Title of thesis: Interactions of dietary polyphenols and their copper complexes with serum albumins: Effects of glycation and binding with DNA

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Abstract

The investigation of protein-ligand interactions under physiological conditions is essential for an understanding of molecular recognition pathways that are crucial in all biochemical processes. Serum albumins have the ability to bind many ligand molecules and can also effectively increase the solubility of ligands in plasma and modulate their delivery to target sites. Over the years, research in natural polyphenols has seen an exponential increase primarily because of their disease preventing capability and expected lower toxicity. Flavonoid interaction with serum albumins has been studied to investigate their affinity towards the albumins and to consider their potency as possible therapeutic agents. This is necessary for the understanding of how specific chemical moieties may affect the binding efficacy of the protein. The thesis investigates how the binding of these flavonoid polyphenols and their copper complexes occurs with serum albumins at a molecular level. This has been extended to a study of the modification of human serum albumin with glucose to investigate how glycation may affect their polyphenol binding affinity.

The interactions of biologically active dietary polyphenols and their copper complexes with the protein serum albumins were investigated by different biophysical techniques such as UV-visible, steady-state fluorescence, circular dichroism (CD), Fourier transform infrared spectroscopy (FTIR), MALDI-TOF spectrometry and molecular docking. UV-vis studies were carried out for the identification of the formation of ground state complexes during the binding processes. Fluorescence spectroscopy was used for the determination of binding constants and thermodynamic parameters. Baicalein, morin and fisetin were found to bind to serum albumins via electrostatic interactions followed by hydrophobic association. The isoflavone genistein binds to BSA via hydrogen bonding and van der Waals forces with hydrophobic association. The binding constants of genistein and baicalein with BSA increased in presence of different metal ions. Denaturation of the protein causes the release of fisetin, baicalein or morin from bound BSA. Three dimensional fluorescence, CD and FT-IR studies indicate conformational changes of serum albumins after binding with the polyphenols.

The interactions of the copper complexes of quercetin, rutin, morin, (-)-epigallocatechin gallate (EGCG) and (-)-epicatechin gallate (ECG) with serum albumins have been investigated. The copper complexes bind to serum albumins mainly via hydrophobic forces. The stability of the protein-ligand complexes in these cases increased with rise in temperature. Copper complexes of the polyphenols, apart from the rutin-Cu(II) complex, led to an increase in the α-helical content of serum albumins. Site selectivity studies revealed that the copper complexes bind to site 1 (subdomain IIA) of the proteins. Glycation of HSA has been characterized by UV-vis, fluorescence, MALDI-TOF and CD spectroscopy. Modification of HSA with glucose was found to alter the binding affinities of the polyphenols. Three dimensional fluorescence and CD results suggest changes in protein conformation after interaction with the ligands.

Natural polyphenols have been found to damage DNA *via* an oxidative mechanism. The study was extended to investigate the interactions of the polyphenols and their copper complexes with DNA. The rutin-Cu(II) complex was found to be an intercalator. Copper complexes of morin and rutin were able to damage DNA via an oxidative pathway. The rutin-Cu(II) and morin-Cu(II) complexes were also found to inhibit the growth of HeLa cells, but morin and rutin do not exhibit such inhibitory effects under similar conditions.

Keywords: Serum albumins; polyphenols; copper complex; fluorescence; binding, energy transfer; docking