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SHORT COMMUNICATION

High Dilutions of Homeopathic Drugs Interact with Human Serum Albumin as Revealed by Electronic Spectroscopy

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Abstract

Homeopathy uses drugs in extreme dilutions that are mostly devoid of the original drug molecules. Drug-induced water structures are thought to be responsible for their therapeutic effect. We have already observed that homeopathic potencies first interact with serum albumin, which is present in the oral mucosa. In this experimental study, we have shown that the homeopathic potencies of three drugs, *Bryonia alba* (Br), *Rhux toxicodendron* (Rt), and *Thuja occidentalis* (Th), initiate their action on Human Serum Albumin (HSA). The potency-HSA complexation has been observed by electronic spectroscopy. The control, HSA plus water, shows only one peak at 216 nm, but the potencies plus HSA show two peaks, one at 205 nm and another around 265 nm. The first peak is due to the peptide bond. The first peak in the control shows a marked red shift. The second peak at higher wavelength is due to the aromatic amino acids. The first peak with the potencies shows a marked blue shift, possibly due to a change induced by the potencies on the peptide bond. Unlike water control the potencies interact with aromatic amino acids. It is evident that the complexes made up of HSA and potency are different from those of the control. This means that homeopathic potencies are not ordinary water. It is concluded that water control interacting with HSA shows a single peak in UV-spectra at lower wavelength, but homeopathic potencies show one additional peak at a higher wavelength besides the peak at the lower wave length. HDs can produce effects on aromatic amino acids. The mother tinctures and their HDs show marked differences from each other in their electronic spectra.

Keywords: Homeopathic Potencies, Human Serum Albumin, Modification of Protein, Water Structure

1. Introduction

Homeopathy uses extremely diluted drugs, which usually do not contain the original drug molecules. We now describe the basic process of preparing HDs. The drugs are prepared by serial dilution with a solvent medium 1:100 followed by mechanical agitation or succussion. These diluted drugs are called potencies. In our earlier experimental study, we reported that the potency interacts with a protein, such as Bovine Serum Albumin (BSA). Using Isothermal Calorimetry (ITC) we have already demonstrated that a homeopathic potency interacts with BSA, Human Serum Albumin (HSA) and insulin[1-4]. Homeopathic potencies are applied to the oral mucosa, which contains many proteins, including HSA[5]. Saliva contains oral mucosal exudates. Salivary glands are surrounded by many capillaries through which molecules exchange[6]. The purpose of the present study is to find out the interaction between homeopathic potencies and HSA with the help of electronic spectroscopy. We tested two potencies, 6 cH and 30 cH of three drugs: *Bryonia alba, Rhux toxicodendron*, and *Thuja occidentalis*. We also tested the interaction between the Mother Tincture (MT)

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of the three drugs with HSA. We have already analyzed the MTs and potencies of the test drugs by electronic and vibrational spectroscopy[7]. In the present study, we simply show the interaction between those potencies and their MTs with HSA. All three drugs are plant products[8].

2. Materials and Methods

2.1 Drugs and Protein

HSA was purchased from Sigma Aldrich, USA (Lot Number-SLBM-7779V). It was dissolved in aqueous solutions at 0.05 mg per ml (fatty acid free) containing phosphate buffer. The pH is 6-7. The molecular weight is 66500 Da. The concentration of buffer was 0.02M. Homeopathic drugs and their solvent medium, 90% ethanol, were kindly donated by Hahnemann Publishing Company (Hapco), Kolkata. Three homeopathic drugs, *Bryonia alba, Rhux toxicodendron*, and *Thuja occidentalis,* were used in this experiment. Two centesimal potencies, 6 cH and 30 cH were tested. The drugs were 90% EtOH. The control consisted of DD water and buffer only. We did not use 90% EtOH as a control because ethanol itself is a homeopathic drug[9]. The pharmaceutical company prepared the drugs in January 2021. The percentage of EtOH in all the test samples was confirmed by the calibration curve prepared with different percentages of ethanol. The test potencies were diluted with DD water 1:100 before mixing with the protein solution. The proportion of the diluted test potencies and protein solution was 1:1 (120 µl drug solution and 120 µl HSA solution).

2.2 Electronic Spectra

UV-Spectra of all test samples were taken in the wavelength range of 200 to 300 nm, scan speed medium and data interval 0.5 minute in our laboratory using a UV-VIS Spectrophotometer (Shimadzu, UV-VIS 1900i, Software Lab Solutions UV-VIS) at room temperature 24°C. The base line was set with phosphate buffer, the solvent medium used for the test samples.

3. Results

The electronic spectra of HSA plus two potencies 6 cH and 30 cH of three drugs, *Bryonia alba* (Br), *Rhux toxicodendron* (Rt) and *Thuja occidentalis* (Th) are presented in Figures 1, 2 and 3.

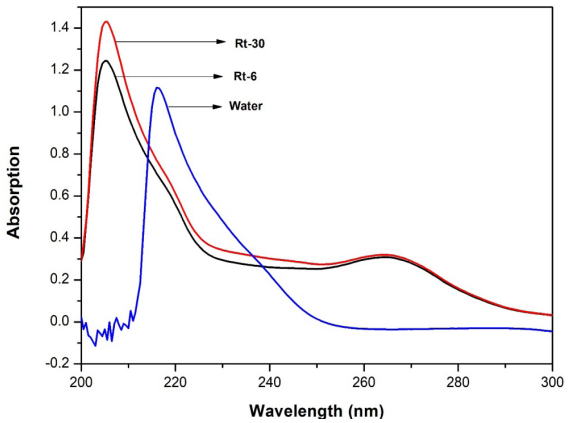

Figure 1. Electronic spectra of HSA plus potencies of *Bryonia alba* and control, water. HSA plus Control shows single peak but the potencies show two peaks.

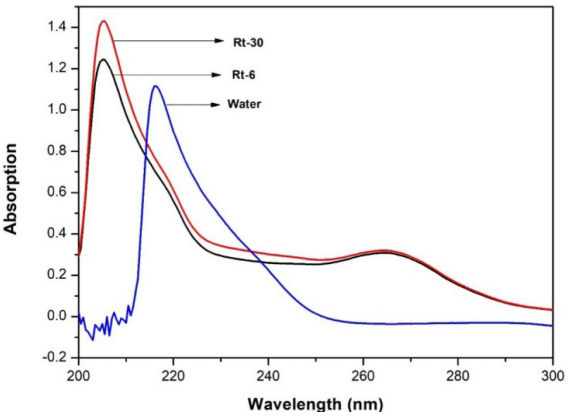

Figure 2. Electronic spectra of HSA plus potencies of *Rhux toxicodendron* and control, water. HSA plus Control shows single peak but the potencies show two peaks.

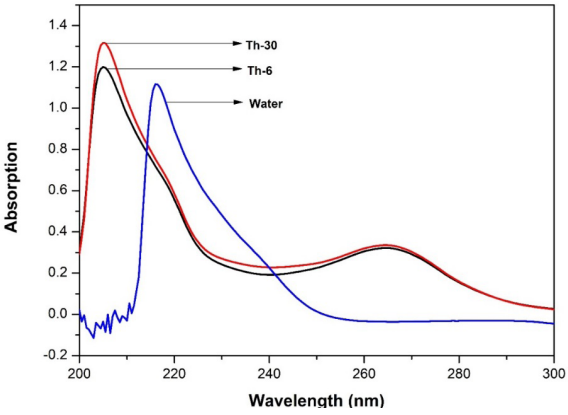

Figure 3. Electronic spectra of HSA plus potencies of *Thuja occidentalis* and control, water. HSA plus Control shows single peak but the potencies show two peaks.

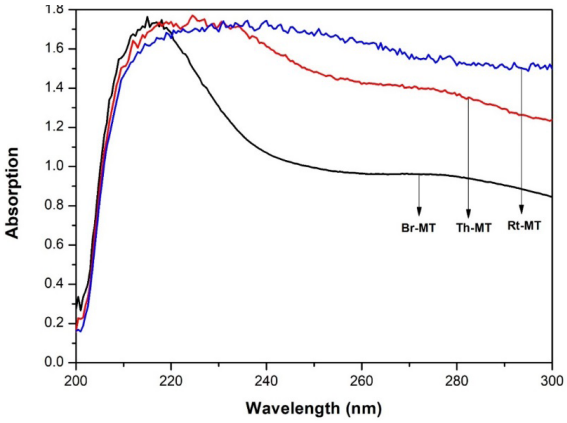

Figure 4. Electronic spectra of HSA plus Mother Tincture (MT) of *Bryonia alba, Rhux toxicodendron* and *Thuja occidentalis*. The intensities are multiplied by 0.5. The MTs show single peak with marked red shift compared to potencies.

The control consisted of HSA plus water only. The control shows a single peak at 216 nm. The potencies of three drugs plus HSA show two peaks, one at 205 nm and another around 265 nm. The absorbance intensities of the first peak lie between 1.2-1.4 and of the second peak around 265 nm, with absorbance intensities between 0.030 to 0.032 (Figures 1, 2 and 3). The MTs show a single peak (Figure 4).

4. Discussion

HSA constitutes 60% of plasma proteins. Two chromophores in a protein give rise to two electronic absorption bands in the UV-region. Peptide bonds linking amino acids absorb around 220-240 nm[10,11]. So the first peak at a lower wavelength is due to the peptide bond. Aromatic amino acids Phenylalanine, Tyrosine, Tryptophan and Histidine absorb around 230-300 nm. So the second peak around 265 nm is due to the aromatic amino acids. The complexes formed of HSA and potencies show a marked blue shift (Figures 1, 2 and 3). So the potencies appear to produce a hyperchromic effect on the protein. Whenever a person suffers from a disease, the cells or tissues in the body become stressed. These cells usually take up albumin as a source of amino acids and energy[12]. In this way, the potency-HSA complexes may reach the sites of the diseased parts of the patient. The single peak in the control with DD water shows a marked red shift, possibly due to nonspecific binding of HSA with water molecules. The MTs show a single peak (Figure 4), indicating their action only on the peptide bond.

Proteins have two hydration shells, and hydration water is more stable than surrounding bulk water. Hydration water interacts with protein and also bulk water, and confers conformational stability of the protein[13]. The transient protein-ligand binding is important in organisms and helps them respond appropriately to any change in environmental and metabolic condition[14]. Proteins have flexible binding pockets lined with amino acids. Internal motion of the protein designs and regulates its binding property[15]. Water molecules bind to various sites of a protein nonspecifically[13]. Homeopathic potencies have two major components: free water molecules and the hydrogen bond strength of water hydroxyl[16-18]. A protein's binding site contains both hydrophobic and hydrophilic residues[4]. Free water molecules in a potency usually bind to the OH groups of amino acids of the protein. This results in HSA modification following interaction with a potency. Water molecules in the solvent of HSA are re-organized when potency is added to it. This reorganized water structure leads to the modification of HSA.

The binding site in a protein is complementary to the ligand in shape, size, charge, hydrophilic and hydrophobic characteristics. So protein-ligand binding is specific. The specificity of a potency is determined by the hydrogen bond strength, free water molecules, number of hydrogen bonds, and charge transfer potential. As a result, the structure of water in a potency is completely different from that of ordinary water. Water molecules play an active role in protein-ligand binding[15]. Unlike ordinary water, the homeopathic potency may specifically bind to the binding pocket of a protein. We previously discovered that a homeopathic potency of *Natrum muriaticum* 200 cH causes an exothermic reaction on multiple sites in a sequential manner of Bovine Serum Albumin, as revealed by isothermal calorimetry[1]. We have also observed that two potencies of sulphur 30 cH and 200 cH interact with the binding site of BSA[2]. Three potencies, 6 cH, 12 cH and 30 cH of Carduus mari interact with the binding sites of insulin[3]. Thus, we can assume that homeopathic potencies initiate their binding interaction with a protein, particularly serum albumin, which is present in the oral mucosa[5].

5. Conclusion

Homeopathic potencies are specific water structures that are different from ordinary water. While ordinary water binds nonspecifically with a protein, potencies do so

specifically with a protein. Potencies are administered on oral mucosa where exist serum albumin. The first interact with HAS, and from complex with HSA. These complexes are observed in the electronic spectra. Complexes with ordinary water show a single peak at a lower wave length due to the peptide bond. But potencies show one additional peak at a higher wave length, interacting with an aromatic amino acid.

6. References

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