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Distribution of urocanic acid isomers between aqueous solutions and *n*-octanol, liposomes or bovine serum albumin

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Abstract

The distribution of urocanic acid (UCA) isomers between aqueous solutions and *n*-octanol, egg yolk phosphatidylcholine (eggPC) liposomes or bovine serum albumin (BSA) has been evaluated. Regarding its partitioning between water and *n*-octanol, the behaviour of both isomers is very similar, and the amount incorporated to the organic solvent is mostly determined by the fraction of the compound that, in the aqueous phase, is present as uncharged species. This implies that the highest hydrophobicity occurs near the isoelectric point.

cis- and *trans*-UCA are readily incorporated into eggPC unilamellar liposomes. A simple pseudophase treatment of ultrafiltration data renders a binding constant of 0.20 ± 0.04 mL/mg for the trans isomer at pH 7.4. The binding constant decreases, by a factor two, at pH 5.0, suggesting that the negatively charged species is more favourably bound to the liposomes than the neutral species, which is mostly present as zwitterions. The *cis*-isomer, at both pHs, is less incorporated to the bilayers.

trans-UCA and *cis*-UCA readily bind to BSA at pH 7.4, with binding constants of 3400 M^{-1} and 6900 M^{-1}, respectively. This result suggests that, as in the octanol/water partitioning, hydrophobic interactions predominate and the degree of binding is determined by the fraction present as uncharged species. A smaller binding constant at pH 5.0 indicates that the charge of the protein is also plying a relevant role.

Keywords: Urocanic acid; Octanol/water distribution; Liposomes; Bovine serum albumin

1. Introduction

Urocanic acid (UCA) is present in the stratum corneum of the epidermis as the *E*-isomer [1]. Upon exposure to UVB radiation, it converts to *Z*-UCA, a compound recognized as immunosuppressor [2] (see Scheme 1). UCA is stable in the skin, but *E*-UCA is metabolized in the liver by the enzyme urocanase, an enzyme that does not recognizes *Z*-UCA. Accumulation of this isomer is mainly controlled by its excretion in sweat. The transport of UCA through the skin and from the skin to the liver is determined by its capacity to penetrate and accumulate in cell membranes and its plasma solubility. This solubility could be modulated by the capacity of UCA isomers to be associated to drug carrying serum albumin. However, very few studies have been addressed to evaluate these properties for both isomers. The protonated and deprotonated forms are extremely soluble in water, while *E*-UCA is not very soluble at its isoelectric point (pH 5). The *cis* (*Z*) isomer is an order of magnitude more soluble that the *trans* (*E*) isomer, both in organic and aqueous solvents [1]. However, no comprehensive study of their partitioning between different solvents has been reported.

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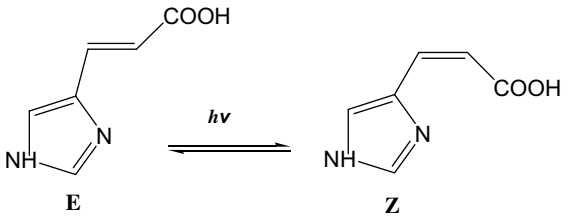

Scheme 1.

Other relevant properties of urocanic acids that can modulate their photoisomerization, distribution and transport are their association to biomolecules and lipidic bilayers. This non-covalent pre-association can also notably increase UCA photobinding to biomolecules *in vivo* [1]. Although evidence of UCA–DNA interaction has been provided [3], no indication of association to native or heat denatured DNA has been detected employing radiolabeled UCA [4]. Similarly, no significant non covalent association between *E*-UCA and BSA has been detected in dialysis experiments [5]. On the other hand, Schwazinger and Falk have reported that the presence of HSA significantly affects the photostationary *trans–cis* distribution [6], and it has been suggested that interaction of UCA with epidermal proteins contribute to a 10 nm bathochromic shift in stratum corneum preparations, increasing so the photoisomerization rate elicited by solar-like irradiations [7].

Data regarding the incorporation of UCA to membrane mimetic systems is also very scarce. This type of data is also of relevance since it has been shown that the efficiency of *trans*-UCA photoisomerization linearly correlates with solvent polarity [8]. Furthermore, transport of UCA is modulated by its capacity to penetrate membranes [9,10]. Barclay et al. have shown that 0.5 M SDS allows to obtain UCA concentrations up to 0.1 M in buffer, pH 7.4, at 37 °C [11]. Sirieix-Plénet et al. have reported the incorporation of *E*- and *Z*-UCA amphiphiles to monomolecular films of DPPC and cholesterol [12]. In the present communication, we report data bearing on the distribution of UCAs between aqueous solutions and *n*-octanol and on their capacity to interact with eggPC liposomes and BSA.

2. Materials and methods

E-UCA was a Sigma product employed as received. No significant impurities were detected by HPLC. Photolysis of *E*-UCA solutions, kept under nitrogen, afforded a mixture of *E*- and *Z*-UCA isomers without significant formation of secondary products. Kept in the dark at low temperature, these solutions remained stable for several days.

2.1. n-Octanol/water partition coefficient

Distribution of UCA between aqueous and octanolic solutions was evaluated by a hand-shaking procedure at 20 ± 1 °C. Solutions containing 0.1–1 mM UCA (mixture of *E*- and *Z*-isomers) were shaken with *n*-octanol for several minutes. Citrate (pH 3.7), citrate–phosphate (pH 5), phosphate (pH 6.8–7.4), or sodium tetraborate, (pH 8.33–9.18), were employed as buffers. The results were independent of the buffer concentration or NaCl (100 mM) addition. After centrifugation, aliquots of both phases were injected to the HPLC and measured the area corresponding to each isomer. The analysis was carried out in a Waters 600 system, equipped with a Waters PDA detector set at 276 nm. A Cyclobond column was employed with acetonitrile: water: phosphate buffer, pH 5.0 (85:10:5 V/V) as mobile phase. The octanol/water partition constant of each isomer was obtained from

$$K_p = [UCA]_{octanol}/[UCA]_{water} \quad (1)$$

2.2. Incorporation to egg yolk phosphatidylcholine (egg-PC) large unilamellar liposomes

Large unilamellar liposomes of nominal diameter 400 nm were prepared by extrusion of multilamellar egg-PC liposomes through polycarbonate membranes (Nucleopore), following the procedure previously described [13]. The multilamellar liposomes were prepared by re-suspending in buffer phosphate (10 mM, pH 5.0 or 7.4) the lipid deposited in the bottom of 50 mL test glass tubes. Deposition was achieved by evaporation in a nitrogen gas stream a solution of the lipids in chloroform (100 mg in 800 μL).

Liposome suspensions (0.5 and 1.0 mg/mL) and mixtures of *E*- and *Z*-UCA (from 5 to 40 μM) were incubated at 37 °C during 30 min. After equilibration, the solutions were ultrafiltered through membranes (cut off at PM 10000; Milipore PM-10). The concentration of UCA was determined by HPLC in the ultrafiltrated and in the original solution. Blanks without liposomes were carried out in order to disregard significant adsorption of UCA to the membranes. Partition constants for a given isomer were evaluated as

$$K_p = ([UCA]_{total} - [UCA]_{free})/([UCA]_{free}[lipids]) \quad (2)$$

that considers the liposome ensemble as a pseudophase. The use of this equation is justified by the fact that, in the above described experimental conditions, the amount of lipids is considerably larger than that of UCA.

Mixtures of isomers were obtained by irradiation of *E*-UCA solutions with a UV-B lamp under nitrogen. The concentrations of each isomer was determined by HPLC. A C-18 μBondapak Waters (3.9 × 300 mm) column and acetonitrile:buffer phosphate 10 mM, pH 7.4 (5:95 V:V) as mobile phase (1 mL/min) were employed in the analysis.

2.3. Association of UCA to bovine serum albumin

The extent of association of *E*- and *Z*-UCA to BSA was evaluated in micro ultrafiltration experiments. A mixture of *E*- and *Z*-isomers was passed through a Microcom membrane and the free acids determined by HPLC in the ultra-