THE UNIVERSITY OF MANITOBA

STRUCTURE - FUNCTION RELATIONS IN THE CALCIUM-DEPENDENT PROTEIN MODULATOR OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASE

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a Ph.D. thesis entitled: "STRUCTURE - FUNCTION RELATIONS IN THE CALCIUM-DEPENDENT PROTEIN MODULATOR OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASE"

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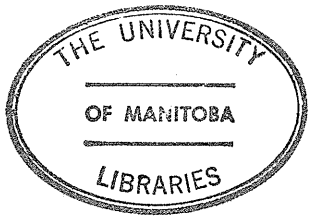
STRUCTURE - FUNCTION RELATIONS IN THE CALCIUM-DEPENDENT PROTEIN MODULATOR OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASE

BY

MICHAEL WALSH

A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY



To Mary, Katy and Emma

"An experiment is never a failure solely because it fails to achieve predicted results. An experiment is a failure only when it also fails adequately to test the hypothesis in question, when the data it produces don't prove anything one way or another".

Robert M. Pirsig in "Zen and the Art of Motorcycle Maintenance", (1974), William Morrow and Company, Inc., New York, N.Y. 10016.

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ABSTRACT

Three aspects relating to the structure and function of the Ca^{2+}-dependent protein modulator of cyclic nucleotide phosphodiesterase were investigated: (1) the mode of interaction between the modulator and phosphodiesterase, (2) the conformational changes induced in the modulator by the binding of Ca^{2+}, and (3) the structural homology between the modulator and troponin C, the calcium-binding subunit of the muscle troponin complex.

Chemical modification of selected functional groups of the protein modulator indicated that the site for interaction with phosphodiesterase is located between the second and third calcium-binding regions and is on the surface of the molecule. The integrity of both methionine and lysine residues is essential for the expression of phosphodiesterase-stimulating activity. It is proposed that interaction with phosphodiesterase occurs via an initial site recognition involving Lys 75 and Lys 77, and possibly other neighbouring charged side chains, followed by formation of a strong binding interaction through hydrophobic interactions involving Met 71, 72 and 76. Chemical modification of histidine, tyrosine and arginine residues, on the other hand, did not affect the stimulation of phosphodiesterase - none of these residues are located in the vicinity of the proposed phosphodiesterase binding domain. Similar studies indicated that the site for interaction with phosphodiesterase is distinct from the site for interaction with troponin I.

A combination of circular dichroism, chemical modification and controlled enzymatic digestion of the protein modulator in the presence and absence of Ca^{2+} provided insight into the conformational changes

occurring as the modulator binds Ca^{2+}. Removal of Ca^{2+} results in a substantial loss of secondary structure: the helical content decreases from 49% to 40%, whereupon the molecule becomes much more susceptible to tryptic digestion and urea denaturation. On a more refined level, His 107, for example, becomes more accessible as the modulator binds Ca^{2+}, as revealed by kinetic analysis of carbethoxylation of this residue. Similar studies revealed altered reactivity of the other functional groups of the modulator upon binding of Ca^{2+}.

The protein modulator and troponin C exhibit approximately 80% sequence homology, taking conservative replacements into account (Vanaman et al, 1977). Circular dichroism studies of the protein modulator indicated the overall secondary structure of the molecule to be very similar to that of troponin C. Furthermore, controlled tryptic digestion of the modulator with structural and functional characterization of the resultant fragments revealed that cleavage occurred at positions homologous to those previously observed (Drabikowski et al, 1977a) in troponin C. The two calcium-binding proteins, therefore, exhibit similar tertiary structures. In addition, they undergo similar conformational changes upon binding of Ca^{2+} ions. The structural differences between the modulator and troponin C are small, but, in functional terms, these subtle differences have profound effects.

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