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Circular dichroism study of the interaction between mutagens and bilirubin bound to different binding sites of serum albumins

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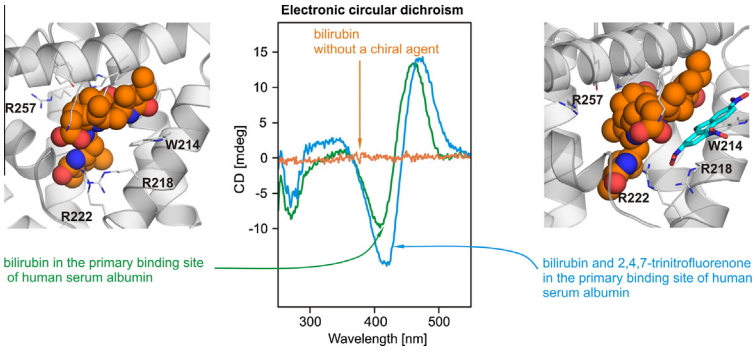
HIGHLIGHTS

- Mutagens with bilirubin bound to serum albumins approximated blood serum.
- Bilirubin bound to primary or secondary sites interacts differently with mutagens.
- 2,7-Diaminofluorene does not interact with bilirubin bound to primary site.
- Studied mutagens partially displaced bilirubin from the secondary binding sites.
- Ligand docking reveals an arrangement of the both bilirubin and mutagens.

GRAPHICAL ABSTRACT



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ABSTRACT

Although recent investigations have shown that bilirubin not only has a negative role in the organism but also exhibits significant antimutagenic properties, the mechanisms of interactions between bilirubin and mutagens are not clear. In this study, interaction between bilirubin bound to different binding sites of mammalian serum albumins with structural analogues of the mutagens 2-aminofluorene, 2,7-diaminofluorene and mutagen 2,4,7-trinitrofluorenone were investigated by circular dichroism and absorption spectroscopy. Homological human and bovine serum albumins were used as chiral matrices, which preferentially bind different conformers of bilirubin in the primary binding sites and make it observable by circular dichroism. These molecular systems approximated a real system for the study of mutagens in blood serum. Differences between the interaction of bilirubin bound to primary and to secondary binding sites of serum albumins with mutagens were shown. For bilirubin bound to secondary binding sites with low affinity, partial displacement and the formation of self-associates were observed in all studied mutagens. The associates of bilirubin bound to primary binding sites of serum albumins are formed with 2-aminofluorene and 2,4,7-trinitrofluorenone. It was proposed that 2,7-diaminofluorene does not interact with bilirubin bound to primary sites of human and bovine serum albumins due to the spatial hindrance of the albumins binding domains. The spatial arrangement of the bilirubin bound to serum albumin along with the studied mutagens was modelled using ligand docking, which revealed a possibility of an arrangement of the both bilirubin and 2-aminofluorene and 2,4,7-trinitrofluorenone in the primary binding site of human serum albumin.

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Abbreviations: AF, 2-aminofluorene; BR, bilirubin; BSA, bovine serum albumin; CD, circular dichroism; DAF, 2,7-diaminofluorene; HSA, human serum albumin; SA, serum albumin; TNF, 2,4,7-trinitrofluorenone; UV–vis, ultraviolet–visible.

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Introduction

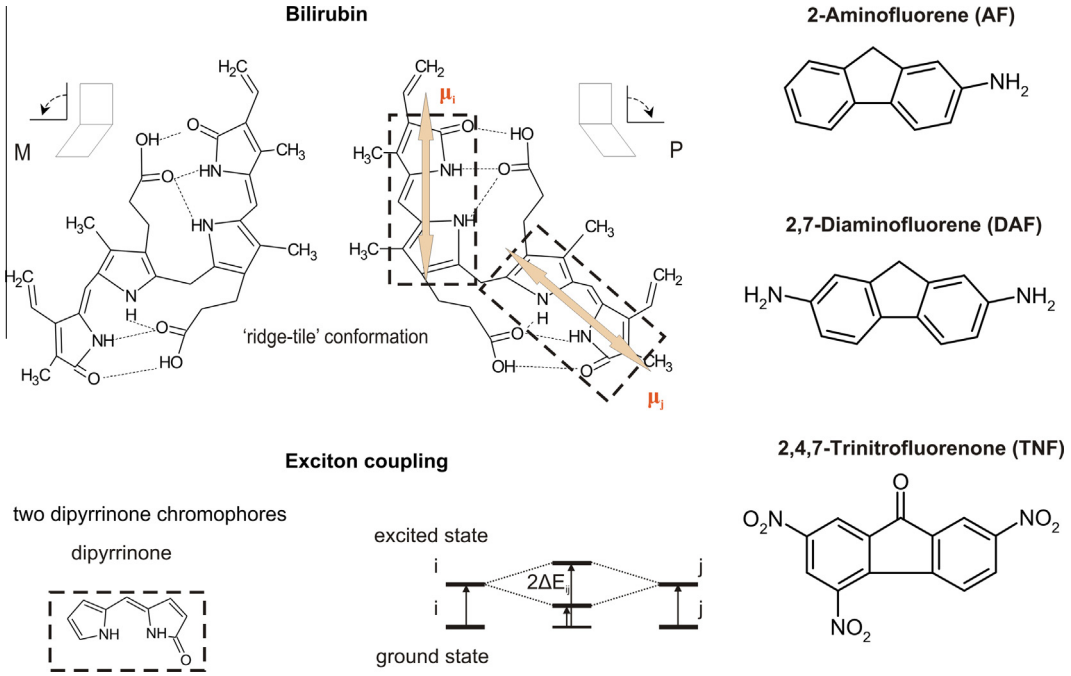
Bilirubin (BR) is an orange yellow pigment of bile, secreted by the liver through a catabolic process. In the first step of heme catabolism it degrades to biliverdin where the enzyme heme oxygenase has caused cleavage of the heme ring. Subsequently, BR is formed by a reduction of biliverdin [1–3]. The main part of the total pigment amount in mammals is bound to transport proteins, mainly to serum albumin (SA). SA binds BR by the formation of noncovalent complexes based on several weak interactions. Such BR is called unconjugated bound BR [4–7].

Previously, it was believed that BR had a negative role on the human organism. BR is regarded as neurotoxic, especially for newborn infants [8]. However, in recent studies it has been shown that bile pigments are not simple by-products of heme catabolism, but also physiologically important molecules which exhibit significant antimutagenic [9–11], antioxidant [1,7,12] and anticomplement [13,14] properties. It was suggested that the antimutagenic activity of the bile pigments is closely related to their 3D structure and type of mutagens [11], but there are no detailed structural studies explaining the mechanism of the interaction between BR and mutagens.

BR in solution adopts a ridge-tile structure existing as two isoenergetic M- and P-helical conformers where the angle between two dypyrrione groups is about 100°, which is stabilized by the presence of intramolecular hydrogen bonds (Scheme 1) [2,15–17]. BR easily racemizes in an isotropic solution and therefore is not optically active. Earlier, human (HSA) and bovine serum albumins (BSA) were used as chiral matrices [18–22], which selectively recognize one of the pigment enantiomers and cause BR to become optically active. The 3D structure of HSA has been determined by X-ray crystallography [23]. It has been suggested that there are two types of binding sites on albumin, one primary binding site of high affinity and two sites of 2–3 orders of lower affinity, which were associated with the subdomains IIA, IB and IIIA [23–26]. The number of binding sites of HSA/BR and BSA/BR and stereoselective preference to bind M- or P-conformers of BR were confirmed in our previous work by chiroptical spectroscopy [27,28]. Until recently, BR bound to the secondary binding sites was considered as not physiologically important because the concentration of SA is about 40 times higher when compared to BR in human plasma [17,29] and therefore BR occupies the high affinity primary binding site. However, the primary binding site can be blocked by other ligands (drugs) which may lead to binding of BR at the secondary sites. Therefore knowledge of the possible interaction between mutagens and bile pigments bound at all binding sites is becoming important. Such a system composed of bile pigments and SAs as matrices approximates a real system for the study of mutagen in blood serum. Considering the optical activity of these complexes, it is possible to characterize them by circular dichroism spectroscopy. Electronic circular dichroism (CD) is a method inherently sensitive to the structure of chiral substances. In combination with molecular docking of both the mutagen and BR, this technique can be useful to reveal the connections between the 3D structure of BR and its antimutagenic effects.

In this paper, we have attempted to clarify the mechanism of interaction between BR bound at different albumin binding sites with the structural analogues of mutagens, aromatic amines 2-aminofluorene (AF) and 2,7-diaminofluorene (DAF) and polycyclic nitroketone 2,4,7-trinitrofluorenone (TNF) (Scheme 1). Aromatic amines AF and DAF, the compounds that form during the distillation of a tar, pyrolysis of proteins, or preservation of wood, are among the most intensively studied of all chemical mutagens and carcinogens [30]. They are not mutagenic per se but products of their sequential N-oxidation are responsible for DNA adduct formation and have been implicated as a critical reactive metabolite in fluorene-induced liver tumours [30]. TNF, a direct-acting mutagen, is a major component of a toning formulation that was used widely in certain photocopy processes [11].

The studied compounds are tightly connected with the antimutagenic properties of the bile pigments [10,11]. The results of this work can be a useful source for the development of methods usable in medical application in the area of mutagens.



Scheme 1. The interconverting, intramolecular hydrogen-bonded enantiomeric ridge-tile conformers of bilirubin IX alpha (BR). The relative orientations of the electric-dipole transition moments of the two dipyrrinone units are shown by the orange arrows. The CD couplets typical for the M- and P-structure are shown. The chemical structures of the model mutagens: 2-aminofluorene (AF), 2,7-diaminofluorene (DAF); and 2,4,7-trinitrofluorenone (TNF) are shown on the right side of scheme.

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