):

$$\begin{aligned} \Delta H - T\Delta S &= -R \ln K, \\ \ln K &= -\frac{\Delta H}{R} \cdot \frac{1}{T} + \frac{\Delta S}{R}. \end{aligned} \quad (3)$$

According to formula (3) the dependence of $\ln K$ on $1/T$ is a line, the tangent of slope angle of which is equal to $-\Delta H/R$. The values of $\ln K$ were determined from those of K for DNA–EtBr and DNA–MTX complexes presented in Tab. 1. Through these experimental points by average square principle a line was passed (see Figure) and the value of ΔH was calculated for DNA–EtBr and DNA–MTX complexes (Tab. 1). It should be mentioned that the value of ΔH determined from the binding isotherms coincides well with that obtained by direct microcalorimetric method for these complexes [1–3].



As it is obvious from Tab. 1, the change of ΔG free energy in the error limit does not depend on temperature. Accepting that the values of ΔH and ΔS do not depend on temperature as well, for ΔH it will be received:

$$\Delta H = \frac{RT_1 T_2 \ln(K_2/K_1)}{T_2 - T_1}. \quad (4)$$

Table 2

The binding enthalpy value determined by formula (4) for DNA–ligand complex-formation

DNA–EtBr						
T, K	295.15	305.15	295.15	315.15	305.15	315.15
$-\Delta H$ kcal/mol	7.45		7.38		7.51	
DNA–MTX						
T, K	308.15	318.15	308.15	323.15	318.15	323.15
$-\Delta H$ kcal/mol	10.5	10.55	10.1	9.7	10.2	10.6

Using the presented in Tab. 1 values for K and choosing two values of K for random two temperatures, ΔH value was calculated by formula (4), which is presented in Tab. 2.

It is followed from Tab. 2 that the values of ΔH determined, through randomly chosen two temperatures for DNA–EtBr and DNA–MTX complexes, do not significantly differ from those determined from $\ln K$ dependence curve on temperature and presented in Tab. 1. If we consider that the error of microcalorimetric measurements exceeds 5% of measured value, it will be possible to insist that there is no necessity to determine values of K at more than two temperatures, then to pass a line through experimental points by average square method and to calculate the value of ΔH . Determining the values of K at two different temperatures the binding enthalpy value may be calculated by formula (4).

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REFERENCES

1. **Babayan Y., Manzini G., Xodo L.E., Quadrifoglio F.** Base Specificity in the Interaction of Ethidium Bromide with Synthetic Polynucleotides. // *Nuclear Acids Res.*, 1987, v. 15, № 14, p. 5803–5812.
2. **Delben F., Quadrifoglio F., Giacotti V., Crescenzi V.** Comparative Microcalorimetric Dilatometric Analysis of the Interaction of Quinacrine Chlorogline and Ethidium Bromide with DNA. // *Biopolymers*, 1982, v. 21, № 2, p. 331–341.
3. **Bhattacharyya J., Basu A., Kuma G.S.** Intercalative Interaction of Anticancer Drug Mitoxantrone with Double Stranded DNA: A Calorimetric Characterization of the Energetics. // *J. Chem. Thermodynamics*, 2014, v. 75, № 8, p. 45–51.
4. **Karapetyan A.T., Babayan Y.S., Torosyan M.A., Shahinyan M.A.** Mitoxantrone Interaction with poly(G). // *Proceedings of YSU. Phys. Math. Sci.*, 2015, № 3, p. 49–54.
5. **Vardevanyan P.O., Parsadanyan M.A., Minasyants M.V.** Ethidium Bromide Interaction with poly(G). // *Biophys. Reviews and Letters*, 2014, v. 9, № 3, p. 239–247.
6. **Babayan Y.S., Manzini G., Quadrifoglio F.** Ethidium Bromide Interaction with Synthetic Double-Stranded Polyribonucleotides. // *Molecular Biologia*, 1988, v. 22, № 4, p. 898–910.
7. **Arakelyan V., Babayan Y., Potikyan G.** Determination of Constant Rats of Adsorption of Ligand on DNA: Analysis of Correlation Functions. // *J. Biomol. Str. Dyn.*, 2000, v. 18, № 2, p. 231–235.
8. **Babayan Y.S., Manzini G.** The Interaction of Antitumoral Preparation Mitoxantrone with Double Helical Nucleic Acids. // *Molecular Biologia*, 1990, v. 24, № 4, p. 1084–1094.
9. **Karapetyan A.T., Torosyan M.A., Babayan Y.S.** Antitumor Drug Mitoxantrone do not Preclude Salt-Driven B→Z Transition of poly(dG-dC). // *Inter. J. Biochem. Biophys.*, 2015, v. 3, № 4, p. 49–53.
10. **McGhee J.D., von Hippel P.H.** Theoretical Aspects of DNA–Protein Interactions. // *J. Mol. Biol.*, 1974, v. 84, № 3, p. 469–489.