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Quantification of salts and cosolvents–DNA interactions in terms of free energies: A study using the pyren-1-carboxyaldehyde as fluorescent probe

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ABSTRACT

Using pyren-1-carboxyaldehyde as a fluorescent probe, alcohol and salt–DNA interactions have been studied and quantified in terms of free energies of binding. This quantification implies the determination of the "*true*" free energy of binding of the probe, after correction through solubility measurements of the equilibrium binding constant of the probe to DNA. According to results, the interaction of alcohols is indirect and is based on the changes that they cause in the structure of water, changes which in turn alter the degree of hydration of DNA. On the other hand, salts interact with DNA through coulombic forces. A linear correlation between the free energy of binding for the different salts and the valency of the cations has been found.

1. Introduction

As it is known, biomolecule–ligand interactions are related to processes of great importance such as enzymatic catalysis [1], heterogeneous catalysis [2], molecular machines [3], molecular electronics [4], trapping of substrates by polyelectrolytes [5] and conformational changes in DNA induced by the binding of solutes [6]. In the case of DNA processes, such as gene therapy [7] or gene transfer [8], they also depend on the interaction of DNA with specific ligands (for example with surfactants [9], but not only with these ones).

The interaction of ligands with a biomolecule depends on the environment of the biomolecule and the ligand because such environment affects the conformational state of the biomolecule and, consequently, its ability to interact with the ligands. On the other hand, the relative free energy of the free and bound ligands also depends on the media. Consequently, a study of the medium effects (salts and alcohols) on the binding ability of biomolecules seems an interesting study. In this line, this paper presents a study of the influence of changes in the environment of DNA caused by the addition of salts and cosolvents (alcohols) on the strength and characteristics (cooperativity) of the binding of a small ligand, the pyren-1-carboxyaldehyde. Using this ligand the measure of the ability of DNA binding is easy, due to changes in the fluorescence intensity of the ligand when it is incorporated into DNA. Moreover, from the data corresponding to the binding of pyrene one can obtain information on the interaction of DNA with cosolutes (salts and alcohols).

According to our results the effect of alcohols on DNA is essentially indirect: alcohols influence the DNA through the changes that they produce on the structure of water, and therefore in the solvatation of the DNA. In the case of salts a direct salt–DNA interaction is found (more specifically the cation of the salt, of course). This interaction has been quantified by following the procedure developed in a previous work [10].

2. Experimental methods

2.1. Materials

Calf thymus DNA was purchased from Pharmacia and used without further purification, because preliminary experiments showed that purification does not produce any changes in the results of experiments. The average number of base pairs by DNA molecule is ca. 3000 [11]. Polynucleotide concentrations were determined spectrophotometrically from the molar absorptivity ($6600\ mol^{-1}\ dm^3\ cm^{-1}$ at 258 nm in order to have the DNA concentration in phosphate units) [12]. $[Co(NH_3)_6](Cl)_3$ was prepared and purified according to published procedures [13]. The other reagents were all Anala. R. grade and were used as purchased. The water used in the preparation of solutions had a conductivity less than $10^{-6}\ S\ m^{-1}$. All the solutions in the presence of salts contained ethanol 8% (in weight) in order to favour the solubility of the pyren-1-carboxyaldehyde.

2.2. Methods

(a) Fluorescence measurements were carried out in a spectrofluorimeter (Hitachi f-2500), interfaced to a PC for the reading

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and handling of the spectra, at 298.2 K. Intensity measurements were performed at [probe] = 5×10^{-7} mol dm^{-3}. The excitation wavelength was 356 nm and the emission wavelength was dependent on the percentage of alcohol. It was checked that the results were independent of the excitation wavelength, provided that this one was in the range from 300 to 425 nm. DNA concentrations ranged from 10^{-5} mol dm^{-3} to 10^{-3} mol dm^{-3} in phosphate units. The temperature was maintained at 298.2 ± 0.1 K.

(b) Solubilities of the fluorescent probe in solutions containing different salts and alcohols were measured by agitating a generous excess of solid with the appropriate solution in a thermostatted (298.2 K) vessel. After a long time for undissolved solids to settle, an aliquot of the saturated solution was removed using a pre-thermostatted pipette and the solution diluted as necessary. Concentrations were measured spectrofluorimetrically. Alcohol and salts concentrations corresponding to solubility measurements are given in Table 1.

3. Results and discussion

Pyrene and its derivatives are less emissive when they are bound to a DNA duplex. According to Nakamura et al., this circumstance arises from the intercalation of pyrene between the base pairs of the duplex [14]. This behaviour is clearly illustrated in Fig. 1, which gives the intensity of emission of pyren-1-carboxyaldehyde versus DNA concentration, for solutions containing 16% of ethanol. Similar behaviour was also observed in the presence of salts and for the other alcohols studied. Experiments were performed at fixed concentrations of the probe and the given concentration of salt or alcohol, and changing, as in the case of Fig. 1, DNA concentration.

In all the cases it was found that the intensity, I, can be fitted to the equation:

$$I = \frac{I_0 + I_{DNA} K[DNA]}{1 + K[DNA]} \quad \text{(a)}$$
$$K = K_{max} \frac{e^t}{1+e^t} \quad \text{(b)} \qquad (1)$$
$$t = \frac{[DNA] - h}{j} \quad \text{(c)}$$

The meaning of these equations is the following: in the presence of DNA, pyrene is partially bound to it:

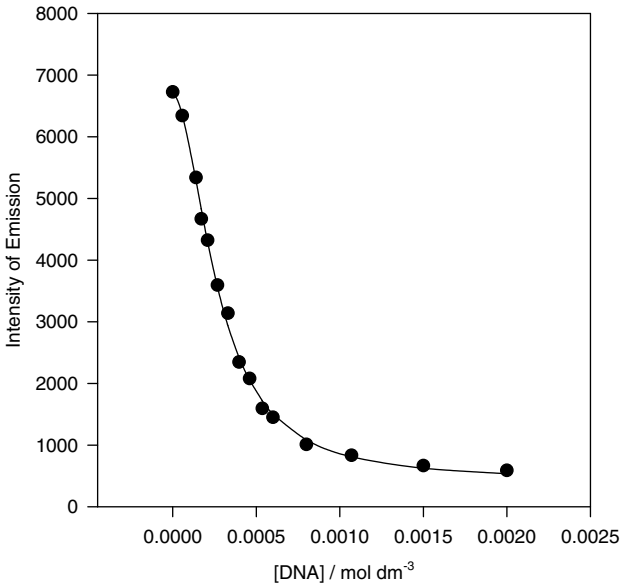

Fig. 1. Intensity of emission of pyren-1-carboxyaldehyde vs. DNA concentration corresponding to ethanol at 16%.

$$\underset{\downarrow I_0}{Py} + \underset{}{DNA} \overset{K}{\rightleftharpoons} \underset{\downarrow I_{DNA}}{Py/DNA} \qquad (2)$$

The free pyrene emits with intensity I_0, and the bound pyrene with intensity I_{DNA}, in such a way that the observed emission is the average given by Eq. (1a). On the other hand, Eq. (1b) shows that the binding constant, K, depends on the relation [pyrene]/[DNA]. In fact, as a fixed [pyrene] was used in our experiments, K is only dependent on [DNA]. It is worth pointing out that this behaviour has been observed in other cases when small ligands bind to DNA [15] and when conformational changes of DNA are present [10]. According to Eq. (1b), K increases as [DNA] does so. This implies that the binding of pyrene to DNA is anti-cooperative. The sigmoidal dependence of K on [DNA] is, in fact, frequently found for anti-cooperative processes [16]. In Eq. (1b), K_{max} represents the maximum (limiting) va-

Table 1
Values of solubility (mol dm^{-3}) of pyren-1-carboxyaldehyde corresponding to different concentrations of alcohols and salts

X_m ethanol	10^6 solubility	X_m 2-propanol	10^6 solubility
0.0201	0.86	0.0221	2.12
0.0264	1.21	0.0254	2.76
0.0328	1.87	0.0291	3.73
0.0693	9.27	0.0382	7.25
0.1141	66.00	0.0559	35.80
0.1430	167.00	0.0800	254.00
0.2310	1350.00	0.0939	984.00
X_m *tert*-butanol	10^6 solubility	10^3 [$NaClO_4$]/mol dm^{-3}	10^6 solubility
0.0078	1.65	10.0	4.46
0.0126	1.61	28.0	3.83
0.0207	3.69	125.0	3.00
0.0362	19.39	250.0	3.11
0.0506	93.07	500.0	4.39
0.0655	498.50	1000.0	12.20
10^4 [$BaClO_4$]/mol dm^{-3}	10^6 solubility	10^6 [$Co(NH_3)_6Cl_3$]/mol dm^{-3}	10^6 solubility
0.5	4.00	5.0	2.30
1.0	3.86	6.0	2.27
5.0	3.41	7.5	2.25
10.0	3.10	10.0	2.22
50.0	2.34	20.0	2.13
100.0	2.04		