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Probing the Hydrogen Bond Involving Acridone Trapped in a Hydrophobic Biological Nanocavity: Integrated Spectroscopic and Docking Analyses

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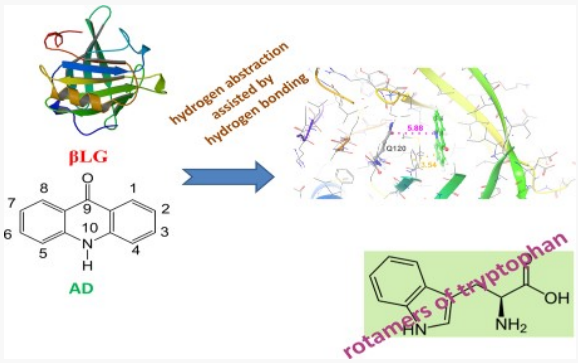
ABSTRACT: Spectroscopic analyses reveal that acridone (AD) penetrates through the structure and enters the hydrophobic cavity of the protein β-lactoglobulin (βLG). Although the protein contains two tryptophan (Trp) residues, AD interacts with only one (Trp-19), which is authenticated by the appearance of a single isoemissive point in TRANES. Alteration in the secondary structure of the protein while AD pierces through βLG is evident from the circular dichroism spectroscopic study. The ground-state interaction between AD and βLG is proven from the UV−vis spectroscopic study and the static nature of quenching of intrinsic fluorescence of the protein by the ligand. The steady-state fluorescence study in varied temperatures indicates the involvement of hydrogen bonding in the ligand−protein interaction. Further, the time-resolved fluorescence anisotropy study gives a hint of the presence of a hydrogen bond in AD−βLG interaction, which possibly involves the rotamers of Trp-19. In fact, the idea of involvement of rotamers of Trp-19 is obtained from the increase in fluorescence lifetime of βLG in the presence of AD. The docking study agrees to the involvement of hydrogen bonding in AD−βLG interaction. The direct evidence of hydrogen bonding between Trp and AD is obtained from the laser flash photolysis studies where the signature of formation of $ADH^{\bullet}$ and $Trp^{\bullet}$ through hydrogen abstraction between Trp and AD, loosely bound through hydrogen bonding, gets prominence. Thus, binding of AD to βLG involves hydrogen bonding in a hydrophobic pocket of the protein.



INTRODUCTION

β-Lactoglobulin (βLG) is a food-based biopolymer, which is the most abundant protein in the whey fraction of milk of cow, sheep, and other mammalians and responsible for transport of hydrophobic nutrients.[1−5] It is a small globular protein (molecular weight 18.3 kDa) containing 162 amino acid residues, folded into a calyx formed by eight antiparallel β-strands and an α-helix located at the outer surface of the β-barrel.[6] Several reports suggest that there are at least two hydrophobic binding sites in the βLG, one in the internal cavity and the other on the outer surface located between the β-barrel and the α-helix.[6] βLG serves as a model protein as its conformation, function, and physiological properties are well-defined. Further, it has two tryptophan (Trp) residues in varied microenvironments, viz. Trp-19 and Trp-61,[6] and thus, the intrinsic fluorescence of Trp may be utilized for the spectroscopic study of the protein.

Acridine derivatives are known to interact with DNA,[7−11] and some of them are recognized as prospective candidates of photosensitizers in photodynamic therapy.[12−14] Thus, study of interactions of such acridine derivatives with exogenous and endogenous drug-delivery vehicles is of pharmacological importance. Previously, we have reported the interactions of an acridine derivative, acridone (AD), as depicted in Figure 1, with two model with two model proteins, human serum albumin (HSA)[15] and bovine serum albumin (BSA).[16] HSA consists of a single Trp (Trp-214) residue, which is housed in a hydrophobic cavity, whereas, BSA contains two Trp residues (Trp-212 and Trp-134) between which Trp-212 resides in a hydrophobic pocket while Trp-134 is solvent exposed. We have observed that in case of AD−HSA interaction, AD directly interacts with Trp-214 while in case of AD−BSA

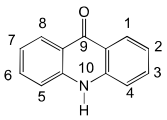


Figure 1. Chemical structure of AD.

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