ABSTRACTS

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Potential anticancer agent hypericin and its model compound emodin: interaction with DNA

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Introduction

Hypericin (HYP) (Fig. 1a) is a natural photosensitizing pigment occurring in plants of the genus *Hypericum.* HYP under light illumination displays anti-proliferative and cytotoxic effects on many tumour cell lines[1, 2]. These properties together with minimal dark toxicity, tumour selectivity and high clearance from the host body make HYP a very promising agent in photodynamic therapy of cancer. Due to its prospective pharmaceutical utilization, this interesting molecule has been the subject of investigation of many scientific research groups[3–6].

Our team has been dealing with the interaction of HYP and its derivatives (such as emodin, quinizarin, danthron, alizarin) with important bio macromolecules to better understand a drug's mechanism of action.

The interaction of the drug with linear calf thymus DNA is the main aim of this contribution. The binding constant and interaction mode have been determined by spectrophotometric methods. The model compound of HYP, emodin (E) (Fig. 1b), was considered in our study for its structural similarity with the HYP molecule. In addition, emodin, a plant derived anthraquinone, was found to be a photosensitizer which possess anti-tumour, anti-bacterial, anti-viral, anti-inflammatory, and myorelaxing activities[7, 8]. Detailed knowledge of the interaction of HYP and E with the DNA at the molecular level is extremely important in determination of their distribution and therapeutic effect in the body.
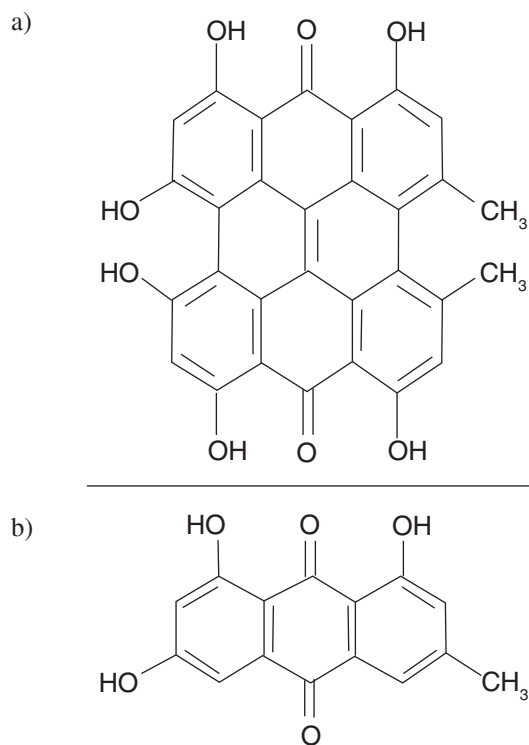
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Fig. 1. Chemical structure of hypericin (a) and emodin (b)

Experimental methods

Hypericin was purchased from KRD (Slovakia), emodin and high polymerized calf thymus DNA were obtained from Sigma-Aldrich (Germany). Stock solutions of HYP (10^{-4} mol/l) and E (10^{-3} mol/l) were prepared in DMSO, which was acquired from Sigma-Aldrich (Germany). Calf thymus DNA ($2 \cdot 10^{-3}$ mol/l) was dissolved in TE buffer (10 mmol/l Tris and 1 mmol/l EDTA), pH = 7.4 by stirring at 4 °C.

Binding constants were determined by fluorescence (fluorimeter RF – 5301 PC Shimadzu) and absorption spectrophotometry (an absorption spectrophotometer UV-VIS 2401 PC Shimadzu) using equilibrium receptor-ligand binding analysis by Copeland[9].

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We used an influence of the drug on thermal denaturation of DNA to determine the mode of its interaction with the macromolecule. For this purpose, an absorption spectrophotometer Specord UV-VIS Analytik Jena connected with Peltier module was used. This experimental arrangement enables continuous increasing of temperature with step 1 °C per minute. Temperature range of measurement was 20–90 °C. Absorption curves of samples were recorded every 2 minutes. The samples were filtrated on MillexGV (0.22 μm) before measurement. Melting curves were fitted by Van't Hoff equation:

$$A = A_{min} + \frac{A_{max} - A_{min}}{1 + e^{[\frac{\Delta H}{R} \times (\frac{1}{T} - \frac{1}{T_m})]}}$$

where A absorbance, A_{min} minimal measured absorbance, A_{max} maximal measured absorbance, ΔH enthalpy of transition, R gas constant, T temperature, T_m melting temperature.

All spectra and figures were evaluated in Origin 6.0 and 8.0 and Grafit programs.

Results and discussion

Binding constants express the strength of interaction between macromolecules and ligands[9]. We used fluorescence spectroscopy for a measurement of dependence of HYP (10^{-6} mol/l) fluorescence intensity on DNA concentration (10^{-8} – 3 · 10^{-5} mol/l) to determine binding constants of the complexes HYP/DNA. Figure 2a shows experimental data fitted by Langmuir isotherm, which gives the equilibrium dissociation constant. Semi-logarithmic graphical representation of equilibrium binding analysis was also used in our calculation to find a more exact binding constant value (Fig. 2b). Considering both graphical approaches, the binding constant of the HYP/DNA complex has been appointed as 4.0 · 10^4 l/mol. Comparing with other drugs which are typical DNA

intercalators such as actinomycin D (binding constant K_b = 1.9 · 10^6 l/mol)[10] or mitoxantron (K_b = 3.9 · 10^5 l/mol)[11] our obtained value does not point to an intercalation mode of the DNA – HYP interaction. In addition, the HYP derivatives danthron and quinizarin interacting with DNA by electrostatic interactions[12] have similar binding constant as HYP[13]. Moreover, another model compound of HYP – emodin has been examined by fluorescence and absorption spectroscopy (see below) and the value of its measured binding constant and thermodynamic parameters point to a non-intercalative mode of interaction with DNA.

The most probable binding sites in the DNA structure for HYP molecule were found by Raman spectroscopy[14]. Vibrational spectra HYP in a complex with polynucleotides show that N7 of purines, preferentially guanine, is included into the interaction. These findings and the value of the binding constant lead us to the supposition of the groove binding mode of interaction.

Model compound of HYP emodin is a molecule which can serve for a better understanding of the binding effect of more complex drugs on macromolecules such as DNA or transport proteins. Fluorescence and absorption spectroscopy were used to measure the binding constant of E/DNA complex, which was 8.1 · 10^4 l/mol.

Fluorescence quenching of emodin as a result of adding DNA, formation of the non-fluorescence complex E/DNA and the value of the binding constant can lead us to assume that emodin interacts with DNA by a non-intercalative but groove binding mode of interaction.

Absorption analysis has confirmed our results obtained by fluorescence measurements. Hyperchromicity and small bathochromic shift of the absorption maximum (not shown) indicate that E and DNA molecules interact through electrostatic forces or hydrogen bindings[15]. Bond by electrostatic forces can be excluded since the value of the binding constant indicates that it is the interactions through hydrogen bonds that are associated with binding to the groove.

In addition, we proved our results by denaturation of pure DNA and E/DNA complexes in ratios 1/1, 2/1, 3/1
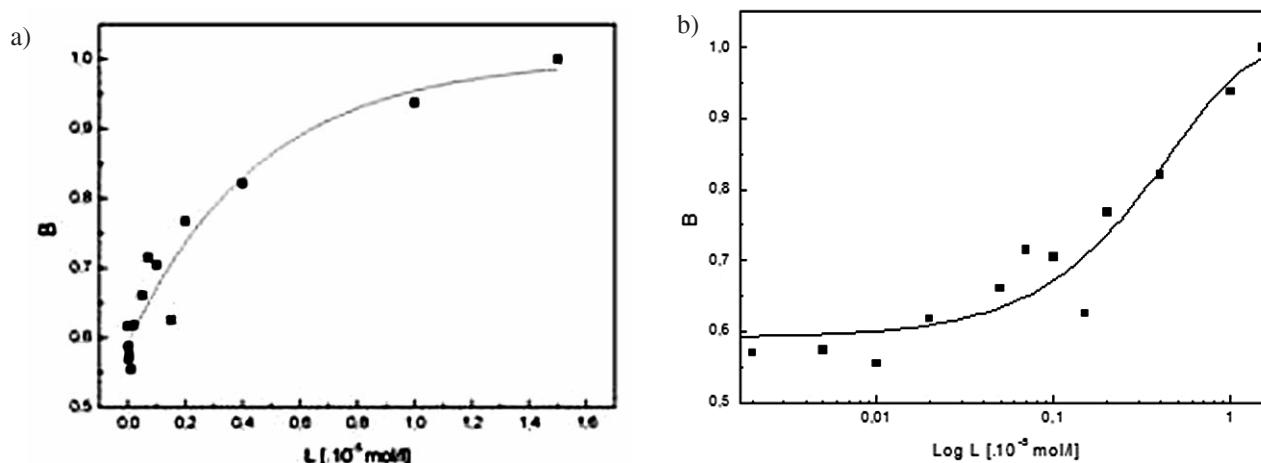
Fig. 2. Langmuir isotherm of HYP/DNA complex (a). L represents the concentration of DNA and B is normalized HYP fluorescence intensity (excitation wavelength: 560 nm). Concentrations are mentioned in the text. Semi-logarithmic representation of binding curve (b). Final binding constant was taken as an average of two ones obtained from a, and b.

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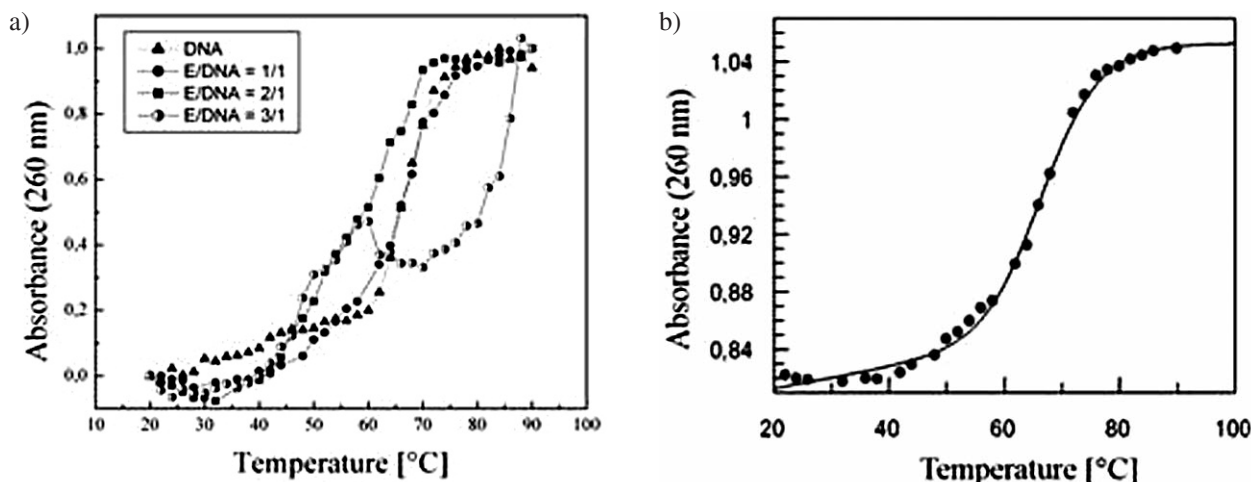
a)

b)

Fig. 3. Normalized melting curves of pure DNA and complexes E/DNA in ratios 1/1, 2/1, 3/1. DNA concentration: $3 \cdot 10^{-5}$ mol/l, emodin concentration: $3 \cdot 10^{-5}$ mol/l – $9 \cdot 10^{-5}$ mol/l (a). Illustration of Van't Hoff fit for complex E/DNA in ratio 1/1 (b).

Table 1. Thermodynamic characteristics determined from the melting curves of pure DNA and the complexes E/DNA in concentration ratios 1/1, 2/1, 3/1

	1st phase					2nd phase	
	T_m (°C)	T_1 (°C)	T_2 (°C)	ΔT (°C)	ΔH (kJ)	T_m (°C)	ΔH (kJ)
DNA	67,5	56,6	75,1	18,5	388		
1/1	66,8	51,7	77,1	25,4	190		
2/1	57,0	36,9	75,5	38,6	126		
3/1	46,7	35,8	59,4	23,6 for AT pairs	325	94,4	121 for GC pairs

by absorption spectroscopy connected with a Peltier module. Melting curves that can be seen in Figure 3a show a destabilizing effect of the drug on calf thymus DNA. They were fitted (Fig. 3b) using Van't Hoff equation to obtain main thermodynamic characteristics (T_m, ΔT, ΔH).

Melting temperature T_m is shifted from 67.5 °C (pure DNA) to 57 °C for the complex E/DNA in 2/1 concentration ratio (Table 1). Also Van't Hoff enthalpy ΔH is decreasing from 388 kJ (pure DNA) to 126 kJ (E/DNA = 2/1), which means that lower energy is needed for denaturation of 50% base pairs of DNA in the complex with emodin. The changes in all thermodynamic parameters lead us to a claim that emodin destabilizes the calf thymus DNA molecule and this destabilization is associated with the groove binding interaction mode[16]. Our findings can be supported by the fact that an intercalative mode of interaction manifests in thermal stabilization of the DNA molecule due to drug binding[16].

It is generally supposed that small molecules like emodin bind preferentially into the minor groove of DNA, especially to regions which are rich in AT base pairs[17].

In our experiments the melting curve of the complex E/DNA in 3/1 (Fig. 3a, Table 1) shows a two-phase character with an expressive destabilization of AT regions in DNA.

Conclusion

The binding constant values of the potential anticancer drugs hypericin and emodin lead us to assume that these molecules do not bind into the DNA structure by an intercalative mode of interaction.

The emodin influence on denaturation of the DNA macromolecule points to the groove binding mode of interaction and the incorporation of the drug takes place probably into the DNA minor groove by hydrophobic or hydrogen interactions. Shahabadi et al.[18] showed that the antidiabetic agent metformin with the binding constant of $8.3 \cdot 10^4$ l/mol binds into the minor groove in the DNA structure. These findings can support our results.

Considering the binding characteristics of emodin as a model compound of hypericin, we can suppose that hypericin binds into the DNA major groove. Results obtained by Kočišová[14] and the largeness of the hypericin molecule lead us to this assumption.

Conflict of interest: none.

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