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MECHANISMS AND PARAMETERS OF THE BINDING OF THE FLAVONOID QUERCETIN TO DNA IN AN AQUEOUS SOLUTION

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Abstract. The interaction of the flavonoid quercetin with the DNA macromolecule in an aqueous solution has been studied through optical spectroscopy - electron absorption and fluorescence. Based on the obtained results, the dependence of the spectral characteristics on the concentration ratio N/c between the DNA base pairs and the ligand molecules was constructed. Using the system of modified equations of Scatchard and McGhee-von Hippel, the binding parameters of quercetin with DNA are determined. The binding parameters to the DNA of other molecules we studied earlier are given for comparison.

Keywords: DNA, quercetin, McGhee-von Hippel binding equation, absorption spectra, fluorescence spectra.
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1. Introduction

The study of the interaction of low-molecular ligands with DNA is important, particularly in the creation of antitumor and immunomodulatory drugs. However, the task of creating low-toxic drugs cannot be successfully fulfilled without understanding the physical mechanisms of these compounds' interaction with DNA at the molecular level.

Quercetin is a plant flavonol of the flavonoid subgroup of polyphenols, which has a wide range of biological effects and therapeutic properties: antioxidant, antitoxic, antiviral, antidiabetic, anticancer, anti-inflammatory, cytoprotective, etc. [1-5]. Due to their plant origin, quercetin and the mentioned alkaloids have a relatively low toxicity. Their antitumor effect is associated with their ability to build into the DNA double helix and, in such a way, to violate and block its functioning.

There are many reports on the study of the interaction of quercetin with DNA [6-16]. These studies were performed using different methods and with different types of DNA. It has been established that quercetin interacts with DNA quite effectively. Certain conclusions were also made regarding the mechanisms of interaction: mostly, these are mechanisms of groove binding or intercalation; there is also a report about external binding. It should be noted that the values of the binding constants differ quite strongly - by orders of magnitude, as given in these reports. At the same time, there are almost no reports on determining binding parameters due to analyzing binding equations using experimental data. Therefore, it seems relevant to determine the binding parameters on the base of the appropriate equations that use experimental data, in particular, the McGhee-von Hippel equations.

This work is a continuation of our previous studies, which dealt with the interaction between a DNA macromolecule and low-molecular compounds, such as celandine alkaloids

berberine, sanguinarine, modified berberine (amitozinoberamid), that reveal the antiviral, antiinflammatory, bactericidal effects, antitumor and other properties (see, f.e., [17-19] and reviews [20-22]). The molecules of ethidium bromide, acridine, and some others were also studied as model molecules to compare with. These investigations were performed by optical methods such as electron absorption, fluorescence, and Raman scattering.

In this work, the main attention is paid to studying the interaction of the flavonol quercetin with the DNA macromolecule in an aqueous solution. Using the system of modified equations of Scatchard and McGhee-von Hippel (see below), the binding parameters of quercetin with DNA were determined. For comparison, the parameters of binding to the DNA of other molecules we studied earlier [23-28] are given. The system of modified equations of Scatchard and McGhee-von Hippel was used since it did not operate with Scatchard variables. The equations written in Scatchard variables are inconvenient for a numerical analysis since these variables are quite complexly related to experimental optical parameters. Besides, the binding parameters determined from the equations in the Scatchard variables have a rather large error since the linearization "by Scatchard" in the general case of nonlinear dependences leads to a significant distortion of the experimental errors and, accordingly, the accuracy of determining the binding parameters, which are better to be determined from the original non-linearized curves.

2. Materials and methods

According to the nomenclature of the International Union of Pure and Applied Chemistry (IUPAC), quercetin is termed as 3, 3′, 4′, 5, 7-pentahydroxyflavanone. Its chemical formula is $C_{15}H_{10}O_7$ [29]. The structure of quercetin molecule contains two aromatic rings A and B linked through a ring C containing oxygen, and a number of hydroxyls OH-groups attached to the positions 3, 3′, 4′, 5 and 7 (see Fig. 1). The substance is poorly soluble in water.

Quantum-mechanical studies of the structure of quercetin have been reported in [30-34]. According to the studies of the spatial structure of quercetin isomers [34], the quercetin molecule is either flat or almost flat for different isomers, while deviation from planarity occurs only for the ring B, which is rotated with respect to the C2–C1' axis. We have determined the optimized geometry of the two stable isomers of the quercetin molecule using the density-functional method at the CAM B3LYP/6-311++G(d,p) level [35]. The spatial structures of the isomers are displayed in Fig. 1.

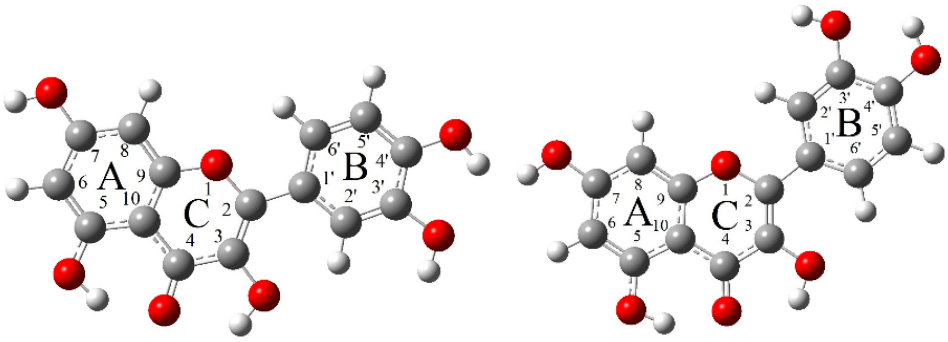

Fig. 1. Optimized spatial structures of the isomers of quercetin molecule, as calculated with density functional CAM method at the level B3LYP/6-311++G(d,p). The numbers of atoms correspond to standard notation. Atoms C are denoted as grey circles, atoms O as red circles, and atoms H as small grey circles [35].

The dependence of the spectral characteristics of quercetin on N/c was obtained in this work (*N/c* is the ratio of the number of DNA base pairs to the number of ligand molecules; we use the designation *N/c* instead of the more common P/D, i.e., Phosphate/Drug). Spectral manifestations of the interaction of quercetin with DNA were described. The data obtained by spectral methods were used to calculate binding parameters using the modified Skatchard and McGee-von Hippel equations system.

This study used the aqueous solutions of the quercetin (Que, IMBG NASU, Ukraine, 302 g/mol) preparations. We also used the DNA of *calf thymus* ("Serva", Germany) with the average molar mass of a base pair of about 650 g/mol and a high purification degree. In auxiliary experiments, the DNA of *salmon sperm* ("Sigma-Aldrich," USA) with a low purification degree (7% of proteins) was used. Water for injection ("Darnitsa", Ukraine) and ethanol (96%, «Medlev», Ukraine), were applied as a solvent. All examined substances were used without their additional purification. Specimens were prepared using mechanical samplers of the MicroPette type ("ULAB," Ukraine). The charge mass was determined using an analytical precision electronic balance ("Vibra," Japan) with an accuracy of 0.01 mg.

Quercetin does not dissolve well in water, so ethanol was used to prepare the stock solution of quercetin. The stock (concentrated) solutions of quercetin and DNA were prepared by dissolving the required quantities of DNA in water and the quercetin in ethanol. Next, the necessary amounts of quercetin and DNA stock solutions were mixed to achieve the desired *N/c* value. Then, the required amount of water was added so that the resulting solution had a fixed volume for the spectrophotometer or fluorimeter cuvette. Since water was added in different quantities to the initial mixture of stock solutions, it was necessary to clarify the actual value of *N/c*.

The water volume fraction in the finished solutions Que+DNA was >99.5%. In such a solution, the fraction of ethanol is very small, so the negative effect of ethanol on DNA during the experiments can be neglected.

It is important to note that if the stock quercetin solution could be prepared once for all experiments because it remained stable and did not degrade over time for several sets of measurements – during approximately two months, the spectra of freshly prepared and two-month-old solutions look almost identical, then the DNA solution had to be prepared for each experiment separately since the DNA solution degraded quite quickly and the use of the old one gave distorted spectral data.

The absorption spectra were registered on a spectrophotometer "Agilent Cary 60 UV-VIS" (Agilent, USA) in a 200–700 nm wavelength interval. The fluorescence spectra were obtained in an interval of 300–800 nm, using a fluorometer "Shimadzu RF 6000" (Shimadzu Corp., Japan).

When the concentration dependences of Que+DNA solutions were obtained, the concentration of quercetin remained unchanged, while the concentration of DNA varied. The ratio of the molar concentrations of DNA and the drug (*N/c*) was expressed as the number of DNA nucleotide pairs per one ligand molecule.

3. Experimental results

3.1. Absorbance

This study's experimental absorbance spectrum of quercetin covers the region of ~230-600 nm. In the absorption spectra of quercetin in ethanol (Fig. 2a), one can see two

complex (at least two-component) intense absorbance bands - near 257 nm and 374 nm; there is also a weakly intense band around 302 nm and some others bands at ~450, and ~525 nm are weak. The position of the absorbance maxima practically does not depend on the concentration. The absorbance spectra agree well with literature data [36-38]. The long-wavelength absorbance line (374 nm) is associated with the absorption of the B ring, and the short-wavelength (257 nm) with the absorption of the A ring [38]. When the concentration of quercetin increases, the absorbance increases, but the position of the band maxima practically does not change. Since the absorbance bands are complex, the spectrum was decomposed into elementary bands, and their changes in the spectra were monitored with increasing quercetin concentration.

It was established that in the working range of concentrations (10-200 μM), a linear dependence of the optical density in the maxima of the absorbance bands on the concentration was observed, i.e., the Bouguer-Lambert-Behr law was fulfilled, the solution was molecular, and the processes of aggregation and reabsorption were negligible. Note that for obtaining absorbance and fluorescence spectra of aqueous solutions of a mixture of quercetin and DNA, the working range of quercetin concentrations was an order of magnitude smaller (10-15 μM).

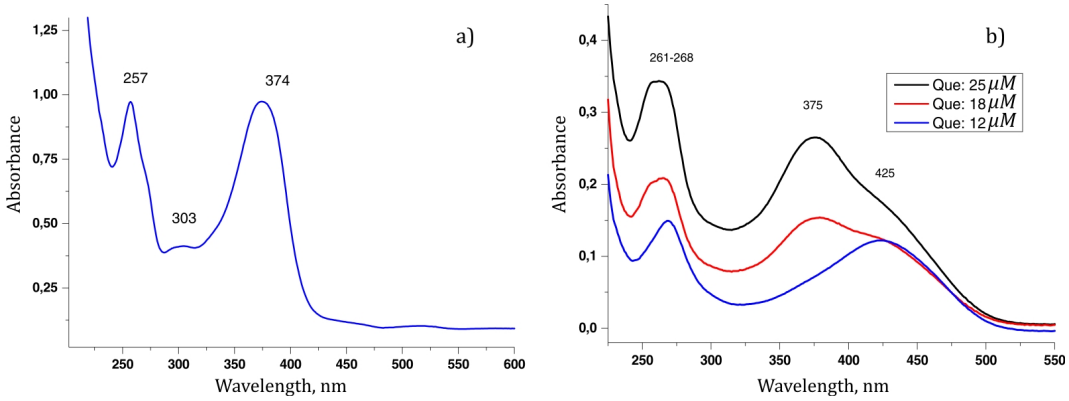

Fig. 2. Absorbance spectra of quercetin in ethanol (a) (c_{Que}: 200 μM) and in water (b) (c_{Que}: 12-25 μM).

In water, the absorbance spectrum of quercetin (actually, of an ethanol solution of quercetin, with >99.5% water) changes slightly: the position of the intense absorption band at ~264 nm, which corresponds to the band at 257 nm in pure ethanol solution, depends slightly on the concentration, the band around ~302 nm is not observed at all. At the same time, the optical density of the 375 nm band is highly dependent on the concentration of quercetin. The intensity of the ~425 nm band, barely visible in pure ethanol, significantly increased in the aqueous solution. Still, its optical density is practically independent of the concentration of quercetin (Fig. 2b).

According to works [39-41], this behavior of the absorption spectrum is related to the deprotonation or detachment of hydrogen atoms from the quercetin molecule. Thus, in [39], different ionic forms of quercetin were studied - depending on the location and number of detached hydrogen atoms, a different ionic form of quercetin is obtained, which has different spectral properties. Each ionic form has its absorption spectrum, but in general, the spectra show four characteristic absorption bands, which correspond to the absorption bands of quercetin in ethanol at 257, 302, 374, and 425 nm. Moreover, if the position and intensity of

the short-wave bands (257 and 302 nm) change relatively weakly, then the region of 350-450 nm of the two long-wave bands of 374 and 425 nm differs significantly for different forms of quercetin. In [40], the spectral properties of quercetin molecules at different pH environments were studied, which is also related to the detachment of external hydrogen atoms. In [41], it is assumed that a long-wavelength absorption band's appearance (or shift) near 425 nm is also related to deprotonation. Still, monovalent complexes are attached instead of detached hydrogen atoms, which causes a rearrangement of the electronic system. Another possible reason for this behavior is the influence of oxygen dissolved in water, which oxidizes quercetin, i.e., depending on the pH, there will be not one form of quercetin in the quercetin solution but a mixture of oxidized and non-oxidized forms, each of which has different characteristics.

The absorption spectrum of an aqueous solution of quercetin+DNA has a slightly different form - the ~257 nm absorption band increases significantly with increasing DNA concentration (because DNA absorbs in this area), the ~302 nm band shifts to the long-wave side ("red" shift) up to ~315 nm with increasing DNA concentration, and its intensity increases slightly; that is, this area is not characterized by non-standard behavior.

The behavior of the two long-wave bands at 374 and 425 nm becomes significantly more complex: the position and intensity of these bands depend both on the concentration of DNA in the solution and the time of interaction between DNA and quercetin in the solution. For example, Fig. 3a shows the absorption spectrum of a quercetin solution with DNA (N/c=0...16), and the spectrum was taken in the first few hours after sample preparation. The band initially at 374 nm increases in intensity with increasing N/c and shifts to the long-wave side. The band initially at 425 nm also shifts to the long-wave side, but its intensity decreases, and a not very clear isosbestic point at ~430 nm can be seen. For samples aged for several days (Fig. 3b), the behavior of the bands in the long-wavelength region becomes somewhat different: the 374 nm band stabilizes and only monotonically increases in intensity with increasing N/c, but now there is an indistinct isosbestic point at ~475 nm between the 425 and 525 nm bands. This behavior is similar to the pH-dependent behavior of these bands in aqueous quercetin solutions [40].

Here, we make only certain assumptions and analogies regarding the behavior of the long-wavelength absorbance bands of quercetin under different conditions; other reasons

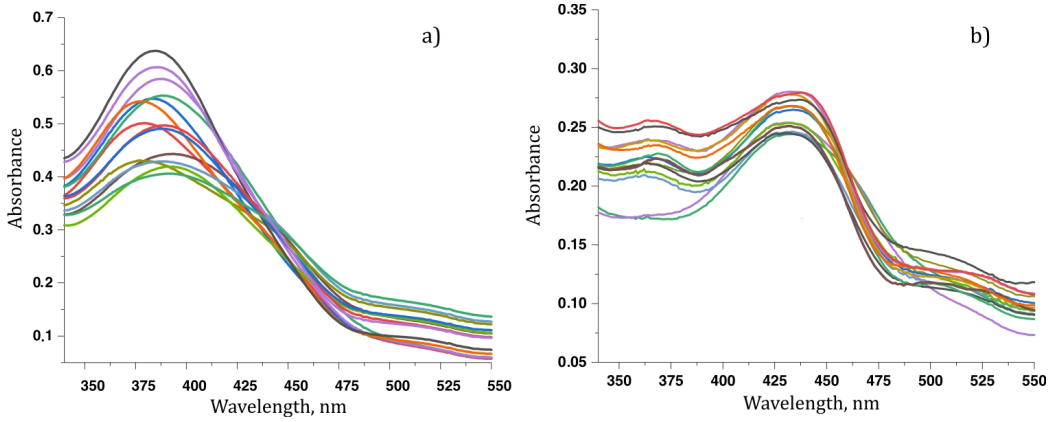

Fig. 3. Absorbance spectra of the Que+DNA solution, long-wave range: the samples aged for (a) several hours, (b) several days.

for such behavior are also possible. The question is complex and requires separate studies; it is not the task of this paper. Our purpose is to study the binding parameters of quercetin with DNA, which requires a detailed study of the fluorescence spectra at different *N/c* since the absorbance spectra, i.e., for quercetin, were unsuitable for such studies. The description of the absorption spectra is given here only for a complete study of the optical properties of quercetin and spectral confirmation of the interaction of quercetin with DNA.

3.2. Fluorescence

The fluorescence spectrum (FS) of the ethanol-aqueous solution of quercetin (the wavelength of excitation λ_{ex}=377 nm) lies in the region of 400-700 nm and consists of a very weak intensity complex undivided band with a maximum at about 538 nm. The optimal excitation of 377 nm was determined from the fluorescence excitation spectra.

The fluorescence spectrum of a mixture of an ethanol solution of quercetin with an aqueous solution of DNA (in the aqueous solution of Que+DNA of ethanol <0.5%) also consists of one visible band, the intensity of which increases greatly (~1500 times) with the increase in *N/c* from 0 to 73 (that is, with an increase in the amount of DNA in the solution), see Fig. 4. At this, the maximum of the FS band shifts significantly with increasing *N/c* to the short-wavelength side ("blue" shift, up to 50 nm, Fig. 4, inset): at small *N/c* (<7) there is a sharp shift in the position of the maximum from 540 nm to 500 nm, further for *N/c* from 7 to 20 the displacement of the maximum slows down - from 500 nm to 490 nm, and at large *N/c* (>20) the position of the maximum reaches the value of about 490 nm and does not change further. Changes in the FS intensity of the Que+DNA mixture have a non-monotonic character depending on *N/c* (Fig. 5); namely, at small *N/c* values (*N/c*≤1), a slight decrease in intensity is observed, with the minimum of the curve at *N/c*~1, further, in the range of *N/c* from 1 to ~20, there is a sharp increase in intensity, after which the increase in intensity slows down and at *N/c*~30 it reaches saturation, which indicates that the maximum possible number of

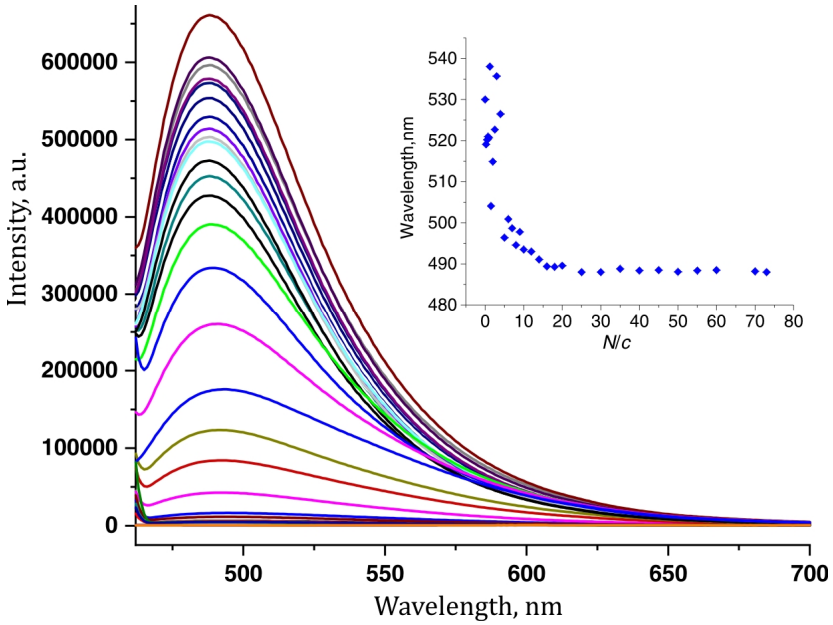

Fig. 4. Fluorescence spectra of an aqueous solution of Que+DNA (part of the set of curves, *N/c*=0...73, quercetin concentration - c_{Que}=11.5 μM, λ_{ex}=445 nm, *N/c*=0...73). Inset: the dependence of the position of the maximum fluorescence intensity of quercetin on the *N/c* of the solution.

ligand molecules is bound. The FS spectrum of Que+DNA for all N/c was excited at 445 nm, and this optimal excitation wavelength was also determined from the FL excitation spectra.

Fig. 4 shows the fluorescence spectra of a mixture of quercetin solutions with DNA for *N/c* values from 0 to 73; a total of >30 samples were made to obtain a large enough number of experimental points for more accurate binding curve construction; in Fig. 4 only part of the curves are shown, all 32.

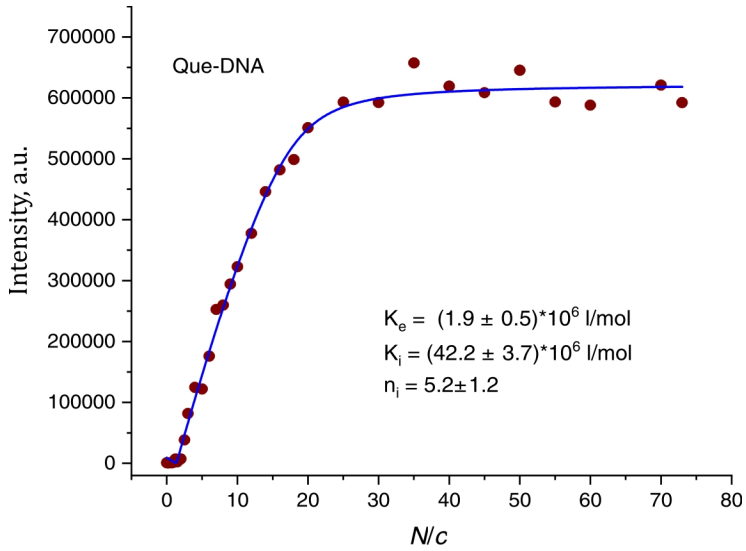

Fig. 5. The binding curve of quercetin to DNA: dependence of the intensity in the maximum of the fluorescence band on *N/c*. Quercetin concentration *c*=11.5 μM, λ_{ex}=445 nm, *N/c*=0...73.

Such a shift of the fluorescence maxima and a change in the fluorescence quantum yield indicate the binding of the small quercetin ligand to the DNA macromolecule, as mentioned above. An increase in fluorescence intensity occurs due to ligand molecules' fixation on the DNA matrix. With the formation of such a complex, the number of vibrational degrees of freedom decreases, leading to a decrease in the probability of non-radiative excitation relaxation; vibrational relaxation during the excited state is, accordingly, less effective. As a result, the fluorescence quantum yield increases.

It is important to note that the fluorescence intensity of such a solution depends not only on *N/c* but also on the time of interaction of quercetin with DNA. Besides, if the stock quercetin solution was stable and did not degrade over time for several series of measurements (the spectra of freshly prepared and two-month-old solutions look almost identical), then the DNA solution had to be prepared for each experiment separately since the use of the old one gave distorted data. In Fig. 4 and 5, the results of not one experiment but the aggregated results of several experiments with different exposure times of the samples are shown - for different *N/c*, the maximum fluorescence intensity was observed at different times after the preparation of the samples. This time was determined experimentally for several iterations of the experiment - for example, for small *N/c* (<5), the maximum intensity was observed in the first couple of hours after preparation. The maximum intensity was observed for large *N/c* (>40) after about a day. This makes working with quercetin very difficult, as several experiments over several days were required to get a good set of points to construct the binding curve.

3.3. Binding parameters

We developed and used the program BindFit to determine the binding parameters. It works with direct experimental data without "linearization" transformations. It allows for increasing the accuracy of parameter determination for processes characterized by a non-linear binding curve even after linearization. This program's principle of operation is approximating experimental data using the binding equations. The general features of the program are described in detail in [27,28]; now, we will mention only the main ones.

The classical Scatchard binding equation $v / c_f = K(1-v)$ was derived for binding small molecules to proteins, and its application to analyze the binding to DNA is almost always incorrect. McGhee and von Hippel [43] extended Scatchard's approach and received the equations for non-cooperative and cooperative binding, respectively, in the form:

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

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

where v is the ratio between the concentration of bound ligands c_b and the total number of binding sites N, c_f is the concentration of free ligands, K is the binding constant for an isolated binding site, n is the number of nucleotides occupied by one ligand molecule, ω is the cooperativity parameter: $\omega = 1$ – non-cooperative binding, $\omega > 1$ – cooperative binding, $\omega < 1$ – binding is anti-cooperative, and $R = \sqrt{(1-(n+1)v)^2 + 4\omega v(1-nv)}$.

The equations written in terms of the Scatchard variables v and v / c_f are not convenient for numerical analysis because those variables are quite complexly related to experimental optical parameters. Besides, the binding parameters are determined from the equations with the Scatchard variables with a rather large error. This occurs because the linearization “according to Scatchard,” in the general case of nonlinear dependences leads to a significant distortion of the experimental errors and, accordingly, the accuracy of determining the binding parameters, which are better determined from the initial nonlinearized curves.

Therefore, to analyze the experimental data and approximate them in experimental variables, Eqs. (1) and (2) were modified [24] and used as equations relative to the variable c_b – the concentration of bound ligands.

For the processes with one type of binding sites, the basic McGhee–von Hippel equations are transformed from their original form (1), (2) to a form (3), (4) that contains only the variables directly related to the experiment:

$$c_b = K(c-c_b)(N-nc_b)\left(\frac{N-nc_b}{N-(n-1)c_b}\right)^{n-1}, \tag{3}$$

$$c_b = K(c-c_b)(N-nc_b)\left(\frac{(2\omega-1)(N-nc_b)+c_b-R'}{2(\omega-1)(N-nc_b)}\right)^{n-1}\left(\frac{N-(n+1)c_b+R'}{2(N-nc_b)}\right)^{2}, \tag{4}$$

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

These equations are implemented in the program and are solved numerically. This makes it possible to work with c_b as the function $c_b = c_b\left(N, c; K, n\right)$.

A system of two equations describes processes with two types of binding sites, and it must take into account the dependence or independence of the binding processes for the ligands occupying the same binding site, i.e., a base pair plus phosphates. If N is the concentration of DNA base pairs, then 2 N binding sites correspond to the binding of the first type (with phosphate), and N binding sites correspond to the binding of the second type (intercalation). A direct transition of bound ligands from type-1 sites to type-2 sites is assumed to be impossible.

For our experimental data, the best approximation results were obtained using a system of modified Scatchard (for external binding) and McGee-von Hippel (for intercalation) equations (5):

$$\begin{cases} c_b^{(1)} = K_1\left(c - c_b^{(1)} - c_b^{(2)}\right)\left(2N - c_b^{(1)}\right)\left(1 - \dfrac{c_b^{(2)}}{N}\right) \\ c_b^{(2)} = K_2\left(c - c_b^{(1)} - c_b^{(2)}\right)\left(N - nc_b^{(2)}\right)\left(\dfrac{N - nc_b^{(2)}}{N - (n-1)c_b^{(2)}}\right)^{n-1}\left(1 - \dfrac{c_b^{(1)}}{2N}\right)^2 \end{cases}. \quad (5)$$

The system describes two interdependent binding processes of ligands occupying one binding site, and intercalation in the gap between base pairs is allowed only if the ligands have not bonded to both phosphates of this gap, and vice versa - binding to phosphates is possible, only if no ligand intercalated into the corresponding gap. Besides, there must be at least n-1 free spaces between the two intercalated ligands. The reduction in the number of external binding sites due to other ions in the solution was also considered; such consideration naturally increases the value of the external binding constant.

To determine the binding parameters, it is most convenient to use data from changes in optical density (in absorption spectra) and changes in fluorescence intensity in the band maximum. In our experiments, sufficiently accurate data were obtained from fluorescence spectra (we wrote about problems with absorption spectra earlier). Accordingly, the approximation results obtained from fluorescence spectra are quite reliable.

The program *BindFit* was used to construct the binding curve (i.e., the dependence of the intensity in the maximum fluorescence band on the concentration ratio) of quercetin with DNA and to determine the binding parameters. The intensity value at the fluorescence maximum was used as an optical parameter. The experimental results obtained by us for quercetin are best described by a system of equations for two-step binding, which corresponds to two types of binding and, accordingly, to two processes - external binding of quercetin to phosphate groups and intercalation; moreover, the program took into account the decrease in the number of (external) binding sites due to other ions present in the solution. The equations describing these processes are the system (5) of the modified Sketchard (for external binding) and McGhee - von Hippel (for intercalation) equations.

The resulting curve of binding of quercetin to DNA is shown in Fig. 5. At the initial segment of the curve (N/c from 0 to 1), a slight depression is formed, the presence of which indicates a noticeable external binding. For a more accurate measurement of this region of values, several samples with a small N/c step in this interval were made because its depth

and width strongly influence the obtained values of the external binding constant. After this minima in the fluorescence intensity, in the *N/c* interval from 1 to 20, there is a sharp increase in the intensity of the fluorescence maxima. The slope of this curve is another parameter that affects the obtained value of the internal binding constant (intercalation, groove); therefore, a sufficiently large number of experimental points with an *N/c* step smaller than on the last part of the curve were also obtained in this interval - this slope accounts for half of all points received. The next part of the curve is a weak increase in the intensity of the maximum at *N/c* from 20 to 30, reaching saturation at *N/c* greater than 30. The intensity of saturation is another parameter that significantly affects the results of the calculations, therefore, in this section of the curve, samples with by a significantly larger step of the N/c change than in the previous sections, since it was important here to make sure that the curve reached saturation.

After analysis of the binding curve and several iterations of calculations with a change of possible binding models, it was found that two types of binding are characteristic of quercetin - intercalation and external binding. To determine the binding constants, a system of modified McGhee-von Hippel and Sketchard Eq. (5) was used, which takes into account two types of ligand binding to DNA, and in which the reduction in the number of (external) binding sites due to other ions is taken into account. As it turned out, for quercetin, both binding processes are manifested - intercalation and external and for small *N/c* (≤2), external binding dominates, and for larger *N/c* - intercalation. The values of the obtained parameters of quercetin binding to DNA are shown in Table 1. There, for comparison, the parameters of binding to the DNA of some other molecules, which we studied by similar methods, are given.

Table 1. Obtained values of binding parameters for quercetin to DNA: K_e – constant for external binding, K_i – binding constant for intercalation, n_i – the average number of binding sites occupied by the ligand during binding. For comparison, the binding parameters of a number of studied ligands [24,26,28] are given for comparison.

Para-meter	Quercetin	Sanguinarine	Berberine	Amitozino-beramid	Ethidium bromide	Acridine
K_e [L/mol]	$(1.9 \pm 0.5) \times 10^6$	$(2.5 \pm 0.4) \times 10^6$	-	$(1.0 \pm 0.1) \times 10^5$	$(7.4 \pm 1.5) \times 10^2$	$(3.0 \pm 1.0) \times 10^5$
K_i [L/mol]	$(42.2 \pm 3.7) \times 10^6$	$(26.0 \pm 5.7) \times 10^6$	$(5.0 \pm 0.2) \times 10^4$	$(3.3 \pm 0.7) \times 10^5$	$(1.2 \pm 0.3) \times 10^5$	$(1.8 \pm 0.2) \times 10^6$
n_i	5.2 ± 1.2	2.3±0.1	1.9±0.1	4.1±0.6	5.7±1.1	3.4±1.1

For comparison, we also give the values of the binding parameters of quercetin calculated by other methods: K_{groove}=17.54× 10^5 L/mol, n=1.39 (groove and external binding found) [6]; K_1=0.43× 10^6 L/mol, K_2=0.016× 10^6 L/mol, n_1=6.0, n_2=1.7 (two intercalation modes - strong and weak) [7]; K=14.24× 10^4 L/mol, n=1.6 [8]; K=(2.2÷9.4)× 10^4 L/mol (external binding) [9].

As can be seen, the binding constant of quercetin for intercalation is an order of magnitude higher than the external binding constant, although the values of both constants are quite high compared to other ligands we have studied (see Table 1). Importantly, in our view, quercetin binds to DNA more effectively than, in particular, berberine, sanguinarine,

and amitoberamide (modified berberine) [24,26,28] which are also known to have antitumor properties; this confirms the results of previous biomedical research and makes quercetin very promising for the creation development of anticancer drugs.

The high value of the external binding constant is caused, in particular, by the exclusion of a part of the binding sites due to other ions present in the solution - up to ~20%. In addition, since the initial part of the binding curve, where the FL intensity is low, is important for determining K_e, the accuracy of determining K_e is relatively lower.

Note that in this case (i.e., for quercetin), the intercalation is incomplete since part of the quercetin molecule (actually, ring B) does not lie in the plane of other rings and is placed not between base pairs but in a groove. In particular, this explains the relatively large value of n_i ccompared to planar or nearly planar molecules, e.g., sanguinarine or berberine (a small value of n_i testifies to the intercalation mechanism of ligand binding to DNA). A similar partial intercalation is observed for ethidium bromide (EthBr), where one of the rings lies perpendicular to the other rings' plane, but EthBr is considered a classical intercalator. At the same time, the similarity of the n_i parameters for quercetin and ethidium bromide is not accidental ($n_i \sim$ 5-6).

4. Conclusions

A series of electronic absorption and fluorescence spectra of aqueous solutions of pure quercetin and its mixture with DNA at different values of N/c (the concentration ratio between the DNA base pairs and the ligand molecules) at different time intervals after sample preparation were obtained. Signs of binding of quercetin to DNA were recorded: changes in fluorescence intensity and short-wavelength shift of the fluorescence maxima.

The rearrangement of the long-wavelength region of the absorption spectrum of the quercetin+DNA solution was recorded (shifts of "old" bands and appearance of new ones). The structure of the spectrum depends on the N/c of the solution and the exposure time of the samples. Assumptions are made about the nature of these changes.

Based on the obtained fluorescence spectra, the binding curve of quercetin with DNA was constructed. Using the special *BindFit* program, the values of the binding parameters (external binding and intercalation constants, as well as the average number of binding sites occupied by the ligand on the DNA matrix) were obtained, and a binding mechanism was established.

It was determined that intercalation is the dominant type of binding for quercetin to DNA. The obtained values of the binding parameters were compared with those we obtained earlier for other ligands. It highlights quercetin's potential for anticancer drug development, and, besides, since the ability of quercetin to directly affect replicative complexes of viruses, leading to either inhibition or complete blocking of virus replication, is primarily due to intercalation mechanisms of interaction, a relatively reliable establishment of this mechanism will be useful in the creation of new antiviral drugs based on quercetin, in particular, against covid, which is still very relevant now.

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Анотація. *Взаємодію флавоноїду кверцетину з макромолекулою ДНК у водному розчині досліджено методами оптичної спектроскопії – електронного поглинання та флуоресценції. На основі отриманих результатів побудовано залежності спектральних характеристик від відношення концентрацій* N/c *між парами основ ДНК і молекулами ліганду. За допомогою системи модифікованих рівнянь Скетчарда і МакГі-фон Гіппеля визначено параметри зв'язування кверцетину з ДНК.* ***Користуючись***

системою модифікованих рівнянь Скетчарда та МакГі-фон Хіппеля, визначено параметри зв'язування кверцетину з ДНК. Для порівняння наведено параметри зв'язування з ДНК інших досліджених нами раніше молекул.

Ключові слова: *ДНК, кверцетин, рівняння зв'язування МакГі - фон Хіппеля, спектри поглинання, спектри флуоресценції.*