Spectroscopic Study of the Interaction of Human Serum Albumin with Steroid Hormones "Progesterone and its parent compound Cholesterol"

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Abstract:

In this study the interaction of steroid hormones (progesterone and its parent compound cholesterol) with human serum albumin at physiological pH have been studied using UV-VIS spectrophotometer, fluorescence spectrophotometer, and FT-IR spectroscopy. The results showed that UV absorption intensity spectra were increased with the increase of progesterone or cholesterol molar ratios in fixed amount of HSA. From UV spectra the binding constants were obtained and equals (6.354×102M-1)for progesterone and (0.2641×104M-1) for cholesterol. Beside that the results that have been obtained from analysis of fluorescence spectra indicated that progesterone and cholesterol have an ability to quench the intrinsic fluorescence of HSA through a static quenching procedure. The values of Stern-Volmer constant were determined to be (6.26×102 L mol-1) for progesterone- HSA complexes and (6.21×102 L mol-1) for cholesterol- HSA complexes. Also the quenching rate constant values obtained were (6.2×1010 L mol-1s-1) for progesterone and (6.21 × 1010 Lmol-1s-1) for cholesterol.

The binding constant from fluorescence spectrum for progesterone- HSA complexes was found to be (6.56×102 M-1). And for cholesterol- HSA complexes was found to be (0.214 × 104 M-1). It was obviously noted that the obtained values agrees well

with the values obtained using UV-VIS spectrophotometer. And that cholesterol binding constant is larger than progesterone binding constant, this refer to the structure of the two compounds which is consistent with that have been reported. FT-IR spectroscopy with Fourier self-deconvolution and second derivative, as well as curve fitting procedures were used in the analysis of amide I, amide II, and amide III regions of HSA to determine protein secondary structure and hormone binding mechanism. It was observed that the intensity of absorption bands decreased as progesterone or cholesterol molar ratios increased. Also all peak positions of the three amide regions were assigned at different progesterone or cholesterol ratios. In addition FT-IR spectra evidence showed that HSA secondary structure has been changed as progesterone or cholesterol molar ratios increased, which was observed in the reduction of α-helices absorption band relative to β-sheets absorption band. The variation in the intensity is related indirectly to the formation of H-bonding in the complex molecules, which accorded for the different intrinsic propensities of α-helix and β-sheets.