

## Chapter 2

# Structure, Thermodynamics and Energetics of Drug-DNA Interactions: Computer Modeling and Experiment

Maxim P. Evstigneev and Anna V. Shestopalova

**Abstract** In this chapter we demonstrate the large usefulness of using complex approach for understanding the mechanism of binding of biologically active compounds (antitumour antibiotics, mutagens etc.) with nucleic acids (NA). The applications of various biophysical methods and computer modeling to determination of structural (Infra-red and Raman vibrational spectroscopies, computer modeling by means of Monte-Carlo, molecular docking and molecular dynamics methods) and thermodynamic (UV-VIS spectrophotometry, microcalorimetry, molecular dynamics simulation) parameters of NA-ligand complexation with estimation of the role of water environment in this process, are discussed. The strategy of energy analysis of the NA-ligand binding reactions in solution is described, which is based on decomposition of experimentally measured net Gibbs free energy of binding in terms of separate energetic contributions from particular physical factors. The main outcome of such analysis is to answer the questions “What physical factors and to what extent stabilize/destabilize NA-ligand complexes?” and “What physical factors most strongly affect the bioreceptor binding affinity?”

---

M. P. Evstigneev (✉)  
Sevastopol National Technical University (SevNTU), Universitetskaya Str., 33,  
Sevastopol 99053, Ukraine  
e-mail: max\_evstigneev@mail.ru

A. V. Shestopalova  
A.Usikov Institute for Radiophysics and Electronics National Academy of Sciences of Ukraine  
(IRE NASU), Ak. Proskura str., 12, Kharkov 61085, Ukraine  
e-mail: shestop@ire.kharkov.ua

## 2.1 Introduction

Rational design of new compounds for therapeutics requires knowledge about their structural stability and interactions with various cellular macromolecules—their molecular receptors or targets. In order to optimize the efficacy of drugs, as well as discover new ones, it is important to fully characterize the drug—bioreceptor (biopolymer) interaction [1].

Nucleic acids (NA) are common targets for antiviral, antibiotic and anticancer drugs that are used in cancer therapeutics [2] and also are viewed as a non-specific target for cytotoxic agents [3]. Many antitumour drugs are considered to exert their cytotoxic effect through DNA-specific interactions, resulting in genotoxic stress and consequent induction of programmed cell death (apoptosis) [4]. Presently, when patients can be provided with a full genome sequence as a part of their medical records, the field of drug design must be adapted and improved in order to meet this challenge [5]. Rational drug design thus requires detailed knowledge of both the structural consequences of ligation and the binding characteristics of the drug. Ideally, such information is required for DNA targets of genomic size and complexity [6].

In this regard it is important to know how small biologically active molecules—drugs or other ligands—will interact with nucleic acids [7]. One can use biophysical techniques to characterize the binding of the drugs with DNA and, based on experimental data, to expand further understanding of the binding process with an aid of molecular modelling or computer simulations. Such approach allows to get different physical parameters of the interaction in the system “molecular target (DNA)—drug” and to use them for the establishment of correlation between these parameters and drug activity *in vitro* or *in vivo* [8, 9].

## 2.2 Biophysical Methods for Studying DNA-Drug Complexation

One of extensively developing trends in molecular biophysics is prediction of pharmacological action of drugs at the molecular level that requires: (1) determination of the structural features of the complexes “target-drug” containing the biologically active ligands and exerting their maximal biological effectiveness; (2) determination of correlations of the physical parameters of interaction in the system “target-drug” and the biological activity of the drugs; (3) obtaining the most probable molecular models of the “target-drug” complexes based on various experimental physical methods and molecular modelling studies. As a practical outcome, one can formulate recommendations for the synthesis of new biologically active ligands with improved pharmacological properties based on information about the biomolecular target and the calculated physical parameters of ligand interaction with the target.

### 2.2.1 *UV-VIS Spectroscopy*

Absorption UV-VIS spectroscopy is one of the most widespread experimental methods used in molecular biology and biophysics for qualitative and quantitative studies of the interaction of biologically active ligands with DNA. This experimental method enables to identify the formation of complexes, to evaluate their complexation constants, to determine different types of ligand states in solution (e.g. free and bound with polymer matrix by various modes) on the basis of the shape and positions of the maxima in the corresponding spectra, to calculate the size of the binding site and the sequence specificity [10–17]. The changes in absorption spectra on addition of drugs to DNA solutions may be used for identification of different types of ligand complexation (e.g. intercalation or major/minor groove binding).

The method of spectrophotometric titration is usually used for detailed analysis of the binding modes and the structures of the molecular complexes [18]. Concentration dependencies obtained during the titration of the DNA—ligand complexes may be used for calculation of the binding parameters. The most important factor in correct determination of these parameters is the choice of the model of complexation, in which all physically possible binding modes (intercalation, binding with one of the DNA grooves, weak binding or electrostatic interactions of the ligand cation with negatively charged phosphate groups of the polynucleotide) should be taken into account. When a suitable model (or the most probable models) is selected, the equations, determining the relation between the equilibrium concentrations of the molecular components and the interaction parameters, may be used for quantitative description of the titration curve [19–21].

Thermodynamic parameters and the process of thermal denaturation of the drug—DNA complexes can be systematically studied by spectrophotometric method. Such approach yields a thermodynamic profile, i.e. standard free energy, enthalpy and entropy changes in ligand-DNA reaction of binding, using the van't Hoff plot based on determining the value of equilibrium binding constant at various temperatures. These thermodynamic parameters allow to further evaluate the enthalpic and entropic contributions to the free energy change in the DNA complexation process [22].

However, the most fruitful outcome can be achieved by a combination of the UV-VIS spectroscopy with other experimental methods.

### 2.2.2 *Infrared and Raman Vibrational Spectroscopy*

Among different physical methods of investigation of specific structural features and intermolecular interactions of nucleic acids with drugs and water, the Infrared (IR) and Raman vibrational spectroscopies occupy very important position. Both of these methods can effectively probe structural details of solution complexes between the drugs and DNA molecules of genomic size, and are used to determine the ligand binding mode, binding affinity, sequence selectivity, DNA secondary struc-

ture, and structural variations of the DNA—ligand complexes in aqueous solution [23–28].

IR and Raman spectroscopies are able to provide information on the formation of hydrogen bonds in solution. It has long been known that the formation of hydrogen bonds between the proton donor (OH, NH, NH<sub>2</sub>, CH) and acceptor (C = O, C–O, C–N, C = N) groups is accompanied by a low-frequency shift, rising in intensity and increase in the half-width of the absorption bands of stretching vibrations in the IR spectrum [29, 30]. At the same time the absorption bands of deformation vibrations (e.g. NH<sub>2</sub>- and OH-groups) experience high-frequency shifts [31]. These spectral features are considered as a direct evidence of H-bonds formation between the interacting molecules in solution [32]. Thus, the groups of atoms involved in stabilization of different types of DNA—ligand complexes can be identified by the vibrational spectroscopy [33–35]. In particular, analysis of Raman spectra allows to identify the atomic groups of the drugs forming hydrogen bonds with donor or acceptor atomic groups of DNA in all possible types of DNA-drug complexes [36, 37], and to determine, for example, the unwinding of double-stranded B-DNA induced by drug intercalation [38] or structural transition of DNA from B- to A-like conformation accompanying the DNA-ligand complexation [39].

### 2.2.3 Hydration

Since the formation of DNA—ligand complex occurs in water environment and brings one of the most significant contributions to stabilization of the DNA-ligand complexes, it is important to carry out analysis of the role, which water plays in the ligand binding processes [40–42]. From a practical point of view, understanding of hydration is valuable for rational design of novel DNA—binding drugs with predictable affinity and specificity to selected sequences of nucleic acid structures [43].

Investigations of the interaction between water molecules and DNA—ligand complexes in diluted solutions face serious difficulties due to the fact that the bound water is present only in insignificant amounts in solution. This fact implies the need to utilize highly sensitive physical methods for the study of water involvement in the complexation process. This is the reason why there is still a lack of reliable information on the distribution of water molecules in the hydration shells of various complexes, although the investigations of water surrounding of nucleic acid—ligand complexes have so far been carried out using numerous methods including X-ray crystallography [44], osmotic stress [43, 45], volumetry [46, 47] and molecular modeling methods [48–50]. In the IR spectroscopy the main difficulties are associated with the strong OH vibration of water molecules. This problem may be resolved by applying this method with respect to DNA—water systems prepared in wet films with changing water content [51–54]. Analyzing the changes in IR spectra which occur in several frequency modes (e.g. the stretching vibrations of OH- or OD-groups, the absorption band of bases—double and multiple bands, the absorption band of sugar-phosphate backbone) with an increase of the relative humidity,

it is possible to observe the atomic groups, which represent hydration centers, to estimate the order and the degree of their filling with water molecules, to determine distinctive features of the formation of DNA secondary structure in the complexes with ligands and the structure of the hydration environment. This procedure enables to control the state of water and the state of individual structural groups of the biopolymer and the ligand as a function of film moistening. Such an approach also gives an opportunity to estimate thermodynamic parameters of hydration and to construct a model of hydration shell of the complexes [55].

In order to reveal the energy contribution of water to stabilization of nucleic acid structures and their complexes, it is necessary to know the thermodynamic parameters characterizing hydration of DNA, the ligands and the DNA-ligand complexes. Various physico-chemical methods may be used to solve this problem experimentally. In particular, a sufficiently sensitive piezomicrobalance or piezogravimetric method based on the use of quartz resonator, allows to obtain hydration isotherms or dependencies of sorption on relative humidity (in moles of water per mole of sorbent) [51, 52]. The isotherms measured for biopolymers or their complexes with ligands give insight into heterogeneity in the energies of interaction between the hydration sites and the sorbed water molecules.

### 2.2.4 *Calorimetry*

Structural studies are crucial for identifying the specific molecular interactions between the host DNA and the ligand, such that the overall three-dimensional shape of the complex and exact position or binding mode can be determined. But structural analysis alone can provide little knowledge on the nature of molecular forces that drive the complex formation in solution, and on the relative energetic contributions of specific molecular interactions. It is therefore essential to complement structural studies with detailed and rigorous thermodynamic analysis to fully characterize bimolecular complex formation. Differential scanning calorimetry (DSC) is one of the most convenient and informative methods for determining the energy parameters of interaction of the ligands with DNA. Direct measurement of heat effects caused by melting of DNA and its complexes enables to determine the full set of thermodynamic binding parameters and the energetic parameters of structural transitions: enthalpy, entropy and free energy changes, melting temperature and melting interval [56–61]. In order to quantify the energetic parameters of the interaction from DSC heat capacity curves, specific theoretical models must be used. The most well-elaborated approaches for the analysis of heat capacity curves have so far been developed only for protein interactions with ligands, because protein unfolding can often be described by simple two-state model [62, 63]. When the DNA-ligand system is being analysed, certain specificities of the complexation and melting of linear polymeric molecules should be taken into consideration. Recently a novel analytical approach for detailed analysis of the DNA-ligand interactions from DSC data was proposed [64]. The DNA macromolecule in this study is represented as an assembly

of cooperative units, which melt according to the two-state model. Explicit account of ligand distribution on polymeric DNA and the temperature dependencies of melting and binding constants, as well as enthalpies, were considered. Such approach enables to extract the binding constant, stoichiometry, enthalpy, entropy, and heat capacity changes from multiple excess heat capacity profiles obtained at varying concentrations of the ligand (i.e. the two-dimensional DSC curves). Comparison of the binding parameters calculated by fitting of two-dimensional DSC curves with the literature data and with that obtained by alternative experimental techniques, had demonstrated that the approach presented in [64] gives satisfactory results.

### 2.2.5 *Computer Modeling*

Binding affinity of the ligands with DNA may be estimated at the molecular level based on shape complementarity of the interacting parts of the ligands and DNA, and by explicit consideration of physical interactions (electrostatic, van der Waals, hydrophobic, specific hydrogen bonding etc.). It allows to determine the extent to which the formation of the complex under investigation is energetically favourable. However, all microscopic details of the interaction cannot be identified in experiment. In such case computer simulations are commonly used as an appropriate complementary tool for modeling atomic-level interactions that produces the data about the structure of the most probable DNA—ligand complexes and on the contributions of different interactions to their stabilization with explicit account of water environment [65–71].

Molecular docking method is one of the most effective computer simulation methods, making possible a fast re-construction of all possible configurations of complexes between biological macromolecule and the ligand of interest. The molecular docking method is commonly used for estimation of specificity of protein–ligand interactions [72–74]. The docking of ligands to DNA molecules is a less frequently used approach. In this approach anticancer drugs are usually taken as the ligands [75]. In order to investigate their complexes by computer simulation, the initial coordinates must be known. If the structure of the complex under study is absent in structural databases, the investigator often faces a difficulty on how to create the binding site. The results of docking of the ligands with different DNA-targets indicate [76] that upon formation of the intercalation site it is usually enough to take into account only the most significant unwinding in one particular helical step or in the adjacent helical step of DNA double helix. The magnitude of the total unwinding of the DNA in the intercalation complex was found to be dependent on the sequence and length of the target DNA.

The application of Monte Carlo method for the study of hydration of nucleic acids, their components [77–80], and hydration of the DNA-ligand complexes (for example, dCpG with proflavine [81] and DNA with azinomycin B [82] intercalated complexes) was described in literature in detail. Monte Carlo simulations enable to evaluate the low energy conformations of various complexes of DNA fragments,

including their complexes with ligands, and to determine the hydration properties of the complexes being formed [54, 55, 83, 84].

Another very valuable tool in arsenal of theoretical investigation of biological molecules is the method of molecular dynamics simulations. This computational method describes the time dependent behaviour of the given molecular system. To date an extensive use of molecular dynamics simulations has resulted in generation of a wealth of detailed information on the fluctuations and conformational changes of proteins and nucleic acids. Such methods are now routinely used to investigate the structure, dynamics and thermodynamics of biological molecules and their complexes [48–50, 65–71, 85].

## **2.3 Results of Experimental Investigation and Computer Simulation of DNA Complexation with New Synthetic Analogues of Anticancer Antibiotics**

### **2.3.1 General Description of New Synthetic Analogues of Anticancer Antibiotics**

As outlined above, in order to provide a scientific basis for rational design of DNA-targeted drugs, it is necessary to understand how the molecules form complexes with DNA. Another important factor is the ability to quantify such complexation in order to make meaningful comparisons of the behaviour of different drugs. This is the focus of the biophysical studies reviewed below.

Here we present the results of investigations of the physical mechanisms of the interaction with DNA of a new series of biologically-active ligands, analogues of anticancer antibiotic Actinomycin D (AMD), obtained using complex approach involving various experimental biophysical methods and molecular computer modeling.

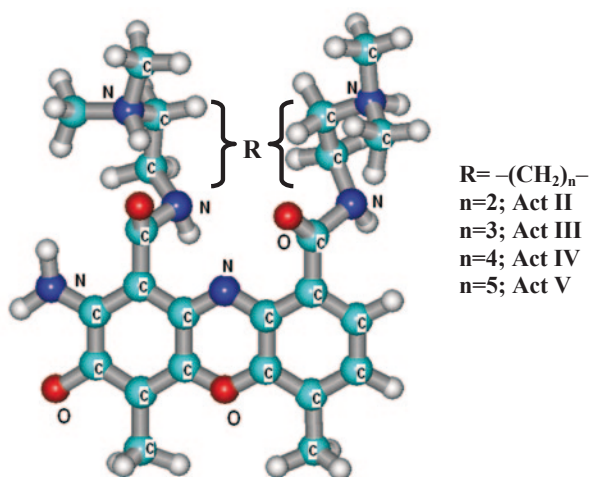
AMD, the synthetic phenoxazone antibiotic, consists of a phenoxazone chromophore substituted with two equivalent cyclic pentapeptide lactone rings. AMD is a DNA-binding drug. Its biological activity is thought to be due to preferential intercalation of the planar phenoxazone chromophore into GC sequence of DNA with the two cyclic pentapeptide rings lying in the minor groove [86].

AMD is an anticancer drug used in treatment of tumours, but its use suffers from induction of negative side effects [87]. With a general aim to reduce the side toxicity of AMD, a new set of drugs with phenoxazone chromophore and dimethyl-aminoalkyl side chains (actinocin derivatives with side chains of different lengths, ActII—ActV, Fig. 2.1) have been synthesized [8].

The cytotoxic effects of the synthetic actinocin derivatives were investigated by examination of the drug-induced apoptosis and cell cycle perturbations in a human leukemia MOLT-3 cell line [88].

Examination of cytotoxic effects in leukemia cells showed that the variation in length of dimethylaminoalkyl side chains of actinocin derivatives leads to signifi-

**Fig. 2.1** Chemical structures of the actinocin derivatives ActII—ActV



cant variation in cytotoxic activity as a function of the number of  $CH_2$  groups in their side chains, with pronounced maximum in cytotoxic activity for the ligand with two  $CH_2$  groups, i.e. ActII. Hence, the antitumour activity in the series of ActII—ActV ligands was found to be very sensitive to minor modifications in the side chains of the AMD derivatives, indicating a direct correlation between structure and activity of the drugs [8].

### 2.3.2 *Free Ligand: Investigation by Experimental and Computer Simulation Methods*

The main goal of the biophysical part of these studies is to understand the nature of specificity interaction between the drugs under investigation and nucleic acids, taking into account the interaction of individual components with water molecules. The following experimental physical methods were used to solve this problem: UV-visible spectrophotometry for the study of different modes of ligand binding with DNA and the corresponding binding parameters, infrared spectroscopy and piezo-gravimetry, giving information on the influence of water on the formation of DNA-drug complexes, and differential scanning calorimetry for obtaining direct data on the thermostability of such complexes. In order to determine the most probable molecular models of the DNA-ligand interactions, the methods of computational analysis (molecular docking, Monte Carlo simulations and molecular dynamics) were used. It is assumed that the results obtained by these methods may be useful for directed synthesis of new drugs with improved medico-biological properties.

The first step of the study was the investigation of the solution behaviour of the synthetic drug molecules alone prior to their complexation with DNA. Investigations of the self- and hetero-association of biologically active compounds

with heterocyclic planar rings (aromatic ligands) in water are interesting from the physico-chemical point of view, resulting in determination of the influence of the structures of the chromophores and side chains on association ability, and estimation of contributions of different interactions to the formation of stable aggregates. Another important issue is the pharmacological aspect, because the self- and hetero-complexations, as well as competitive binding of the drugs with bioreceptor, may influence their activity.

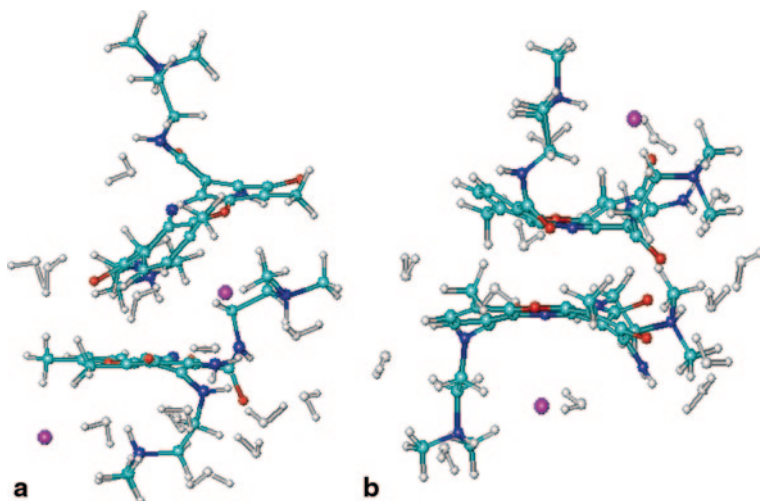
Distinctive features of the self-association of the actinocin derivatives were determined experimentally (by UV-VIS spectrophotometry, piezogravimetry and IR-spectroscopy) and using computer simulation (by Monte Carlo method and molecular dynamics modeling).

Analysis of both spectral and thermodynamic parameters obtained from UV-VIS spectrophotometric data for the set of synthetic actinocin derivatives enabled us to conclude that the drugs experience strong tendency to aggregate in solution and the aggregation is appreciably higher in solutions of high ionic strength. The dimerization parameters depend slightly on the number of methylene groups in the side chains of phenoxazone antibiotics. Dimerization of the investigated ligands in aqueous solution leads to significant changes in the spectral characteristics of the antibiotics ActII-ActV, which needs to be taken into account in any studies of drug complexation with DNA [89].

Formation of DNA complexes with actinocin derivatives is accompanied by hydration changes for both the DNA molecule and the intercalated ligands. In order to evaluate the energy contribution of water molecules to stabilization of these complexes, it is necessary to obtain experimental data on the energies of interaction between water molecules and the free ligands. An investigation of the adsorption of water in the films of actinocin derivatives was performed using quartz crystal microbalance (piezogravimetry). In order to identify the hydration-active centers, the IR absorption spectra of wet and dry films of the actinocin derivatives were recorded in the spectral range  $900\text{--}1700\text{ cm}^{-1}$ , in which the DNA molecules can be characterized by the nitrogen base absorption region ( $1500\text{--}1700\text{ cm}^{-1}$ ) and by the region of sugar phosphate absorption ( $900\text{--}1300\text{ cm}^{-1}$ ). The main conclusion of this stage of investigation was that in contrast to the DNA molecule, the investigated actinocin derivatives demonstrate very weak absorption in the spectral region  $950\text{--}1250\text{ cm}^{-1}$  and, thereby, analysis of the IR spectra of the DNA-drug complexes can be carried out without taking into account the drug absorption in this IR region [55].

Computer simulation of the hydrated environment of actinocin derivatives in aqueous clusters by Monte Carlo method allows to determine the most energetically favourable “ligand-water” configurations, the number and the positions of water molecules forming hydrogen bonds with actinocin derivatives or their hydrated active sites. Comparative analysis of the simulation data and the results of IR-spectroscopic and piezogravimetric studies of the actinocin derivatives’ hydration had demonstrated their complementarity and general agreement.

With an aim to investigate the molecular mechanisms of actinocin derivatives complexation in water solution, the molecular dynamics simulation of both monomer and dimer forms of the ligands was carried out [90]. The hydration properties



**Fig. 2.2** Two stable forms of ActII dimers with the nearest water molecules (*white*) and  $\text{Na}^+$  ions (*violet balls*). **a** Stable form I: phenoxazone chromophores are tilted. **b** Stable form II: phenoxazone chromophores are parallel

of the monomer and the aggregated forms of the actinocin derivatives were determined (Fig. 2.2). The calculated values of interaction energies of the monomers in dimers show that the aggregation of these compounds in aqueous solutions is an energetically favourable process. The aggregates were stabilized by the van der Waals, electrostatic and hydrophobic interactions, and also due to formation of intermolecular hydrogen bonds [91].

In summary, the results of the first step of the investigation gave insight into the nature of the state of actinocin derivatives in aqueous solution and their interaction with solvent molecules. These results are needed for building molecular models of binding of these ligands with DNA. In particular, positions of the hydration centers indicate the sites of possible interactions of the ligands with atomic groups of DNA double helix. The information on the dimerization of the actinocin derivatives in water solution is necessary for estimation of the amount of drug molecules available for binding with DNA.

### 2.3.3 Investigation of the Ligand-DNA Complexation

The second step in the investigation of the activity of new synthetic anticancer antibiotics (actinocin derivatives) at the molecular level is a detailed study of their complexation with DNA.

UV-VIS spectrophotometry was used to investigate the parameters of DNA-actinocin complexation. It was shown that two types of complexes are being formed in DNA-drug solutions, viz. binding of the drug with DNA phosphate groups and

**Application of Computational Techniques in Pharmacy  
and Medicine**

Gorb, L.; Kuz'min, V.; Muratov, E. (Eds.)

2014, XIII, 550 p. 172 illus., 80 illus. in color., Hardcover

ISBN: 978-94-017-9256-1