Physicochemical and kinetic properties of purified arginase from Camel and Bovine liver cytosol

A thesis Submitted by

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This Thesis has not been submitted to this or any other University.

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Dedication

Special thanks and gratitude to my beloved parents, brother and my beloved husband who are behind every successful aspect of my life. If every one is as kind as them, earth would be an utopia. Special thanks, love and care to my lovely son Abdullah. Finally, I want to dedicate this thesis to every member in my family especially to my grandfather and my grandmother (God rest their soul).

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ABSTRACT

Rasha Elsherif Hassan Ibrahim, Physicochemical and kinetic properties of purified arginase from camel and bovine liver cytosol.

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In the present study, camel liver was chosen as a source of arginase, since no information is available about this enzyme from this species, so this is the first time to purify arginase from camel liver cytosol. Arginase was also purified from bovine liver cytosol and some of the physicochemical and kinetic properties of the purified arginase from the two sources were studied and compared.

Arginase was isolated and purified from camel and bovine liver using heat denaturation followed by ammonium sulphate fractionation, DEAE-cellulose column chromatography, SP-Sepharose column chromatography and gel filtration using Sephadex G-100-120 and Sephacryl HR-300 columns.

It was observed from the present investigation that there is a similarity between the purified camel and bovine liver arginases in some properties including the non-adsorption on anion exchanger column, the oligomeric structure of the

enzyme, the non-cooperativity of subunits, the efficiency constant, the suspectability to the inhibition by some amino acids, the requirement for metal ions as cofactor, the alkaline optimum pH, the stability at 4^{o}C, the cold lability through freezing and thawing cycle and storage at -20^{o}C. On the other hand, purified camel liver arginase differs from the purified bovine liver enzyme in its chromatographic behaviour on the cation exchanger column, higher molecular weight (174-180 kDa) versus (133-140 kDa), slightly alkaline p*I* value (7.7) versus the slightly acidic to neutral p*I* values (5.9, 7), higher carbohydrate content 2.7% versus 0.6%, affinity towards arginine with K_m value 7.1mM versus 14.3mM of bovine liver enzyme, its inhibition by some metal ions as Sr^{+3} and Zn^{+2}. Also, it was found that camel liver arginase is more thermostable with an optimum temperature of 70^{o}C and E_a of about 11,752cal/mole versus an optimum temperature of 55-60^{o}C and E_a of about 10,420cal/mole for bovine liver arginase which demonstrates that camel liver enzyme has a relative structure rigidity. Finally, although purified camel liver arginase was affected by freezing at -20^{o}C and by repeated freezing and thawing it proved to be more stable than the purified bovine liver enzyme.

Key words: ***Camel, bovine, liver, arginase, purification, physicochemical and kinetic properties.***

LIST OF FIGURES