

INTERACTION OF FLAVONOIDS: MORIN, QUERCETIN AND RUTIN,
WITH DNA

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Studies of interaction of flavonoids: morin, quercetin and rutin, with DNA at 0.002 M ionic strength solution have been carried out by square wave voltammetry (SWV) method. SWV curves of the mentioned flavonoids and their complexes with DNA were obtained. Based on titration curves coordinates of r and C_f (Scatchard's coordinates) were obtained and the curves of r/C_f dependence on r were constructed. Nonlinear binding curves were obtained, which were interpreted from the point of view of existing at least two modes of binding the mentioned flavonoids with DNA. From the curves for two modes of interaction, values of the binding constant (K) and the number of DNA base pairs (n) per one binding molecule of flavonoid were determined.

Keywords: DNA, flavonoid, quercetin, rutin, morin, binding curves.

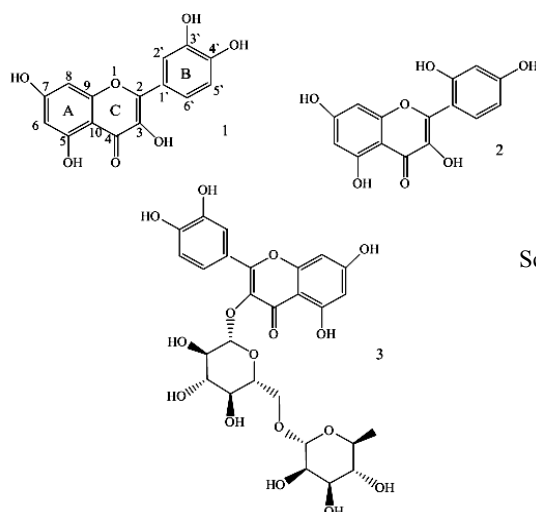
Introduction. DNA is one of cellular targets for different natural or coming from outside synthesized compounds that surround it. These compounds, having significantly low molecular weight, may be immediately bound with it and influence on its functions, including replication and gene expression (on transcription level). Such substances may show antiviral, antibiotic, anticancerogeneous or cancerogeneous as well as mutagenic activities. From this point of view the interaction of biologically active low-molecular compounds with DNA is important for designing and synthesis of new preparations possessing pharmacological value [1, 2]. Low-molecular compounds interact with DNA covalently or non-covalently: in the last case these substances are called ligands which may be bound by intercalation mechanism becoming inserted into the plane between DNA base pairs in the minor or major groove (groove binding) or from the external side of DNA helix [2].

Recently the interaction of natural substances such as flavonoids that show biological activity in many mammalian cell systems *in vitro* and *in vivo* with DNA has been investigated [3–7]. Flavonoids: quercetin, morin and rutin (see Scheme) are strong chain breaking antioxidants since they directly scavenge free radicals [8–10]. Flavonoids also inhibit the hydroxyl radical production by chelating the transition metals [11–12]. They may also associate oxidable substrates like DNA, protein, cell membranes to prevent direct hydroxyl radical damage. Thus, they

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protect the body against free radicals like reactive oxygen species (ROS) such as singlet oxygen, superoxide, peroxy, hydroxyl and reactive nitrogen species (RNS) like peroxynitrite [13–16].



Scheme. Chemical structures of flavonoids: quercetin (1); morin (2) and rutin (3).

The interaction of flavonoids with DNA was investigated by different electrochemical and voltammetric modes by many authors [6, 7, 17]. The electrochemical investigations of DNA binding to these molecules can provide a useful complement to spectroscopic methods (for non-absorbing species). Electrochemical methods differ by their sensitivity, simplicity and universality and yield information about the mechanism of binding. The square wave voltammetry method is the most sensitive among electrochemical methods. It makes possible to investigate the interaction of DNA–ligand complexes at significantly low concentrations of initial substances and low ionic strength of the solution.

The purpose of the present work is the study of different modes of binding quercetin, morin and rutin with DNA by the method of SWV and the determination of a binding constant (K) and a number of bases corresponding to one site of the binding (n).

Materials and Methods. In the present work Calf Thymus DNA (ultrapure, “Sigma”, USA), quercetin-3-rutinoside (rutin), 3,3',4',5',7-pentahydroxyflavon (quercetin), 2',3',4',5',7-pentahydroxyflavon (morin) (with high purity), ethanol, NaCl (“Sigma”, USA) were used. Solutions of flavonoids were prepared in 96% ethanol with $2 \cdot 10^{-4}$ M. Ethanol was purified according to the accepted mode [18]. Solutions of flavonoids in electrochemical tubes were diluted by NaCl water solution (0.002 M ionic strength) 100 times. Solution of NaCl was prepared in deionized water (electric resistance >16 M Ω /cm at 25°C).

Apparatus. SWV curves of rutin, quercetin and morin complexes with DNA were obtained by triple-electrode system through voltammetric complex (Bioanalytical system, BAS-100B/W, USA) at $37 \pm 0.1^\circ\text{C}$. Carbon-glassy electrode with 3 mm diameter has served as working electrode, which has been purified by aluminum oxide dust and eluted by deionized water before measurements. Ag/AgCl (3 M NaCl water solution) served as a comparative electrode and platinum

wire served as an auxiliary electrode. Voltammetric diagrams were registered from 0 to 1600 *mV* interval. Square-wave parameters were: step potential 2 *mV* and pulse amplitude 25 *mV*. Square-wave voltammograms were recorded at 25 Hz.

Procedure of DNA–Flavonoid Complex-Formation Studies. The initial concentration of flavonoids in the solution was $2 \cdot 10^{-6}$ *M* and it was titrated by DNA solution with $1.06 \cdot 10^{-3}$ *M* concentration. During titration DNA solution with 20 *mL* volume (automatic pipettes Ependorf were used (USA), 0.5–10 μL and 5–50 μL volumes with application of single-time tips) was added each time to solutions of flavonoids in electrochemical thermostating bottle (BAS, USA). The initial volume of titrated solution was 3 *mL*, the terminated volume was 3.6 *mL*. Decreasing of flavonoid concentration was registered through measurements of current force (330 ± 20 *mV* for rutin, 305 ± 20 *mV* for quercetin and 296 ± 20 *mV* for morin) corresponding to anode oxidation formal potential. Direct linearity (correlation rates $R^2 > 0.99$) dependence of the electric power corresponding to anode oxidation formal potential on concentration of each of flavonoids was checked. Before starting the titration by DNA solution, the correspondence of initial concentrations of flavonoids to calculated ones was determined from volt-amperegrams. Linearity dependence of electric power of anode oxidation potential peak on rutin concentration change was presented in Fig. 1 as an example.

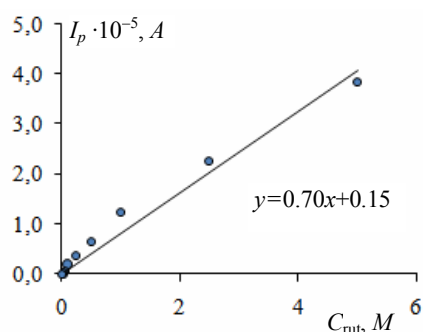


Fig. 1. Linear dependence of electric power peak of rutin anode oxidation potential on its concentration change.

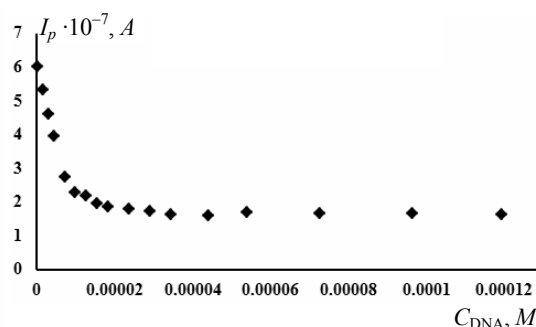


Fig. 2. Electric power dependence of rutin solution on C_{DNA} at 0.002 *M* ionic strength of the solution: $C_{\text{rut}} = 2 \cdot 10^{-6}$ *M*; $C_{\text{DNA}} = 1.06 \cdot 10^{-3}$ *M*.

Determination of Binding Parameters of Flavonoids with DNA. As it is obvious from Fig. 2, adding of DNA to flavonoid solution a decreasing of electric power occurs. Dependence of electric power decreasing obtained for rutin from added DNA concentration (similar changes have been registered for other flavonoids as well) is presented in Fig. 2. Isotherms of flavonoids adsorption on DNA were constructed in Scatchard's coordinates (r/C_f on r , $r = C_b/C_p$, where C_b is a concentration of bound molecules of ligand with DNA and $C_b = C_0 - C_f$, C_0 is total concentration of ligand, C_f is concentration of free molecules of ligand in a solution, C_p is concentration of phosphate groups of DNA). The experimental binding curves were analyzed by the method of regression analysis of theoretical curves obtained from Crothers's equation for the case of two types of ligand–DNA interactions (see [19]):

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

and linearized by Arakelyan and co-authors [19]:

$$r/C_f = K[1 - (2n - 1)r]. \quad (2)$$

The values of K and n were determined from adsorption isotherms according to the Eq. (2).

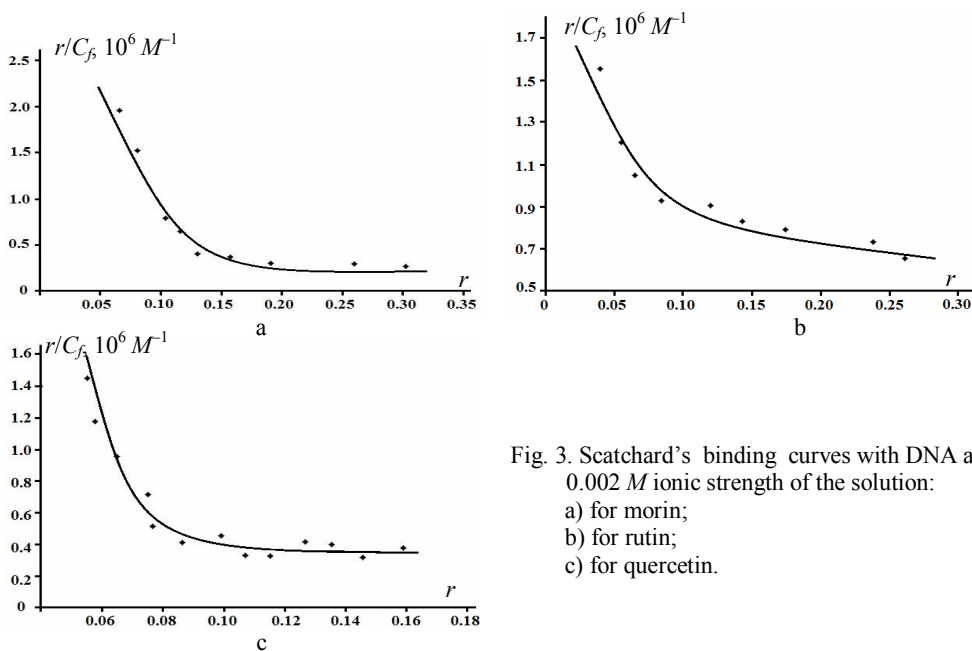


Fig. 3. Scatchard's binding curves with DNA at 0.002 M ionic strength of the solution:
 a) for morin;
 b) for rutin;
 c) for quercetin.

Binding constants (K_1 , K_2) and number of base pairs (n_1 , n_2) corresponding to one binding molecule of ligand

Flavonoids	$K_1, 10^6 M^{-1}$	$K_2, 10^6 M^{-1}$	n_1	n_2
Rutin	0.2 ± 0.05	0.0016 ± 0.0005	5 ± 0.5	1.2 ± 0.3
Morin	0.24 ± 0.5	0.0073 ± 0.0003	4 ± 0.5	1.5 ± 0.3
Quercetin	0.43 ± 0.5	0.016 ± 0.005	6 ± 0.5	1.7 ± 0.3

Results and Discussion. Using the data of square wave voltammograms (SWV) of titrating solutions (Fig. 2), binding curves were built (Fig. 3, a–c). The obtained results indicate that it is possible to find out interaction of flavonoids with DNA by SWV method as well as to carry out a quantitative analysis and to determine parameters of interaction (K and n). From the titration curves using the Eq. (2) the experimental values of r/C_f and r , were obtained and the binding isotherms were built in these coordinates (Fig. 3, a–c). Fig. 3, a–c show, that there are at least two types of binding. For this reason the parameters of binding corresponding to two types are received according to Eq. 2, which are presented in Table. It is followed from obtained data that one of these binding modes of aforementioned flavonoids with DNA is strong compared with others and corresponds to intercalation mode [15–16]. It is indicated by the values of K_1 and particularly n_1 as well, since a high value of n is the result of limit of the binding centers by this mode. It is known that at intercalation almost 2–3 pairs of bases

neighboring above and under the intercalation center become excluded [20–23]. It is obvious from the table data, that in the case of weak binding mode, a number of base pairs per one bound molecule of ligand is less and it may be maintained that stoichiometry of saturation approximates to 1:1 relation (one ligand molecule per one pair of bases). This result, most probably, indicates that at weak binding mode molecules of flavonoids are bound from the external side of DNA.

Thus, our obtained data indicate that at low ionic strength of the solution, molecules of studied flavonoids are bound to DNA by two modes: strong-intercalation and weak. Moreover, the values of K_1 are higher almost by one order compared to literature data, which may be conditioned by low ionic strength of the solution (DNA molecule in solutions with 0.002 M NaCl is more untwisted which supports intercalation [20]). Obtained data reveal also, that quercetin shows the highest affinity to DNA whereas the lowest affinity has rutin, this is most probably conditioned by its side groups.

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