Bioorganic Chemistry 36 (2008) 252–254

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Preliminary Communication

Enantiodiscrimination of bilirubin-IXα enantiomers in biomembrane models: Has chirality a role in bilirubin toxicity?

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ARTICLE INFO

Article history:
Received 27 February 2008
Available online 1 July 2008

Key words:
Bilirubin
Deracemization
Bio-membrane model
Chiral recognition
Enantioselection
Cicular dichroism

ABSTRACT

Simple biomembrane models, namely micellar aggregates formed by enantiopure sodium *N*-acylprolinates, are able to convert the racemic mixture of bilirubin-IXα into an enantiomerically enriched mixture, thus suggesting a possible role of chirality in bilirubin toxicity due to the perturbation of neuron membrane dynamics. The length of alkyl chain does not influence the extent of equilibrium displacement, however, it affects the conformation of bilirubin, thus confirming the role of lipid structure in the membrane/bilirubin interaction, and suggesting a non-superficial main site of association.

1. Introduction

Bilirubin is a yellow-orange lipophilic bile pigment produced in mammals by catabolism of heme proteins, principally from haemoglobin of red blood cells. It is an unsymmetrically substituted linear tetrapyrrole dicarboxylic acid consisting of two dipyrrole halves joined by a methylene group. However, the most stable conformation in solution is a folded ridge-tile structure stabilized by intramolecular hydrogen bonds [1]. The engagement of the polar groups in the hydrogen bonds renders the moiety lipophilic and has important implications for biological and methabolic functions. In fact, due to its poor solubility in aqueous media, bilirubin is intrinsically unexcretable by living organisms where it is transported to the liver as an association complex with albumin, and there enzymatically converted to water-soluble glucuronides that are promptly secreted into bile [1]. Impaired excretion of the glucuronides and bilirubin is manifested in jaundice. In some cases abnormal accumulation of bilirubin in the central nervous system can cause irreversible brain damages, a condition known as bilirubin encephalopathy. It has been proposed that bilirubin neurotoxicity may be due to cell death by apoptosis and that the perturbation of membrane dynamics in neurons might be an early event in the bilirubin induced apoptosis [2,3]. In other words, the perturbation of cell membrane structure, lipid polarity and fluidity, protein order, redox status, are immediate components of the apoptotic pathway triggered by bilirubin.

It is well known that the folded ridge-tile conformation of bilirubin is dissymmetric and that bilirubin is a racemic mixture of conformational enantiomers that interconvert with a barrier of 18–20 kcal/mol [4], and it is also known that displacement of the equilibrium toward one of the enantiomers takes place in the presence of chiral selectors such as albumins [5,6], chiral amines [7], cyclodextrins [8].

1.1. Bilirubin structure

Though the potential role of chirality in bilirubin methabolism had been suggested previously [8], in particular in the enzymatic glucoronidation probably involving diastereoselective complexation, at the best of our knowledge, the possibility that chiral recognition might have a role in the destabilization of membrane organization, and thus in bilirubin toxicity, has not been taken into consideration. However, the components of cell membranes are chiral and enantiopure and therefore it is possible that chiral recognition might play a role in the interaction of bilirubin with cell membrane and in its destabilization by bilirubin.

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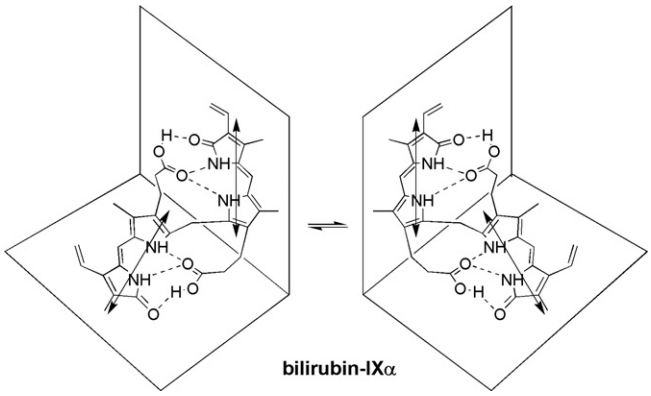
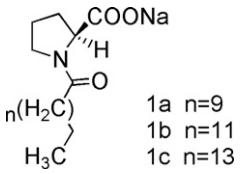
doi:10.1016/j.bioorg.2008.05.001



Here, we report a circular dichroism, CD, investigation on the chiral recognition of bilirubin in a membrane model that we have largely investigated and that features a high extent of organization [9–11], namely micellar aggregates of sodium *N*-acyl-L-prolinates, **1**. We also explored the role of the length of alkyl chain in the interaction of bilirubin with the micellar aggregates.



2. Materials and methods

Sodium *N*-acyl prolinates were prepared and purified as previously described [12].

The CD spectra of 10 μM bilirubun-IXα in aqueous solution of aggregates formed by surfactants **1** were recorded on a Jasco spectropolarimeter J-715, using a 1 cm quartz cuvette. Experiments were performed at pH 9, at the same aggregate concentration, i.e. taking into account the different critical micellar concentration (cmc), and the different aggregation number (n) [11], summarized in Table 1, therefore, aggregate concentration = ([1] − cmc)/n.

3. Results and discussion

All CD spectra of bilirubin in aqueous **1** show a conservative bisignate band of the same intensity. In this kind of system, lacking additional chromophores adsorbing in the same region of the spectrum as bilirubin, a CD bisignate band had been reasonably ascribed to the deracemization of bilirubin [9,13]. In the bichromophoric bilirubin structure, the interaction of the locally excited states of the twin dipyrrinone chromophores yields exciton splitting that, in the CD spectra, leads to two transitions with oppositely signed Cotton effects (CEs), that, however, can only be observed if one of the two enantiomers is in excess in solution. The possibility of a difference between the CD spectra of diastereomeric complexes of aggregates with equal amounts of both bilirubin enantiomers is unlike, in fact, it had been reported that spectra of diastereomeric bilirubins feature very similar intensities [13]. In Fig. 1, we report, as an example, only the spectrum of 10 μM bilirubin in aqueous 100 mM **1a**, because the position, signs and intensity of CD bands do not change with the length of the hydrophobic chains in **1a–c**, indicating that the corresponding aggregates apparently generate the same enantiomeric imbalance in terms of both molecular chirality and extent of enrichment.

The observed negative bisignate band correspond to an enantiomeri excess of the left-handed (M) enantiomer. The value of obtained CD CEs ($|\Delta\varepsilon| \sim 40$ L mol^{-1} cm^{-1}) is in the average of values (20–50 L mol^{-1} cm^{-1})more typically seen in tight, highly enantioselective heteroassociation complexes between a chiral selector and bilirubin [7,8]. The extent of deracemization cannot be determined without quantitative CD for the pure diastereomeric complex bilirubin enantiomer/aggregate, however it can be estimated ∼15% on the basis of reported data relative to a computed CD spectrum of bilirubin [7]. Note that this value is underestimated because $\Delta\varepsilon$ obtained in aggregating conditions were calculated by normalizing for the analytical concentration of bilirubin rather than for the concentration of bilirubin bound to the aggregates.

The UV–vis spectra obtained in the different aggregates (Fig. 2) suggest that bilirubin responds to variations in the micellar environment by changing its conformation (but retaining the overall chirality). In particular the UV–vis spectrum of bilirubin in aggregates of 1c**,** showing the two transition of the exciton splitting of comparable intensity, corresponds to a conformation where the dihedral angle between the dipyrrinone planes of bilirubin is $\theta \sim 100°$, *whereas* the spectra of bilirubin in **1a** and **1b** that show the higher energy transition of exciton splitting as more intense, correspond to conformations featuring smaller θ values [1]. The different conformations observed in the diverse aggregates and the dianionic form of bilirubin at the experiment pH (pH 9) are in favor of a site of binding far from the negatively charged surface of the aggregate.

On the other hand, bilirubin is scarcely deracemized by sodium *N*-acyl-L-prolinates in non aggregating conditions, in fact, a methanol solution of 10 μM bilirubin and 100 mM **1a** and an aqueous solution of 10 μM bilirubin and 100 mM sodium *N*-acetyl-L-prolinate show CD bands of only modest intensity (Fig. 3), thus demonstrating its very modest affinity for the head group fraction as such. This last result demonstrates that the deracemization observed in aqueous **1** occurs, for the most,

Table 1
Aggregation parameters of surfactants **1** measured at 298 K

Surfactant	cmc, M	n
1a	$(9.6 \pm 0.8) \times 10^{-2}$	46 ± 2
1b	$(7.1 \pm 0.11) \times 10^{-4}$	60 ± 1
1c	$(2.4 \pm 0.12) \times 10^{-5}$	84 ± 5

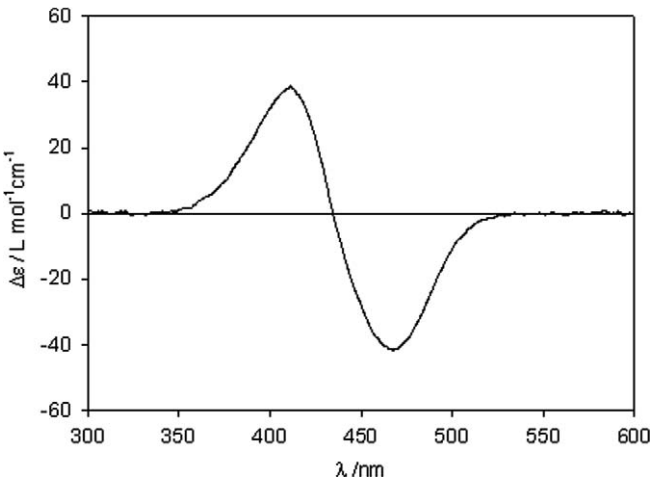


Fig. 1. CD spectrum of 10 μM bilirubin in aqueous 100 mM **1a**.

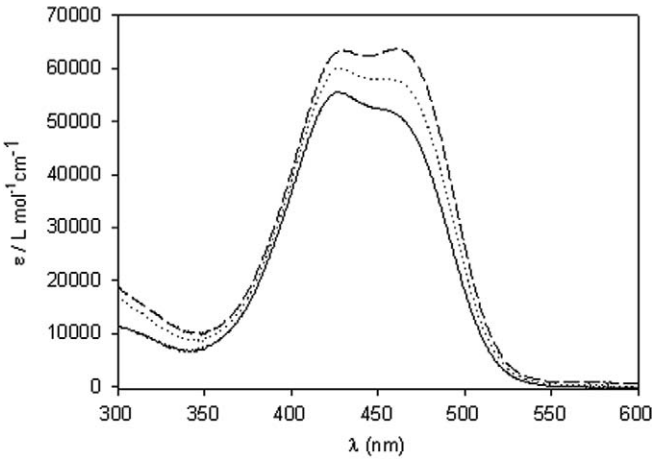


Fig. 2. UV spectra of aqueous bilirubin (10 μM) in 100 mM **1a** (solid line); 135 mM **1b** (dotted line); 189 mM **1c** (dashed line).

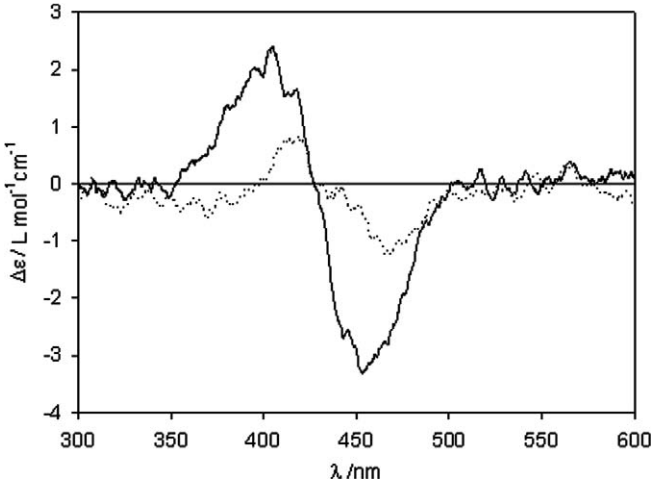


Fig. 3. CD spectra of 10 μM bilirubin in: (a) a methanol solution of 100 mM **1a** (dotted line); (b) aqueous 100 mM sodium *N*-acetyl-L-prolinate (solid line).

in the aggregates and therefore that their chirality has a role in the interaction with bilirubin.

4. Conclusions

Indeed, the induction of a certain conformation in correspondence of a specific molecular structure confirms the finding, reported previously [3], that the interaction of bilirubin with cell membrane is controlled by the lipid molecular structure. The stereochemical information of chirality is part of the molecular structure and, as such, it might control the lipid/bilirubin interaction; actually the enantioselection of bilirubin in these simple membrane models support this hypothesis and raises new perspectives in the investigation and in the comprehension of the mechanism of bilirubin toxicity.

Acknowledgments

Authors acknowledge financial support from CNR, Dipartimento di Progettazione Molecolare, and FIRB contract No. RBPR05NWWC_003.

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