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Study of the unfolding of bovine serum albumin induced by N-methyl phenyl pyridinium derivatives

Manindu N Peiris, Rohan P Perera*, V N P Liyanage, M D P De Costa
Department of Chemistry, University of Colombo, Colombo 03, Sri Lanka

N-methyl-4-phenyl pyridinium salt (MPP^+) and its methoxy derivatives (Scheme 1) act as an excellent substrate for vesicular monoamine transporter (VMAT) in dopamergenic neurons. It is important to study the interaction of these compounds with available protein models such as bovine serum albumin (BSA) to understand the mode of interaction of MPP^+ with multiprotein complexes.

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Scheme 1: The chemical structures of N-methyl-4-phenyl pyridinium salt (**1**, MPP^+) and N-methyl-4(4-methoxyphenyl) pyridinium salt (**2**, $4OCH_3MPP^+$)

The interaction of $4OCH_3MPP^+$ and BSA was studied in aqueous buffer solution at physiological pH (7.45) by fluorescence and UV-visible spectroscopy at three temperatures, 298K, 303K and 308 K and the binding mode, the binding constant and protein structure changes have been evaluated. The quenching constants K_q , K_{sv} and the association constant K were calculated by using Stern-Volmer equation. The thermodynamic parameters, the enthalpy (ΔH) and the entropy change (ΔS) were obtained using van't Hoff equation and summarized in the Table 1. Quenching spectra of the BSA shows a red shift which indicates the changes of secondary structure of the protein upon binding of quencher.

Table 1: Thermodynamic and quenching parameters for quenching of BSA by $4OCH_3MPP+$ at pH 7.45

Temperature (K)	K_{sv} ($\times 10^4$ L mol^{-1})	K_q ($\times 10^{12}$ L mol^{-1} s^{-1})	Binding constant K ($\times 10^4$ M^{-1})	ΔG^o (kJ mol^{-1})	ΔH^o (kJ mol^{-1})	ΔS^o (J mol^{-1} K^{-1})	# of binding sites (n)
298	1.78	1.78	1.26	-23.11			0.97
303	1.67	1.67	2.12	-25.66	128.44	508.58	1.04
308	1.54	1.54	6.80	-28.19			1.15

The negative sign for ΔG^0 indicates that the binding process is spontaneous. For drug–protein interaction, positive entropy is frequently taken as the evidence for hydrophobic interaction, but it has been pointed out that positive entropy may also be a manifestation of electrostatic interaction. Furthermore, the main source of ΔG^0 value is derived from a large contribution of ΔS^0 term with no contribution from the ΔH^0 factor, so the main interaction is by hydrophobic contact, but the electrostatic interaction cannot be excluded.

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*rohan@chem.cmb.ac.lk Tel: 011-2503367