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BIOPHYSICAL CHEMISTRY

Dependence of the Solubility of Natural Flavonoids in Water on the Concentration of Miramistin, Polyvinylpyrrolidone, and Human Serum Albumin

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Abstract—In organized media of the cationic surfactant miramistin and the polymers polyvinylpyrrolidone and human serum albumin, the solubility of natural flavonoids quercetin and rutin increased by one or two orders of magnitude. The increase was more significant for hydrophobic quercetin than for hydrophilic rutin. The solubility also depended on the structure and self-organization of molecules in organized media and the site of flavonoids in them. The calculated binding constants increased in the series polyvinylpyrrolidone < miramistin < human serum albumin.

Keywords: rutin, quercetin, miramistin, polivinylpyrrolidone, human serum albumin, organized media, solubility

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INTRODUCTION

Flavonoids (Fl) belong to a large group of natural polyphenol compounds and have a broad spectrum of pharmacological activity [1, 2]. Many natural flavonoids have extremely low solubility in aqueous media and body fluids. This leads to certain difficulties in creating highly effective medicines, while the solubility is one of the major biopharmaceutical characteristics that largely determines the drug's bioequivalence and the possibility of creating drug forms with an effective dose and effective absorption rate and completeness [3].

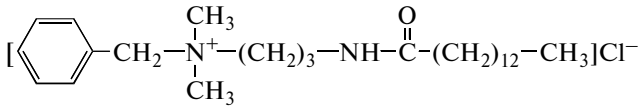
To improve the solubility of drugs, various methods were developed and are used in pharmaceutics: solubilization, preparation of solid disperse systems with soluble and insoluble matrices, inclusion in liposomes, nanocapsules, and others [3]. Earlier [4–6], we found that the solubility of the flavonoids quercetin (Qu) and rutin (Ru) increases substantially in aqueous solutions of β-cyclodextrin, biopolymers (BPMs) polyvinylpyrrolidone (PVP), and human serum albumin (HSA) due to the formation of supramolecular complexes and that the spectral and protolytic properties of flavonoids in these organized media also change. Another type of organized media are self-organized supramolecular micellar systems based on surfactants, in which the solubility of hydrophobic substances can increase substantially due to solubilization [7, 8]. A distinction of organized media from homogeneous solutions lies in the fact that the key role in them is played by the local effect [9]: the hydrophilic and hydrophobic molecules dissolve in the void of the receptor molecule or in the micellar phase.

Benzyldimethyl[3-(myristoylamino)propyl]ammonium chloride known under the commercial name Miramistin (MR) is one of the therapeutically most effective surfactants. It is used as an antiseptic with a broad spectrum of activity including the antimicrobial, antifungal, and antiviral activities [10].

The aim of this work was to study the influence of miramistin on the solubility of flavonoids and to determine the constants of their binding with miramistin, polyvinylpyrrolidone, and serum albumin. This will allow objective evaluation of the solubilizing properties of these organized media in developing and using drugs based on such systems in medical practice.

EXPERIMENTAL

The cationic surfactant miramistin (Infamed) and quercetin and rutin (Sichuan Xieli Pharmaceutical, Korea) were used.



(miramistin)

The electronic absorption spectra of solutions were measured on a Specord M-40 spectrophotometer (Carl Zeiss Jena, Germany). The influence of background on the analytical signal obtained by recording the absorption spectra of the solutions was eliminated

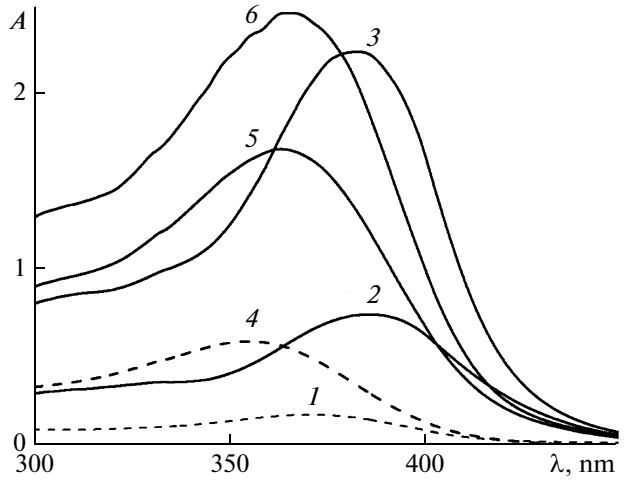


Fig. 1. Absorption spectra of (*1–3*) quercetin and (*4–6*) rutin dissolved in water (*1*, *4*) and aqueous solutions containing miramistin at concentrations c_{Mr} (10^{-3} M) of (*2*, *5*) 4.48 and (*3*, *6*) 11.2; $l = 0.5$ (*1*) and 0.1 cm (*2–6*).

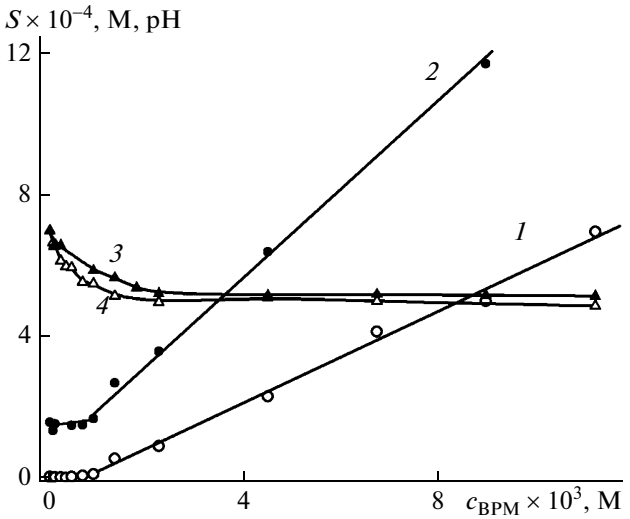


Fig. 2. Dependences of the (*1*, *2*) solubility and (*3*, *4*) pH of the equilibrium solution of (*1*, *3*) quercetin and (*2*, *4*) rutin on the miramistin concentration in solution.

by heterochromatic extrapolation at two wavelengths [11].

To study the solubility of flavonoids in organized media, we prepared a series of aqueous solutions with (0–6) × 10^{-4} M PVP or HSA or (0–1.2) × 10^{-2} M miramistin. An excess of dry quercetin or rutin was added. The mixture was shaken on a shaking apparatus until equilibrium was achieved (24 h) and centrifuged for 10 min at 2000 rpm. The absorption spectra and pH of the resulting solutions were measured. The temperature was constant (293 K) in all experiments. The solubility of quercetin and rutin in aqueous solutions and organized media was calculated from their light absorption data. For this, we used the molar absorption coefficients of the flavonoids, which were preliminarily determined for each concentration of the solubilizer taking into account the evaluated pH of the equilibrium solution.

The increase in the solubility of flavonoids in miramistin solutions relative to their solubility in aqueous solutions was used to determine the thermodynamic constants of their binding (K_{bnd}) with surfactant micelles. The constants were calculated by the method described in [12] using the linear equation

$$S/S_w - 1 = K_{bnd}(c_{Mr} - CMC), \quad (1)$$

where S_w and S are the solubilities of the flavonoid in water and organized medium, respectively; c_{Mr} is the total concentration of miramistin; and CMC is the critical micelle concentration of miramistin. The binding constant of the supramolecular complex Fl–Mr was determined as the slope of the line plotted as $(S/S_{bnd} - 1)$ vs. $(c_{Mr} - CMC)$. Since the formation of organized media by biopolymers is not related to their concentration (i.e., CMC = 0), in contrast to the case of surfactants, Eq. (1) is transformed into

$$S/S_w - 1 = K_{bnd}c_{BPM}. \quad (2)$$

Accordingly, the binding constant of the Fl–Bpm supramolecular complexes was determined as the slope of the line constructed in coordinates $(S/S_w - 1)$–c_{BPM}.

RESULTS AND DISCUSSION

Figure 1 shows the spectra of the equilibrium solutions of quercetin and rutin after their dissolution in water and miramistin solutions of different concentrations. A comparison of these spectra shows that the light absorption intensity characteristic for flavonoids increases significantly as a result of dissolution at higher concentrations of miramistin; the bathochromic shift of the absorption bands of quercetin and rutin in surfactant solutions relative to their bands in water suggests supramolecular interactions in these organized media.

A drastic increase in the solubility of flavonoids was observed at $c_{Mr} > 1 \times 10^{-3}$ M (curves *1* and *2*, Fig. 2). The dissolution of quercetin and rutin was accompanied by a decrease in pH of solution from 7 ($c_{Mr} = 0$) to 5 (curves *3* and *4*, Fig. 2). This was taken into account in the spectrophotometric determination of their concentration in equilibrium solutions. Both concentration dependences (of solubility and pH) have an inflection at $c_{Mr} = 1 \times 10^{-3}$ M, which corresponds to the CMC of this surfactant. The increase in the solubility of quercetin and rutin in miramistin solutions relative to their solubility in water was used to determine the thermodynamic constants of their binding with surfactant micelles. It was found experimentally that the dependence of $(S/S_w - 1)$ on $(c_{Mr} - CMC)$ is linear for both flavonoids and described by the equations shown in Table 1.

Table 1. Parameters of the equation $y = a + bx$ that describes the dependence of $S/S_w - 1$ on c_{Mr}–CMC or $c_{PVP(HSA)}$ for the dissolution of flavonoids in these organized media

Compound	Quercetin		R	n
	a	b		
Miramistin	-3.6 ± 2.3	$(12.9 \pm 0.4) \times 10^3$	0.997	8
Polyvinylpyrrolidone	0.53 ± 0.24	$(8.0 \pm 0.8) \times 10^3$	0.977	7
Human serum albumin	2.9 ± 1.3	$(101.8 \pm 5.4) \times 10^3$	0.986	12
	Rutin			
Miramistin	0.27 ± 0.06	$(7.8 \pm 0.1) \times 10^2$	0.999	7
Polyvinylpyrrolidone	0.02 ± 0.01	$(6.6 \pm 0.3) \times 10^2$	0.990	9
Human serum albumin	0.17 ± 0.06	$(24.3 \pm 2.2) \times 10^2$	0.970	9

Table 2. The binding constants ($\log K$) of flavonoids with miramistin, polyvinylpyrrolidone, and human serum albumin

Flavonoid	Miramistin	Polyvinylpyrrolidone		Human serum albumin	
Quercetin	4.11 ± 0.02	3.89 ± 0.02 [5]	3.91 ± 0.03	5.04 ± 0.02 [5]	5.01 ± 0.03
Rutin	2.89 ± 0.02	2.92 ± 0.01 [6]	2.82 ± 0.03	3.45 ± 0.01 [6]	3.39 ± 0.01

The binding constants of flavonoids with miramistin in logarithmic form are given in Table 2.

A comparison of the constants shows that the solubility of quercetin in miramistin solutions increased significantly compared to the constants for water solutions. The solubility of rutin increased less dramatically evidently because of the presence of a large hydrophilic substituent rutinose in its molecule. A conventional parameter that characterizes the lipophilic properties of substances is the coefficient of their distribution in the water–*n*-octanol system ($\log P$) [13]. The literature values of $\log P$ of quercetin ($\log P = 1.480$) and rutin ($\log P = -2.020$) [14] indicate that quercetin is a moderately hydrophobic compound ($1 < \log P < 3$), while rutin is hydrophilic ($\log P < 1$).

Note that the binding constant found for the Qu–Mr system is comparable to the binding constant of quercetin with micelles of another cationic surfactant, cetyltrimethylammonium bromide ($K_{bnd} = 24615$ L/mol, $\log K_{bnd} = 4.39$) [15], but significantly exceeds the binding constants of quercetin with the nonionic surfactant Triton X-100 ($K_{bnd} = 1540$ L/mol, $\log K_{bnd} = 3.19$) [16] and the anionic surfactant sodium dodecyl sulfate ($K_{bnd} = 1279$ L/mol, $\log K_{bnd} = 3.11$) [15]. This is explained by the fact that interactions of quercetin with the neutral and negatively charged micelles of surfactant are mainly hydrophobic, while its binding with the positively charged micelles of cationic surfactants mainly occurs by electrostatic attraction, especially in the pH range of the dissociation of hydroxyl groups.

The larger binding constant of quercetin compared with that of rutin is explained by the higher hydrophobicity of quercetin, which is known [13] to correlate with the binding constant in series of structurally related compounds.

Similar tendencies were found when we studied the solubility of flavonoids in organized PVP and HSA media [5, 6] and analyzed the formation constants of Fl–PVP and Fl–HSA supramolecular complexes, which were calculated on the basis of a 1 : 1 ratio of the components found (Table 2). For a reliable comparison of the effects of organized media of different types on the solubilization of quercetin and rutin, the solubility of flavonoids in PVP and HSA solutions was studied under the same conditions as those used for miramistin. The experimental data in the coordinates $(S/S_w - 1)$ vs. $c_{PVP(HSA)}$ were linearized by the least-squares method with correlation coefficients R of 0.97–0.99 (Table 1).

The estimated constants of binding of flavonoids with PVP and HSA (Table 2) generally coincide with the parameters of the constants calculated previously [5, 6]. This makes it possible to objectively compare the stabilities of the supramolecular complexes of flavonoids in the organized media of various types under study. The two flavonoids showed the same tendency

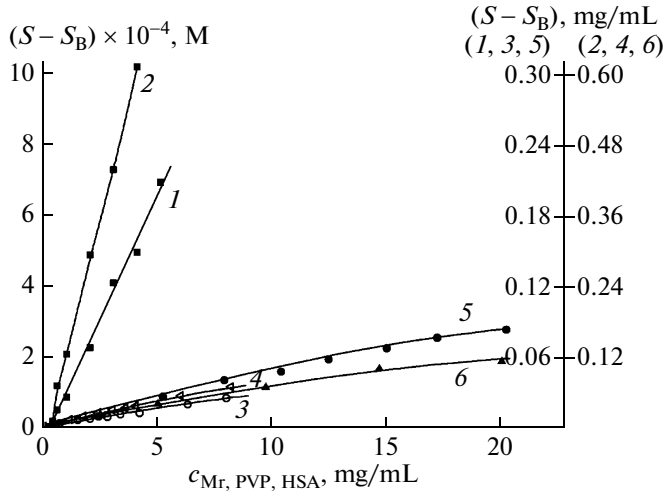


Fig. 3. Dependences of increased solubility (S–S_w) of (*1*, *3*, *5*) quercetin and (*2*, *4*, *6*) rutin on the concentration (c, mg/mL) of (*1*, *2*) miramistin, (*3*, *4*) polyvinylpyrrolidone, and (*5*, *6*) serum albumin relative to their solubility in aqueous solution.

in the variation of the binding constant: PVP < Mr < HSA. This is evidently explained by the structural features of these organized media.

Thus, according to [17, 18], flavonoids, including rutin and quercetin, are localized in the hydrophobic void of HSA domain IIA. A similar mechanism can be adopted for dilute aqueous solutions of PVP, in which PVP has the form of a static coil [19] where the hydrophobic cavity can exist. Based on the estimated binding constants we can conclude that the localization of flavonoids in the fixed hydrophobic cavity of HSA is more advantageous than in the hydrophobic loop of the static PVP clew.

Unlike biopolymers, surfactants form organized media (supramolecular assemblies called micelles) only when their concentration in solution exceeds CMC and are characterized by the volumetric capacity; i.e., they have cavities that may be filled with other molecules without loss of the thermodynamic stability of the system. At miramistin concentrations in the range $c_{Mr} = 1.0 \times 10^{-3}$–$1.2 \times 10^{-2}$ M, the solubility of flavonoids increased linearly (Fig. 2); this points to constant solubilization capacity and is typical for the formation of spherical micelles according to [8]. Polar hydrophobic organic substances including flavonoids are solubilized by accommodating their molecules in the surface layer of micelles, with polar groups directed into the aqueous phase because the energy of the binding of polar groups with water prevents full penetration of solubilizate molecules into the micelle nucleus [20].

The differences in the stability of supramolecular complexes with miramistin, serum albumin, and polyvinylpyrrolidone, namely, the higher binding constants of quercetin compared to those of the more hydrophilic rutin suggest that hydrophobic interactions play a significant role in the solvation of structurally related flavonoids in organized media.

The binding constant is not a single criterion for choosing a solubilizer for flavonoids in pharmaceuticals; another important criterion is the absolute quantity of solubilizer required for an effective increase of solubility. Thus, according to the dependence of the solubility of flavonoids on the concentration of the surfactant, PVP, and HSA expressed in mg/mL (Fig. 3), miramistin is clearly a more promising solubilizer for the development of new drugs.

CONCLUSIONS

To summarize, it was found that in the organized media of PVP, HSA, and miramistin, the solubility of flavonoids increased by one or two orders of magnitude, and the increase was more significant for hydrophobic quercetin than for hydrophilic rutin. The solubility of quercetin and rutin was shown to depend on the structure and self-organization of molecules in organized media (PVP (static clew), HSA (globule with hydrophobic cavities), and miramistin (spherical micelle)) and on the localization site of flavonoids; the calculated binding constants increase in the series PVP < miramistin < HSA.

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