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SIMULATIONS OF SOME BIOMEMBRANE INTERFACIAL PHENOMENA. I. SPECIFIC MOLECULAR INTERACTIONS BETWEEN BOVINE SERUM ALBUMIN AND MELATONIN

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ABSTRACT. This study was designed to investigate the effect of melatonin on the bovine serum albumin (BSA) at the air/aqueous solutions interfaces. Melatonin is a known secretory hormone of the pineal gland with multiple actions "in vitro" and "in vivo", namely, it clearly protects macromolecules from oxidative damage. This hypothesis is supported by our experimental data which show that melatonin specifically interact with bovine serum albumin (BSA). Our findings indicate that melatonin increases the surface pressure of BSA adsorbed films exerting a substantial stabilizing effect on BSA interfacial self-assembled films at the air/water interface. This effect suggests that melatonin might act as a protective agent of macromolecules "in vitro" and "in vivo" through physicochemical specific interactions with bio-molecules, such as BSA, and/or with their biologically active assemblies. As a consequence, melatonin may facilitate the inhibition of the peroxidation damage of bio-molecules by increasing their supramolecular assemblies stability. Our results confirm both the involvement of melatonin in specific interactions with BSA and its remarkable effect on the stabilization of biological compounds at fluid interfaces.

Key words: adsorption at liquid interfaces, melatonin, bovine serum albumin, interfacial pressure, ring du Noüy method.

INTRODUCTION

Melatonin (N-acetyl-5-methoxy-tryptamine; Fig. 1), the main secretory hormone of the pineal gland of vertebrates including the humans, it is also produced by extrapineal tissues and edible plants and it has multiple actions "in vivo". Recently, melatonin was reported to efficiently protect biomolecules against oxidative damage and, consequently, against carcinogenesis [1,2]. Melatonin's function is substantially consistent with a general protection action, upon fatty acids [3,4] lipids, proteins and DNA. It is evaluated as being an effective hydroxyl free radical scavenger and an efficient antioxidant [5,6]. This non-receptor related action might shed light on several of melatonin activities on cell membrane function, that is largely committed to cellular membrane integrity. The multiple actions make melatonin a potentially useful compound in the treatment of various diseases generated by the oxidative damage "in vivo" [7-9].

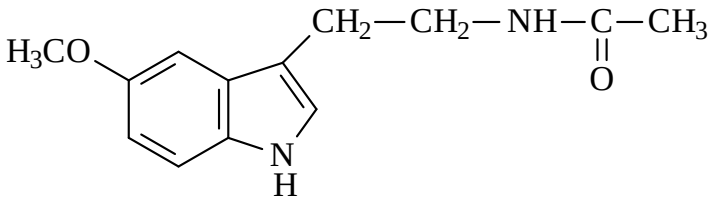

Fig. 1 Molecular structure of melatonin

To our knowledge, no data are available about the effects of melatonin on proteins at fluid interfaces. The goal of this study was to determine the effects of melatonin on the bovine serum albumin (BSA) self-assembled films adsorbed at the air/aqueous solution interface, near the physiological pH. Our results indicate that melatonin interacts with BSA and stabilizes the supramolecular structure of BSA self-assemblies formed at fluid interfaces. Consequently, melatonin might modulate some biological phenomena by having its ability to stabilize macromolecules from oxidative stress both "in vitro" and "in vivo".

EXPERIMENTAL

Melatonin was purchased from Sigma Chemical Co. and used without further purification. Bovine serum albumin (BSA) was obtained from Merck and used also without further purification. All other chemicals were of the highest grade available commercially. Other chemicals used were of analytical grade and obtained from commercial sources. Twice distilled water was used in all experiments. In order to ensure identical working conditions, all BSA solutions were prepared in phosphate buffer (pH 7), made up from phosphate salts (6.6×10^{-2}M) in twice distilled water.

Surface tension σ (dyn/cm) measurements were performed at the interface between the air and phosphate buffer solutions, in the presence of melatonin (ranging from 10^{-4} M to 10^{-2} M) both in the absence of BSA and in the presence of the constant concentration of BSA of 0.4% w/v, at 20^0 C, by using the ring du Noüy method, with an accuracy of ± 0.1 dyn/cm. The experimental details have already been given in one of our previous work [10].

RESULTS AND DISCUSSION

The equilibrium adsorption of melatonin at the air/aqueous phosphate buffer interface, in terms of surface tension as a function of time, was reached in about 60 minutes, in the absence of bovine serum albumin (BSA), and in 40 minutes in the presence of BSA at a constant concentration in BSA of 0.4 % w/v. The more rapid attainment of the adsorption equilibrium for melatonin at the air/water interface in the presence of BSA is probably the result of the interaction of melatonin with BSA within the interfacial self-assembled films.

These results clearly show that both melatonin and BSA are surface active bio-molecules and both act by lowering the surface tension of the pure air/water interface. The simultaneous adsorption of melatonin and BSA at the air/water interface is a complex phenomenon difficult to be fully analyzed.

However, the two compounds show a synergistic effect when adsorbed together from water phase to the fluid interfaces being incorporated into interfacial mixed self-assemblies. The molecular association of melatonin and BSA in the interfacial self-assembled films or adsorbed membranes at fluid interfaces is particularly noteworthy and might explain some biomembrane phenomena.

Further, the equilibrium values of the surface tension were given versus logarithm of the melatonin concentrations in Fig. 2, both in the absence and in the presence of BSA. By analyzing these isotherms a linear dependence of $\sigma = \sigma(\log c)$ is found in the range of large concentrations of melatonin.

The maximum adsorption coefficient (Γ_{max}) and molecular area (a_o) for melatonin in adsorbed films at the air/water (pH 7) interfaces, both in the absence and in the presence of bovine serum albumin (0.4% w/v) in aqueous phase, were determined from the slope of the linear portions of the isotherms (Fig. 2) by using the Gibbs adsorption equation. These values are given in Table 1.

Table 1

Maximum adsorption coefficient (Γ_{max}) and molecular area (a_o) for melatonin in adsorbed films at the air/water (pH 7) interfaces, both in the absence and in the presence of bovine serum albumin (0.4% w/v) in aqueous phase.

Interface	Aqueous solution of melatonin (pH=7)/air	Aqueous solutions of melatonin and bovine serum albumin (BSA: 0.4% w/v; pH 7)/air
Γ_{max} , moles/cm^2	2.6×10^{-10}	1.02×10^{-10}
a_o , Å^2/molecule	63.85	162.78

From the data given in Table 1 we can observe that the melatonin film is more expanded in the presence of BSA due to the penetration of biopolymer into the melatonin film, in a similar case with the drug penetration from water phase into the model membranes at the air/water interface [11-14].

In order to obtain an image of the possible interactions in the interfacial mixed film of melatonin and BSA, in Table 2, are listed the data related to the variation of the equilibrium interfacial tension with the melatonin concentration (column I), both in the absence (column II) and in the presence of BSA (column III), respectively. By comparing column II with column III, it can be seen that the presence of BSA lead to a supplementary decrease of the interfacial tension, respectively to an increase of the pressure in the adsorbed melatonin film.

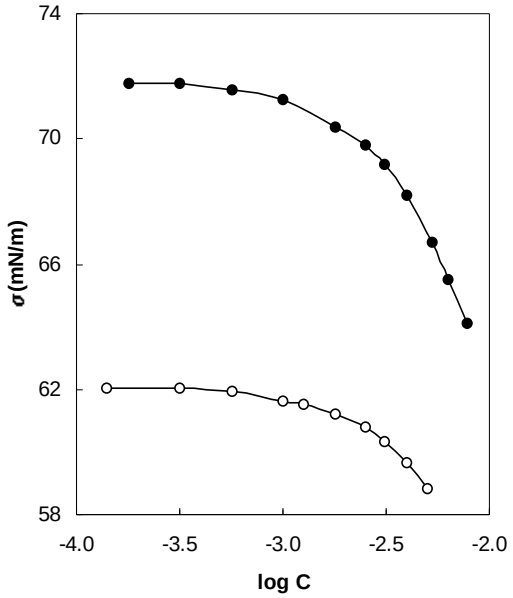

Fig. 2. *Dependence of static (equilibrium) interfacial tension, as a function of logarithm of melatonin concentration in aqueous buffer phosphate pH 7, both in the absence of bovine serum albumin (BSA) (•) or in the presence of BSA (o), at the air/water interfaces.*

Column IV contains the interfacial pressures of pure melatonin film. In colum V of the Table 2, the contributions of the BSA to the interfacial pressure of the mixed melatonin- BSA film are given, in considering the melatonin contribution (column IV) as constants. The BSA contribution was evaluated from the difference between the values in columns II and III for each melatonin concentration. It can be seen that the interfacial pressure due to BSA (column (V) is lowered as the melatonin concentration increases. In other words, the contribution of each component to the interfacial pressure of the melatonin-BSA film is not independent. This fact also suggests that the interaction between melatonin and BSA is a strong and specific one.

Furthermore, one considers the adsorption of BSA as being constant at the air/water interface with the value of σ = 62.50 dyn/cm for the interfacial tension (see column III). The last column VI of Table 2 indicates the interfacial pressure increment ($\Delta\ \pi$, dyn/cm) due to the melatonin adsorption and it is calculated with the values in column III. The increase of interfacial pressure is more pronounced as the concentration in melatonin is increased. The interaction between melatonin and BSA is thus evidenced by the decrease of the interfacial tension, respectively, by the increase of the interfacial pressure at the air/water interface.

Table 2

The effect of melatonin and of bovine serum albumin (BSA) on the surface tension and the surface pressure at the air/aqueous buffer (pH 7) interface.

Melatonin Conc. [M] 10^4	σ dyn/cm (without BSA)	σ dyn/cm (with BSA 0.4 % w/v)	π dyn/cm (for pure melatonin films)	π dyn/cm (BSA: 0.4 % w/v)	$\Delta\ \pi$ dyn/cm (melatonin and BSA)
I	II	III	IV	V	VI
0.00	72.75	62.50	0.00	10.25	0.00
1.00	71.85	62.05	0.90	9.80	0.45
3.10	71.76	62.03	0.99	9.73	0.47
5.60	71.57	61.96	1.18	9.61	0.54

Melatonin Conc. [M] 10^4	σ dyn/cm (without BSA)	σ dyn/cm (with BSA 0.4 % w/v)	π dyn/cm (for pure melatonin films)	π dyn/cm (BSA: 0.4 % w/v)	$\Delta\ \pi$ dyn/cm (melatonin and BSA)
10.00	71.28	61.64	1.47	9.64	0.86
17.70	70.58	61.21	2.17	9.37	1.29
31.00	69.20	60.31	3.55	8.89	2.19
39.00	68.22	59.67	4.53	8.55	2.83
50.00	66.72	58.85	6.03	7.87	3.65

Consistent with our data are the findings on the interaction of bovine serum albumin with various soluble surfactants [15–17] showing a good stability of molecular associations of surfactants to BSA.

The mechanism of specific interaction between melatonin and BSA is unknown. Melatonin probably interact with the hydrophobic regions of the protein and also with the hydrophilic groups of the protein, taking into account its hydrophilic and hydrophobic structure (Fig.1). Consequently, the melatonin molecules might be covalently attached to proteins. These experimental findings should bring insights on the binding of soluble compounds to biomembranes and might simulate and explain some biomembrane phenomena [18].

Taking into account that melatonin decreases significantly the surface tension of BSA adsorbed films at fluid interfaces, our data are, certainly, an important contribution to the knowledge of the interfacial action of melatonin on bio-macromolecules stabilizing their interfacial supramolecular structures. This binding of melatonin with macromolecules is likely to be of a physiological significance, but future studies will be design to systematically evaluate the melatonin capacity to stabilize the cellular membranes.

CONCLUSIONS

Melatonin has a hydrophilic and a hydrophobic character, possessing a methoxy group in the 5-position and an acetyl side chain that can orient the molecule at the air/water interfaces. Our findings show that melatonin associates with and increases the stability of BSA interfacial adsorbed layers. These results confirm the involvement of melatonin in specific interactions with soluble proteins.

Melatonin effect on the stabilization of biopolymers might provide a general protection of biological compounds against oxidative damage "in vitro" and "in vivo". The detailed mechanism of melatonin action on BSA interfacial self-assemblies and for its stabilizing effect is not clear at the present time and it is a research subject of future interest in our laboratories.

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