BINDING PARAMETERS OF ALKALOIDS BERBERINE AND SANGUINARINE WITH DNA

V.G. GUMENYUK,[1] N.V. BASHMAKOVA,[1] S.YU. KUTOVYY,[1] V.M. YASHCHUK,[1] L.A. ZAIKA[2]

[1]**Taras Shevchenko National University of Kyiv**
(6, Academician Glushkov Prosp., Kyiv 03127, Ukraine; e-mail: lns@univ.kiev.ua)

[2]**Institute of Molecular Biology and Genetics, Nat. Acad. of Sci. of Ukraine**
(150, Academician Zabolotnyi Str., Kyiv 03143, Ukraine)

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We study the interaction of berberine and sanguinarine (plant alkaloids) with DNA in aqueous solutions, by using optical spectroscopy methods (absorption and fluorescence). The dependences of alkaloid spectral characteristics on the concentration ratio N/c between the DNA base pairs and alkaloid molecules in the solutions are considered, and the manifestations of the alkaloid–DNA binding are revealed. The character of binding is found to depend on N/c. The parameters of the binding of berberine and sanguinarine with DNA are determined, by using the modified Scatchard and McGhee–von Hippel equations.

1. Introduction

The creation of effective low-toxic antineoplastic preparations on the basis of natural alkaloids is an important problem of modern medicine. Alkaloids are actual for these preparations because of their property to be selectively accumulated in tumor cells and their capability to form non-covalent complexes with nucleic acids, by blocking the processes of transcription and replication of the latter.

This work continues our researches [1] aimed at studying the interaction between deoxyribonucleic acid (DNA) and celandine alkaloids, berberine and sanguinarine. The latter two are included into the content of the antineoplastic preparation, amitozine, created at the Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine. Berberine and sanguinarine (Fig. 1) belong to the isoquinoline group. The structural formulas of berberine and sanguinarine molecules are $C_{20}H_{19}NO_5$ and $C_{20}H_{15}NO_5$, respectively.

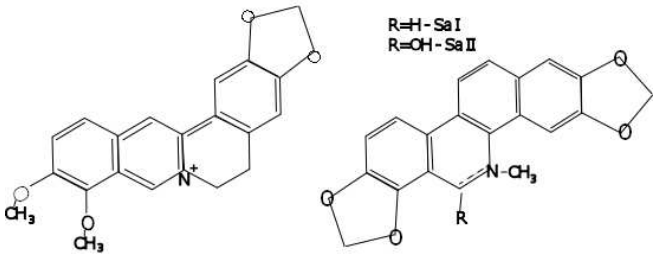

Fig. 1. Formula structures of berberine (a) and sanguinarine (b). The dashed line marks a double bond that exists in the SaI form

Usually, isoquinoline alkaloids interact with DNA as intercalators, or they are arranged in a small groove; their external binding with phosphate groups is also possible. However, the binding mechanisms have not been definitively determined—neither for berberine, nor for sanguinarine—despite that rather intensive researches of this issue have been carried out.

The interaction between berberine and nucleic acids was studied using spectral methods in a number of works, but conclusions concerning the way of their binding are ambiguous. Some results testify to the intercalation (complete [2] or partial [3–5]), whereas the others to groove binding [6, 7]. In work [8], two independent types of binding with different affinities were proposed, and the association constant was found for each of them.

The interaction between sanguinarine and DNA was also studied in a number of works (see review [9]). Sanguinarine is known [9] to exist in aqueous solutions in two forms, imine (SaI, pH < 6) and alkanolamine (SaII, pH > 8.5). The former is a cation, the latter is neutral (see Fig. 1). In work [10], the formation of complexes partially intercalated into DNA was shown for both sanguinarine forms, imine and alkanolamine. However, this conclusion was denied by the results of work [11], the authors of which asserted that only one form, imine, interacts with DNA. At the same time, the intercalation as a way for sanguinarine to bind with DNA was confirmed in work [12].

In this work, we obtained the dependences of the spectral characteristics of berberine and sanguinarine on the ratio N/c between the number of DNA base pairs and the number of alkaloid molecules. We also specified man-

ifestations of the binding between those alkaloids and DNA. A special attention was given to the analysis of the Scatchard and McGhee–von Hippel equations and their application to the numerical approximation of experimental data.

2. Experimental Specimens and Technique

We used alkaloids berberine (Be, "Alps Pharmaceutical", Japan) and sanguinarine (Sa, Ivan Franko Lviv National University) fabricated in the form of microcrystalline powders. The latter were dissolved in water for injections at a temperature within the interval of 60–70 °C. The experimental alkaloid concentrations ranged from 12.5 to 50 μM. Reabsorption or concentration effects are insignificant at such concentrations. We used the DNA of chicken erythrocytes (DNA CE) treated with ultrasound and the DNA of calf thymus (DNA CT), both were obtained from Serva (Heidelberg, Germany). The average molar mass of a nucleotide pair was about 650 Da. When measuring the concentration dependences for the solutions Be + DNA and Sa + DNA, the concentration of an alkaloid remained constant, but the concentration of DNA varied. The ratio between the molar concentrations of DNA and an alkaloid (N/c) was expressed in terms of the number of nucleotide pairs per one alkaloid molecule.

The absorption spectra were registered on a Specord UV VIS spectrophotometer in the range of 200–700 nm. The spectral resolution was 1 nm. Fluorescence spectra were obtained making use of a Cary Eclipse fluorometer in the range of 300–800 nm. The spectral width of a slit for fluorescence measurements was 5 nm.

3. Experimental Results

The interaction of alkaloids with DNA is characterized by such phenomena in absorption and fluorescence spectra as the hypochromism in alkaloid absorption bands; the "red" and "blue" shifts of maxima in the absorption and fluorescence, respectively, spectra; and the variation of the fluorescence quantum yield. These and other manifestations of the binding of berberine and sanguinarine with DNA observed in optical spectra and their dependences on N/c will be analyzed in detail elsewhere. Here, we only describe them in brief.

3.1. Berberine

The absorption spectrum of berberine lies in the spectral interval $\lambda < 500$ nm and consists of four two-component

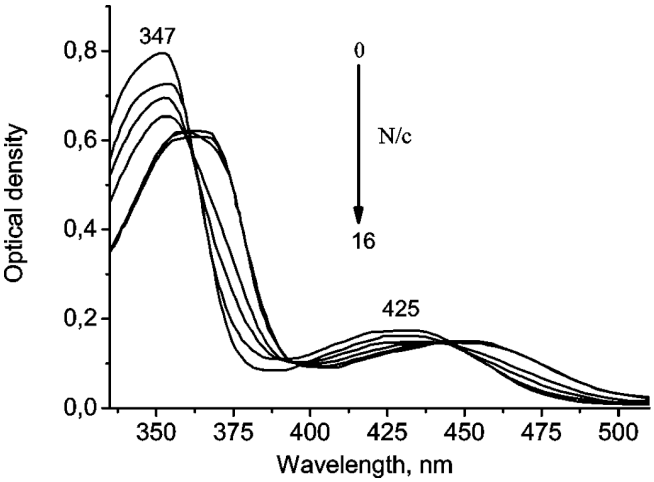

Fig. 2. Absorption spectra of the aqueous solution of berberine and complex Be + DNA CT at various values of N/c. $c_{\mathrm{Be}} = 2.5 \times 10^{-5}$ M

bands with the maxima at 425, 347, 264, and 229 nm. When DNA molecules are added to the berberine solution, a substantial hypochromism (up to 30 %) and the shift of maxima toward long waves (up to 22 nm) are observed for 345 and 425-nm bands in the alkaloid absorption spectrum, which testifies to the binding of berberine with DNA (Fig. 2).

The dipole moments of planar aromatic molecules is oriented in the molecular plane [13]. Therefore, the spatial arrangement of the dipole moments of those molecules corresponds to that of the molecules themselves. If the dipole moments of molecules are oriented in parallel (the "sandwich" structure), the spectra demonstrate hypochromism. In this case, hypochromism, as a consequence of the interaction between the π-systems of molecules oriented in parallel to one another, testifies to the intercalation or external stacking as probable binding mechanisms.

The fluorescence spectrum of berberine has one band with a maximum at about 556 nm. The fluorescence quantum yield of berberine is very low at room temperature. The fluorescence spectra of the complex Be–DNA are characterized by a very considerable amplification of their intensity (up to a factor of 200, depending on the excitation λ, the concentration, and the specimen type) and a blue shift of the fluorescence maximum (up to 26 nm) in comparison with the free berberine case (Figs. 3 and 4).

The changes in the fluorescence spectra are caused by the fixation of berberine molecules on the DNA matrix. Namely, the probability of the radiationless excitation relaxation diminishes at the complex formation,

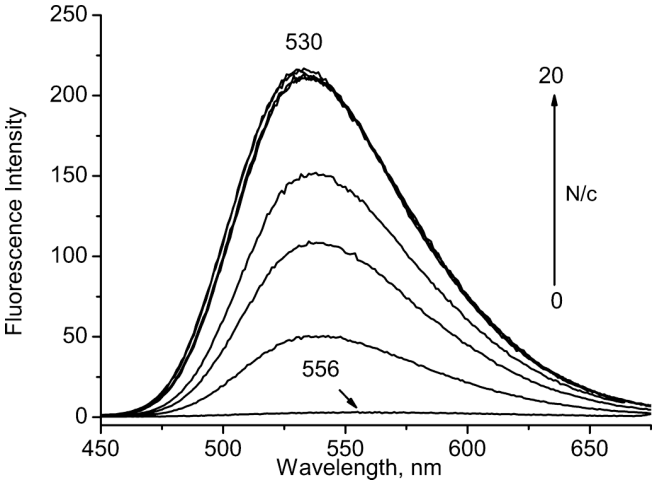

Fig. 3. Fluorescence spectra of berberine and complex Be + DNA CT at various values of N/c. $c_{\mathrm{Be}} = 4.1 \times 10^{-5}$ M. Excitation at 450 nm

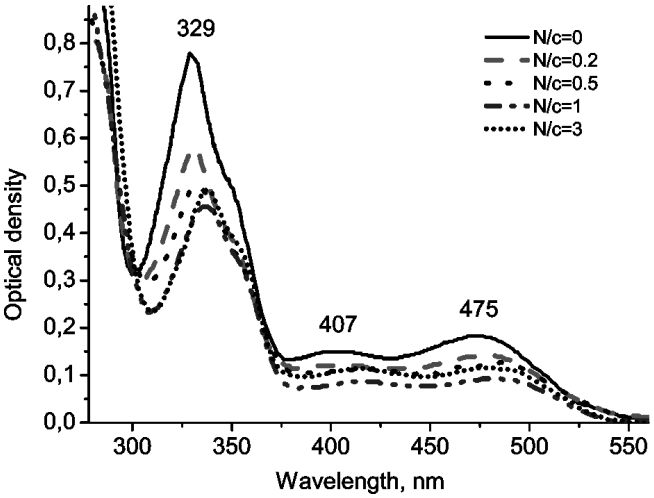

Fig. 5. Absorption spectra of a solution Sa + DNA CE at various values of N/c. $c_{\mathrm{Sa}} = 5 \times 10^{-5}$ M

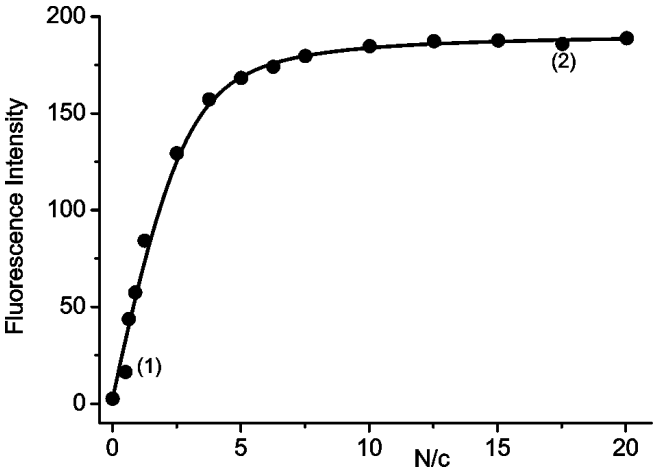

Fig. 4. Dependence of the fluorescence intensity of complex Be + DNA CT on N/c. $c_{\mathrm{Be}} = 4.1 \times 10^{-5}$ M. Excitation at 450 nm. As for points (1) and (2), see section 4.5

because the interaction of vibrations with one another is less effective. Moreover, the energy transfer to solvent molecules is also less effective now. Accordingly, the fluorescence quantum yield grows. The "blue" shift of the fluorescence maximum is mainly associated with variations in the polarity of the fluorophore molecule environment: DNA is a less polar medium for alkaloid molecules than water. In addition, bound alkaloid molecules are partially screened from solvent molecules. As a result, the effect of solvent relaxation, i.e. the interference between the dipole moments of excited alkaloid and solvent molecules [14] becomes insignificant.

Following the procedure of work [15], we found the intersection point of the fluorescence band and the first absorption band plots presented in terms of I/ν^4 and ε/ν units, respectively, to determine the dependence of the frequency of the first electron transition (0-0) in the system Be + DNA on N/c.

In whole, all the mentioned dependences (the "red" and "blue" shifts, variations of the fluorescence intensity and the hypochromism degree, a change of the 0-0 transition frequency) are characterized by a mutual behavior of the type depicted in Fig. 4: a rather quick growth of the corresponding parameter at the beginning followed by a saturation at certain, close to one another, N/c-values.

3.2. Sanguinarine

As was mentioned, the sanguinarine molecule can exist in the imine (at pH < 6) or alkanolamine (at pH > 8.5) form. In our experiments, pH was about 7, so that both forms were available. The presence of two Sa forms in the solution is characterized by a complicated shape of the absorption curve. The latter includes bands typical of two Sa forms (Fig. 5). The fluorescense spectrum consists of two bands with the maxima at 587 and 419 nm (Fig. 6), which different excitation spectra correspond to.

When adding DNA, the absorption and fluorescence spectra of sanguinarine, similarly to those of berberine, also demonstrate the band shift, hypochromism, and a variation of the fluorescence intensity. However, in contrast to berberine, variations of the optical density and

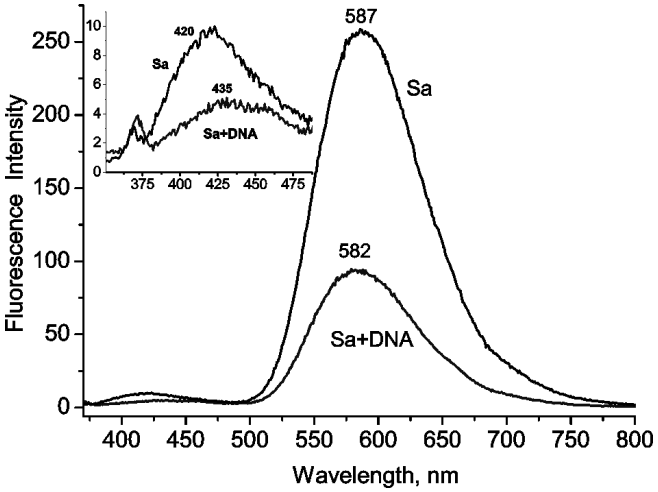

Fig. 6. Fluorescence spectra of sanguinarine and complex Sa + DNA CT at $N/c = 1$. $c_{\mathrm{Sa}} = 5 \times 10^{-5}$ M. Excitation at 330 nm. The corresponding spectra of the SaII form are shown in the inset

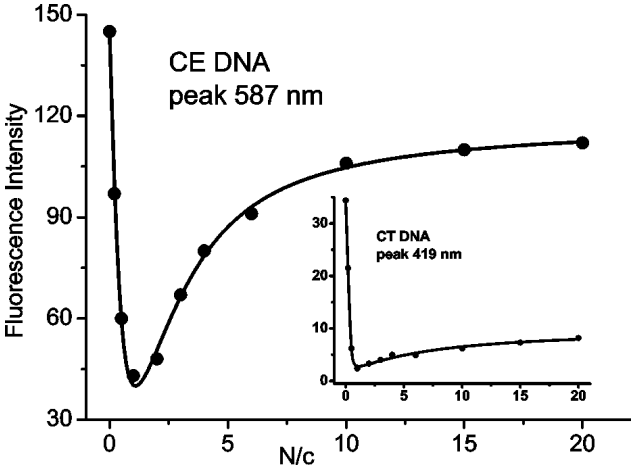

Fig. 7. Dependence of the fluorescence intensity on N/c for the 587-nm band of complex Sa + DNA CE. Excitation at 470 nm. The same but for the 419-nm band and the excitation at 330 nm is shown in the inset. $c_{\mathrm{Sa}} = 5 \times 10^{-5}$ M

the fluorescence intensity, as well as the frequency of the first electron transition (0-0), depend on the ratio N/c for sanguinarine in a nonstandard way. Namely, the corresponding curves have a minimum (see the fluorescence spectrum in Fig. 7), and such a behavior is observed for both sanguinarine forms.

The minimum in the fluorescence intensity contradicts the data of work [19]. A similar dependence was found only in work [16], but no relevant explanation was given. This atypical variation of the optical parameter of alkaloid can be explained as a manifestation of two types of the binding of sanguinarine to DNA: an external one and the intercalation. The minima in the dependences at $N/c \leq 1$ correspond to the most compact arrangement of sanguinarine molecules on the DNA matrix, which brings about the hypochromism in the absorption bands (by about 40–50%) and the fluorescence quenching (by about 70–80%). Since the intercalation mechanism of binding is characterized by the values $N/c \geq 2$, the most probable mechanism of binding at $N/c < 1$ is the mechanism of external "stacking". When the DNA concentration grows, the number of binding sites also increases, and alkaloid molecules can be arranged at a larger distance from one another along the DNA chain, which is observed as an increase of the optical parameter. In this case, the most probable for sanguinarine is the intercalation way of binding. At certain concentration ratios, the dependences saturate.

Note that, although the character of the intensity variation is similar for the fluorescence bands at 419 and 587 nm, the minimum for the former band is observed at larger N/c-values. This fact evidences the interaction between SaII and DNA, which is more effective at larger N/c-ratio values in comparison with that for the SaI form.

As the main reason of the fluorescence yield reduction in the case of sanguinarine, we consider an effect similar to the concentration quenching. In particular, by binding with DNA (it is especially valid for the external binding), alkaloid molecules are arranged relatively close to one another in comparison with free molecules, which is responsible for some fluorescence quenching.

We also note that all the mentioned dependences for the DNA CE and DNA CT have the same character, but, in the DNA CT case, they attain the corresponding maximum or minimum (or saturate) at smaller N/c-values. It looks as if the alkaloid interacts more effectively with DNA CT, which was no subjected to the ultrasonic treatment. The corresponding calculations for binding parameters (see below) confirmed this assumption.

4. Calculation of Binding Parameters

4.1. Theory

To determine the parameters of small ligand binding with DNA, we wrote a computer program BindFit. Its feature is the operation with direct experimental data, i.e. the absence of any coordinate transformations known as "linearization". It allowed us to improve the accuracy of the parameter determination for processes

described by nonlinear plots even after the linearization (see section 4 4.5).

A classical equation for the determination of binding parameters (more specifically, the association constant) is the Scatchard equation [17],

$$\frac{\nu}{c_f} = K(1-\nu) \tag{1}$$

Here, ν is the ratio between the concentration of bound ligands c_b and the total concentration of binding sites N, c_f is the concentration of free ligands, and K is the association constant. According to Eq. (1), the dependence of the ratio ν/c_f on ν must be linear, and the association constant can be determined as the slope of the obtained straight line. For some reasons, this dependence can be nonlinear, in particular, if a ligand molecule occupies more than one binding site (namely, n sites) in the DNA matrix. Then, the equation looks like $\nu/c_f = K(1-n\nu)$. However, its use is not always correct. Owing to the underestimation of the number of empty binding sites, the binding parameters are determined incorrectly. In particular, the association constant can be overestimated by a factor of about $2n$ [18].

McGhee and von Hippel [18] developed Scatchard's approach, by extending it onto the case $n > 1$. Taking advantage of the probability theory methods, they correctly made allowance for that fact that extended ligand chains can occupy more than one binding site. The McGhee–von Hippel equations are as follows:

– for the non-cooperative binding,

$$\frac{\nu}{c_f} = K(1-n\nu)\left(\frac{1-n\nu}{1-(n-1)\nu}\right)^{n-1} \tag{2}$$

– for the cooperative binding,

$$\frac{\nu}{c_f} = K(1-\nu)\left(\frac{(2\omega-1)(1-n\nu)+\nu-R}{2(\omega-1)(1-n\nu)}\right)^{n-1} \times$$

$$\times \left(\frac{1-(n+1)\nu+R}{2(1-n\nu)}\right)^2. \tag{3}$$

Here,

$$R = \sqrt{(1-(n+1)\nu)^2 + 4\omega\nu(1-n\nu)},$$

and ω is the parameter of cooperativity ($\omega > 1$ for cooperative, $\omega = 1$ for non-cooperative, and $\omega < 1$ for anti-cooperative binding).

In most cases, the plots are nonlinear in the Scatchard coordinates $(\nu, \nu/c_f)$. However, in terms of those variables, the binding equation can be written down in an explicit form, which is impossible to be done in experimental variables. That is why the Scatchard variables found a definite application.

4.2. Connection with the optical parameter

The concentrations of bound or free ligands are not experimentally observable quantities. Experimentally, the dependences of the optical parameters of a solution on the concentration of its components are measured. Usually, the optical parameter is calculated as a sum of contributions made by ligands in both states, bound and free.

4.2.1. Two states of ligand (one type of binding sites)

Provided that the ligand can be in only one of two states (free or bound), the expression for the optical parameter is rather simple, and it allows the concentrations of free and bound ligands to be determined straightforwardly:

$$A = \frac{c_f}{c}A_f + \frac{c_b}{c}A_b, \tag{4}$$

where A is the optical parameter of the mixture, c the total concentration of ligands, A_f the optical parameter for free ligands, c_f the free ligand concentration, A_b the optical parameter for bound ligands, c_b the bound ligand concentration, $c = c_f + c_b$. Knowing the parameters A_f and A_b (the former is determined for the solution of a pure ligand, the latter could be determined using the solution of a ligand with a considerable excess of DNA), it is possible to determine c_f and c_b, and then change to the Scatchard coordinates:

$$\nu = \frac{(A-A_f)c}{(A_b-A_f)N}, \quad \frac{\nu}{c_f} = \frac{A-A_f}{(A_b-A)N}. \tag{5}$$

For the approximation of direct experimental data, those procedures are redundant.

4.2.2. Three states of ligand (two types of binding sites)

Provided that the ligand can be in only one of three states (free, bound to a site of the first type, and bound to a site of the second type), the expression for the optical parameter A looks like

$$A = \frac{c_f}{c}A_f + \frac{c_b^{(1)}}{c}A_b^{(1)} + \frac{c_b^{(2)}}{c}A_b^{(2)}, \tag{6}$$

where $A_b^{(1)}$ and $A_b^{(2)}$ are the optical parameters of ligands bound to sites of the first and the second type,

respectively; and $c_b^{(1)}$ and $c_b^{(2)}$ are the corresponding concentrations of those ligands. In this case, it is difficult to determine the concentration of ligands in each state (and, hence, to change to the Scatchard coordinates), because both $A_b^{(1)}$ and $A_b^{(2)}$ cannot be determined by direct measurements, so that the number of equations in the system used for the determination of the concentrations of bound and free ligands is smaller than the number of variables. This problem does not arise at a straightforward approximation of experimental data, because those quantities are calculated together with other parameters.

4.3. Model equations

For numerical analysis, Eqs. (1)–(3) written down in terms of the ν and ν/c_f variables are not convenient, because those quantities are connected with experimental optical parameters (the fluorescence intensity, the optical density, and others) in rather a complicated way. In addition, the equations for the binding parameters written down in terms of the Scatchard variables give solutions with a relatively high error. It is associated with the fact that, in the general case of nonlinear dependences, the linearization "following Scatchard" gives rise to a considerable distortion of experimental errors and, respectively, the determination accuracy for binding parameters, which should better be determined from the initial, non-linearized curves. Therefore, in order to analyze and to directly (i.e. in terms of experimental variables) approximate experimental data, the indicated equations were modified and applied in the form of equations with the independent variable c_b , i.e. the concentration of bound ligands.

4.3.1. One type of binding sites

In this case, basic are the McGhee–von Hippel equations transformed from their original form to that including only the variables directly related to the experiment. As a rule, what is experimentally measured is the dependence of a certain optical parameter of the solution on the concentration ratio between the dissolved components; normally, it is the ratio between the total concentration of binding sites to the total concentration of ligands, N/c, i.e. the quantity reciprocal to ν. It is the concentrations of components rather than those of bound and free ligands that are known. Therefore, such variables as the total concentrations of ligands, c, and binding sites, N, would be more expedient for computerized processing. In this case, there remains only one unknown variable in the equation, which can be determined numerically.

Carrying out a series of simple transformations (we multiply the "canonical" equations (2) or (3) by the factors c_f and N, and make some changes in the variable notations), the McGhee–von Hippel equations for the non-cooperative (Eq. (2)) and cooperative (Eq. (3)) bindings are reduced to the following sought expressions, which include the variable c_b:

$$K(c-c_b)(N-nc_b)\left(\frac{N-nc_b}{N-(n-1)c_b}\right)^{n-1}-c_b=0, \quad (7)$$

$$K(c-c_b)(N-nc_b)\left(\frac{(2\omega-1)(N-nc_b)+c_b-R'}{2(\omega-1)(N-nc_b)}\right)^{n-1}\times$$

$$\times\left(\frac{N-(n+1)c_b+R'}{2(N-nc_b)}\right)^2-c_b=0, \quad (8)$$

where

$$(R'=\sqrt{(N-(n+1)c_b)^2+4\omega c_b(N-nc_b)}).$$

It is those equations which were implemented in the program and were solved numerically (we did not manage to obtain their analytical solutions). It enabled us to operate with the quantity c_b as with the function $c_b = c_b(N, c; K, n)$.

A separate remark should be made on the parameter n. In the case $0 < n < 1$, the substitutions $n = 1$ and $N' = N/n$ are made by force in the equation. In other words, such parameter values are interpreted as a hint that, actually, there are more binding sites than N. This situation can be realized, e.g., if N stands for the concentration of DNA base pairs, and binding occurs with phosphate residues, the concentration of which is $2N$. However, the aforementioned n-values can also testify to the cooperative binding. In this case, the process is described by the cooperative equation (8) with $n = 1$, with the compulsory condition $\omega > 1$. At $\omega \to 1$, the denominator in Eq. (8) tends to zero. If the corresponding passage to the limit is done, the equation transforms into a non-cooperative one. This situation is handled programmatically by changing from the cooperative equation to the non-cooperative one at $\omega = 1$. The parameter n is processed in the same way, as for the non-cooperative equation.

4.3.2. Two types of binding sites

Processes with two types of binding sites can be schematically designated as $c_b^{(1)} \Longleftrightarrow c_f \Longleftrightarrow c_b^{(2)}$, i.e. the process

of direct transition by bound ligands from sites of type 1 to sites of type 2 is impossible. These processes are described by a system of two equations, which must take into account whether the processes of binding of the ligands that occupy one binding site (i.e. the base pair and phosphates) are interdependent or not. If the parameter N stands for the concentration of DNA base pairs, then $2N$ binding sites correspond to the first type of binding (with a phosphate) and N binding sites to the second type (intercalation).

Among the implemented schemes, the simplest is the system of two modified Scatchard equations, which describes two independent processes of binding of the ligands that occupy one binding site:

$$\begin{cases} c_b^{(1)} = K_1(c - c_b^{(1)} - c_b^{(2)})(2N - c_b^{(1)}), \\ c_b^{(2)} = K_2(c - c_b^{(1)} - c_b^{(2)})(N - c_b^{(2)}). \end{cases} \tag{9}$$

Other combinations of the Scatchard and McGhee–von Hippel equations are also possible.

For our experimental data, the best approximation results were obtained making use of a system of modified Scatchard (for external binding) and McGhee–von Hippel (for intercalation) equations. The system describes two interdependent processes of binding of the ligands that occupy one binding site. The intercalation into the interval between the base pairs is allowed only if both phosphates in this interval are not connected with ligands and *vice versa*, i.e. the binding with phosphates is possible only if no ligand has intercalated into the corresponding interval. In addition, there can be not less than $n-1$ free intervals between two intercalated ligands. This model brings about the following system of equations:

$$\begin{cases} c_b^{(1)} = K_1(c - c_b^{(1)} - c_b^{(2)})(2N - c_b^{(1)}) \left(1 - \frac{c_b^{(2)}}{N}\right), \\ c_b^{(2)} = K_2(c - c_b^{(1)} - c_b^{(2)})(N - nc_b^{(2)}) \times \\ \times \left(\frac{N - nc_b^{(2)}}{N - (n-1)c_b^{(2)}}\right)^{n-1} \left(1 - \frac{c_b^{(1)}}{2N}\right)^2. \end{cases} \tag{10}$$

It is important that an additional multiplier emerges in the equations for interdependent processes. The mechanism of its appearance is as follows. Any equation describing the binding looks like $c_b = Kc_f N_f$, i.e. the concentration of bound ligands is equal to the product of the free ligand concentration, the concentration of empty binding sites, and the association constant. It is the factor N_f that generates additional multipliers, because this quantity ultimately acquires the form $N_f = (N - nc_b)P_f$, i.e. the concentration of binding sites is equal to the concentration of unoccupied sites times the probability that no factors that prohibit the binding are actual for any arbitrarily selected empty binding site. For interdependent processes, this factor is selected to be the presence of a bound ligand at the neighbor binding site, provided that this ligand belongs to the different type. The probability that this factor does not interfere the binding is equal to the probability that all neighbor binding sites are free, i.e.

$$P_f = \left(1 - \frac{c_b^{\text{other}}}{N^{\text{other}}}\right)^s,$$

where s is the number of neighbor binding sites. In this case, this quantity is determined as follows:
– for the binding with a phosphate, it is a fraction of phosphates belonging to the unoccupied interval,

$$p_a = 1 - \frac{2c_b^{(2)}}{2N} = 1 - \frac{c_b^{(2)}}{N};$$

i.e. every occupied interval forbids the binding with two phosphates;
– for the intercalation, it is a probability that both phosphates in this interval are free,

$$p_b = (P_{\text{ph.free}})^2 = (1 - P_{\text{ph.occupied}})^2 = (1 - \frac{c_b^{(1)}}{2N})^2;$$

i.e. the probability that the phosphate is busy, $P_{\text{ph.occupied}}$, is equal to the probability that one of $c_b^{(1)}$-ligands became bound with one of $2N$ phosphates.

Note that

1. The processes, in which a direct transition of bound ligands from the sites of type 1 to sites of type 2 is possible, is not considered here, because several equations and about a dozen parameters are needed for their description. It is too much for the parameters to be determined with a satisfactory accuracy.
2. Since the solution describes the concentration of bound ligands, certain restrictions are imposed on it: the concentration of bound ligands must be a non-negative number, it cannot exceed the total ligand concentration, and it is confined from above by the concentration of binding sites.

Generally speaking, the equations given above have a number of solutions. However, it turned out that only one of them satisfies the indicated restrictions in the working range of parameters.

3. Among the algorithms applied to solve the equations, the method of binary search turned out to be the best, i.e. it produced stable results at the highest calculation rate. Every parameter was approximated separately.

4.4. Parameters of the complex formation for berberine and sanguinarine

To determine the binding parameters, the most convenient is to use the data on variations of both the optical density in the absorption spectra and the fluorescence intensity. In our experiments, more exact data were obtained from the fluorescence spectra; accordingly, results of the corresponding approximation turned out a little more accurate.

4.4.1. Berberine

For the approximation of experimental data, we used the modified McGhee–von Hippel equations (7) and (8) for the non-cooperative and cooperative, respectively, bindings. It turned out that the best results were obtained, if the binding was considered to be cooperative, but the degree of cooperativity was small. For DNA CT, we obtained the values quoted in Table 1. The table demonstrates a very good agreement between the parameters determined by approximating the data obtained in independent experiments (fluorescence and absorption). The value $n \approx 2$ means that one alkaloid molecule occupies two DNA base pairs, which evidences the intercalation model of berberine binding to DNA. A small cooperativity can testify to a certain untwisting of the DNA helix at intercalation sites.

For the sake of comparison, note that the values $K = 3.54 \times 10^4$ and $n = 2$ were obtained in work [19] on the basis of analysis of other equations (but in the Scatchard coordinates). Hence, we have good agreement with those results, taking into account the difficulties faced when obtaining exact experimental and calculation data.

4.4.2. Sanguinarine

Since the dependences of the optical density and the fluorescence intensity on the DNA concentration in the solutions Sa + DNA are nontrivial (their behavior is similar to what is shown in Fig. 7), the McGhee–von Hippel equation cannot be applied directly to this case. As turned out, the relevant experimental results are better described by a system of equations, which takes two interdependent processes of ligand binding into account; these are the external binding with phosphates (type 1) and the intercalation into the DNA double helix (type 2). In addition, the process of direct transition by bound ligands from sites of type 1 onto sites of type 2 is impossible (condition 1), and there must be not less than $n-1$ free intervals between two intercalated ligands (condition 2). This model gives rise to the system of equations (10), in which conditions 1 and 2 are taken into account. The number of base pairs occupied by one alkaloid at the external binding was considered to be equal to 0.5; at the intercalation, n was one of the equation parameters. The concentration of external binding sites was adopted to be about $2N$ and that of intercalation sites to be N.

By approximating the experimental dependences with the systems of equations (10), we obtained the values for the association constant (in terms of M^{-1} units) and the parameter n, which are presented in Table 2.

4.5. Characteristic features of Scatchard coordinates

The usage of the Scatchard coordinates requires that the concentrations of bound and free ligands be determined directly from experimental data, which is not always possible. However, even if it is possible, this operation can distort the results uncontrollably. We intend to illustrate the expediency of the used programmatic approach on a specific example. For the comparison between the results obtained (namely, the K- and n-values) to be more correct, we used the McGhee–von Hippel equation for the non-cooperative binding, because the parameter ω cannot be determined at all from the Scatchard equation.

In Fig. 4, the raw experimental data for the dependence of the fluorescence intensity on the ratio N/c obtained for the complex Be + DNA CT and the results of their direct approximation are shown. The approximation curves calculated using the equations for both the cooperative (Fig. 4) and non-cooperative bindings practically coincide. The differences become noticeable between the numerical values of the parameters determined making use of the corresponding equations. For the non-cooperative binding, we obtained $K = (7.69 \pm 3.18) \times 10^4\ \mathrm{M}^{-1}$ and $n = 2.11 \pm 0.23$.

T a b l e 1. Parameters of the binding of berberine with DNA

	From fluorescence spectra	From absorption spectra
K	$(5.2 \pm 0.2) \times 10^4$	$(5.7 \pm 0.7) \times 10^4$
n	1.85 ± 0.1	1.8 ± 0.3
ω	1.3 ± 0.2	1.45 ± 0.4

T a b l e 2. Parameters of the binding of sanguinarine with DNA

	SaI + DNA CE	SaI + DNA CT	SaII + DNA CT
K_1	$(6.7 \pm 0.4) \times 10^4$	$(3.5 \pm 0.3) \times 10^5$	$(7.9 \pm 0.3) \times 10^5$
K_2	$(5.5 \pm 0.7) \times 10^5$	$(1.1 \pm 0.4) \times 10^6$	$(1.0 \pm 0.5) \times 10^5$
n	3.4 ± 0.3	1.5 ± 0.4	12 ± 3

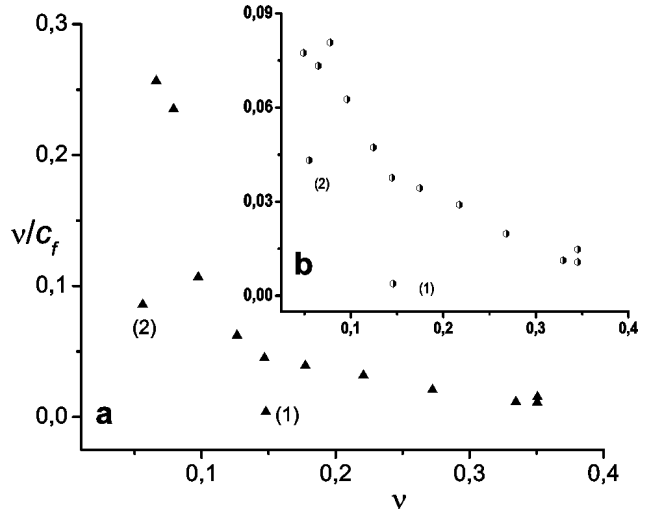

Fig. 8. The same data as in Fig. 4, but in the Scatchard coordinates

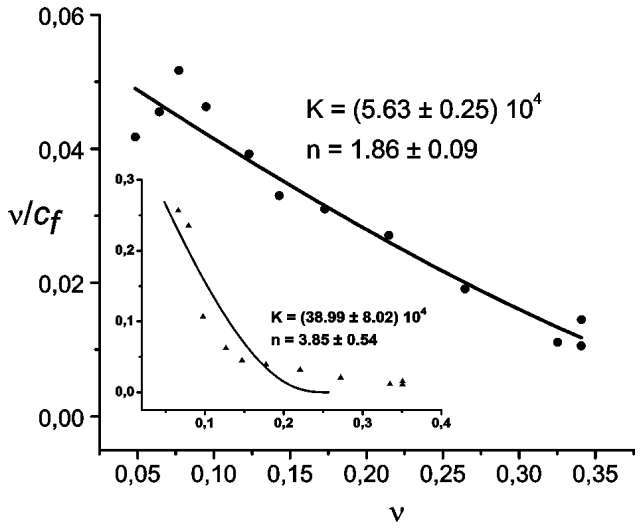

Fig. 9. Approximation for the non-cooperative binding by the McGhee–von Hippel equation and in the Scatchard coordinates. The same, but in the case where A_b was determined without the approximation of initial data, is depicted in the inset

When transforming the experimental data to the Scatchard coordinates (5), there arises a problem concerning the determination of the bound and free ligand concentrations: which value should be taken for the optical parameter for bound ligands? First, let us use the maximal value (188.9) of optical parameter among all experimental points; the result is shown in Fig. 8,*a*. It is evident that the spread of points considerably increased after the linearization.

If the optical parameter for bound ligands is taken from the approximation results (Fig. 4), we obtain a value of 191.8, which is rather close to the used one. The corresponding Scatchard plot is depicted in Fig. 8,*b*. One can see that the plot changed substantially. Namely, the variation of A_b by less than 1.5% gave rise to the multiple change of the ratio ν/c_f. Moreover, two points, (1) and (2), drop out of the general tendency. We excluded them and carried out the direct approximation of the remained data once more to obtain $K = (5.64 \pm 0.35) \times 10^4$ M^{-1} and $n = 1.86 \pm 0.07$. Both parameters evidently changed considerably. The determination accuracy for the parameter increased at that, i.e. the omitted points really inserted a substantial error. We also obtained a corrected value, $A_b = 194.3$. It is already clear that the Scatchard plot essentially depends on this quantity. Therefore, we replotted it again and found the binding parameters (Fig. 9) $K = (5.63 \pm 0.25) \times 10^4$ M^{-1} and $n = 1.86 \pm 0.09$.

For the sake of comparison, let us approximate the initial Scatchard plot (Fig. 8,*a*) with omitted points (1) and (2), of course (see the inset in Fig. 9). Hence, the value obtained for A_b as a result of the direct approximation of experimental data provides a better agreement between the theoretical curve and the experimental points plotted in the Scatchard variables (Fig. 9) than the value obtained without this approximation (the inset in Fig. 9). The magnitude of association constant strongly depends on the determination accuracy for A_b. In our case where A_b changed from 188.9 to 194.3, i.e. the relative variation of this quantity was less than 3%, which was close to its determination error, the association constant changed by an order of magnitude (see the parameter values in Fig. 9). The error rescaled into the Scatchard coordinates does not take the above-mentioned fact into account and, therefore, has not any reason.

If the transition to the Scatchard coordinates is fulfilled with a sufficient accuracy, the results practically coincide with those obtained at the direct approximation. However, this "sufficient" accuracy demands for rather a laborious treatment of initial data.

An impression might arise (Fig. 8) that the Scatchard coordinates allow "bad" experimental points to be excluded easily. However, at the direct approximation, there are no difficulties in detecting the points that insert a suspiciously large error. In addition, an advantage of the program is its equally simple operation with data producing both linear and nonlinear Scatchard plots.

5. Conclusions

Hence, the analysis of the absorption spectra and the fluorescence manifestations of the interaction between alkaloids berberine and sanguinarine, on the one hand,

and DNA, on the other hand, testifies to the binding of alkaloids with DNA. The binding character was shown to depend on the concentration ratio between alkaloid molecules and DNA base pairs. The parameters of the binding of berberine with DNA were determined with the help of a modified McGhee–von Hippel equation for the cooperative binding. The obtained values evidence the intercalation mechanism of binding. We also determined the parameters of the binding of sanguinarine with DNA. Two types of binding turned out to be characteristic of the sanguinarine–DNA interaction: the external binding prevails at $N/c < 2$ and the intercalation at $N/c > 6$.

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ПАРАМЕТРИ ЗВ'ЯЗУВАННЯ АЛКАЛОЇДІВ БЕРБЕРИНУ ТА САНГВІНАРИНУ З ДНК

В.Г. Гуменюк, Н.В. Башмакова, С.Ю. Кутовий, В.М. Ящук, Л.А. Заїка

Р е з ю м е

Досліджено взаємодію рослинних алкалоїдів берберину та сангвінарину з ДНК у водному розчині методами оптичної спектроскопії (поглинання, флюоресценція). Розглянуто залежності спектральних характеристик алкалоїдів від співвідношення концентрацій пар основ ДНК та молекул алкалоїду (N/c), визначено прояви зв'язування алкалоїдів з ДНК. Показано, що характер зв'язування залежить від N/c. За допомогою модифікованих рівнянь Скетчарда та МакГі–фон Хіппеля визначено параметри зв'язування берберину та сангвінарину з ДНК.