

Effect of the Structure of Bile Salts and Guests on the Binding Dynamics of Guests with Bile Salt Aggregates

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Bile salts are important biological molecules that form aggregates in aqueous solutions due to their amphiphilic nature. It is known that at low concentrations, primary aggregates consisting of a small number of monomers are formed and thus contain a hydrophobic binding site. Previous studies have shown that higher concentrations of these steroidal derivatives lead to secondary aggregation and consequently to the addition of a different, more hydrophilic, binding site. The main focus of this study was to investigate how the structure of the bile salt monomer changes the fast reversible binding of small non-polar guests to the binding sites in the aggregates.

The guest binding dynamics were studied with the use of polyaromatic hydrocarbons as probe molecules (**A-D**). The guest 1-NpOH binds to the secondary aggregates whereas the other guests all bind to the primary aggregate. Four different bile salts (**I-IV**) were employed. The trihydroxy derivatives (**I** and **III**) are more soluble in water than the dihydroxy compounds (**II** and **IV**) and for this reason, the latter form aggregates at lower monomer concentrations.

Steady-state and time-resolved fluorescence was employed to follow the access of quenchers to the excited states located within each binding site. Laser flash photolysis was used to measure the dynamics of the triplet states of the guests. Experiments were performed at different bile salt concentrations to determine if the binding dynamics of **B-D** to the primary aggregates changed with the progressive formation of the secondary aggregates at the higher bile salt concentrations. Fluorescence quenching experiments showed that the protection of the guests within the primary aggregates was dependent on the guest structure. In addition, the protection efficiency for the guests in the primary aggregates was higher for **II** and **IV** when compared to the trihydroxy bile salts.

This result may be related to the fact that dihydroxy bile salts form more rigid aggregates. The least protection was observed for aggregates of taurocholate. A much lower protection efficiency, which is less dependent on the structure of the bile salt, was observed for **A** bound to the sites in the secondary aggregates. Preliminary laser flash photolysis experiments indicate that the dissociation rate constants for the guests parallel the observations from the fluorescence quenching experiments.

