Deanship of Graduate Studies
Al-Quds University

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

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M.Sc. Thesis

Jerusalem – Palestine

1431/ 2010

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"A thesis Submitted to the Faculty of Science and Technology, Al-Quds University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Physics"

1431 / 2010

Al-Quds University
Deanship of Graduate Studies
Physics Department

Thesis Approval

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Dedication:

I dedicate this thesis to the brightness of hearts, watercress's and remedy of chests, mister prophet Mohammad "peace and blessings of Allah be upon him". To my parents, Hamed and Najla thank you for every prayer, and bean of perspiration ripple from your brows, fatigued for me. My wife Dalal my soul mate and confidant, for always being there for me. Thank you for your continual love, support, and patience as I went through this journey. I could not have made it through without you by my side. My brothers and sisters; Taha, Yousef, Belal, Asma', Assem, Yasser, and Maysa' for their love and support throughout the years, thank you for the laughing, the fighting, and everything in between. To my Future daughter … "Baby Daddy desirous to enfold you".

Jafar Hamed Taha Ghithan

Declaration:

I certify that this thesis submitted for the degree of Master is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Signed: _____

Jafar Hamed Taha Ghithan

Date: / / 2010

Acknowledgements

After my Great commendation and Thanks to Allah.

This thesis arose in part out of months of research that has been done since I came to Al-Quds University. By that time, I have worked with a great number of people without whom this thesis might not have been written, and to whom I am greatly indebted.

First for providing the inspiration to embark on my master candidature my deepest thanks go to my supervisor who shepherded me through the bulk of the work, Dr.Musa Abu Teir, his kindly but rigorous oversight of this thesis constantly gave me the motivation to perform my maximum ability. I was very fortunate to have been able to work with him and hearing his jokes. I must also thank my co supervisor prof. Mahmoud Abu hadid, his detailed and constructive comments were vital to the development of this thesis, his advice and patience is appreciated.

Special thanks to whom spotlight my way in his glowing science Dr. Saqer Darwish who has made invaluable comments about the thesis.

A special thanks also goes to Sawsan Abu Sharhk who greatly enriched my knowledge with her experience in bio physics lab.

I would also like to thank the staff at Nano-Technology lab in Al-Quds University for their tireless efforts.

My parents who have supported me all the way since the beginning of my studies. And my beloved wife who has been a great source of motivation and inspiration. Thank you for your tremendous encouragement.

My friend Said Qshoua', for helping me preparing for my defense and printing my thesis, thank you for all what you have done for me.

ABSTRACT

It was found that the distribution and metabolism of many biologically active compounds in the body whether drugs or natural products are correlated with their affinities toward serum albumin. Thus, the study on the interaction of such molecules with albumin is of imperative and fundamental importance. Extensive studies on different aspects of drug–HSA interactions are still in progress because of the clinical significance of the processes.

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 ($6.354\times10^{2}M^{-1}$)for progesterone and ($0.2641\times10^{4}M^{-1}$) 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 (6.26×10^{2} L mol^{-1}) for progesterone- HSA complexes and (6.21×10^{2} L mol^{-1}) for cholesterol- HSA complexes. Also the quenching rate constant

values obtained were (6.20×10^{10} L $mol^{-1}s^{-1}$) for progesterone, and (6.21×10^{10} $Lmol^{-1}s^{-1}$) for cholesterol.

The binding constants from fluorescence spectrum for progesterone- HSA complexes was found to be (6.56×10^{2} M^{-1}), and for cholesterol- HSA complexes was found to be (0.214×10^{4} 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.

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