FOLDING AND CONFORMATIONAL STABILITY OF PORCINE GROWTH HORMONE

A thesis submitted by

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TABLE OF CONTENTS

SUMMARY

The large scale production and purification of recombinant-derived porcine growth hormone (pGH) and mutants thereof, bream growth hormone (brGH) and human growth hormone (hGH) was carried out. Each of the protein products were analysed by various physicochemical means, in order to verify their identity and homogeneity.

The equilibrium denaturation of pGH using the chemical denaturant guanidine hydrochloride (GuHCl), was monitored by a variety of spectroscopic and hydrodynamic probes. The denaturation of pGH resulted in absorbance- and fluorescence-detected transitions which were coincident, whereas far-UV circular dichroism- and hydrodynamic radius-detected transitions occured at higher concentrations of GuHCl, indicative of a greater stability to denaturation. Conformations intermediate to the folded and fully unfolded states were found to be stable at equilibrium under partially denaturing conditions. These intermediate forms have the characteristics of *molten-globule* or *compact denatured states;* a compact structure with considerable helix content, yet possessing a tertiary structure similar to that of the fully unfolded state. At concentrations above 10μM, the intermediate was shown to aggregate, forming a stable associated intermediate. These results suggest that pGH does not follow a simple two-state folding mechanism, but is consistent with the framework model of protein folding.

The conformational stabilities of pGH, ten site-directed mutants of pGH, and wild-type bream and human GH, were determined using GuHCl-induced equilibrium denaturation under a standard set of conditions. Single amino acid changes in the sequence of pGH were shown to have different effects on (*i*) the conformational stability, (*ii*) the cooperativity of the denaturation transition, i.e., m_{GuHCl} and (*iii*) the midpoint of the denaturation transition, i.e., $[GuHCl]_{½}$. Bream GH was shown to have a stability similar to that of wild-type pGH whereas human GH, in accordance with previously published values [Brems, D. N., Brown, P. L., and Becker, G. W.

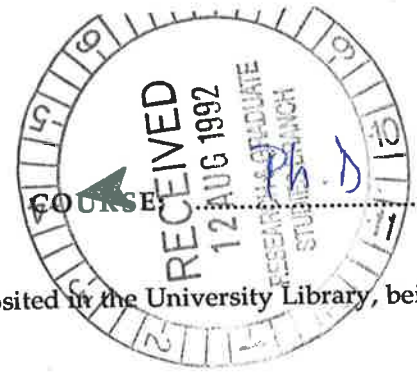
(1990) *J. Biol. Chem.* *265*, 5504-5511], was found to be significantly more stable than pGH and brGH.

One mutant in which a methionine residue was replaced by a tryptophan [pGH(M8)], was found to be significantly more stable than wild-type pGH, due to an increase in both m_{GuHCl} and $[GuHCl]_{½}$. The coincidence of the UV-, fluorescence- and hydrodynamic radius-detected equilibrium denaturation curves and the absence of significant amounts of associated forms suggests that pGH(M8) folding/unfolding is more closely approximated by a two-state mechanism than wild-type pGH.

STATEMENT

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of my knowledge, it contains no material that has been previously published or written by another person except where due reference is made in the text.

Stan Bastiras



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Publications and Conference Presentations Arising from Thesis Research.

Bastiras, S., & Wallace, J. C. (1992).
Equilibrium Denaturation of Recombinant Porcine Growth Hormone
Biochemistry 31, (in press).

Bastiras, S., Sawyer, W. H., Wallace, J. C., & Wells, J. R. E. (1992)
Equilibrium Denaturation of Recombinant Porcine Growth Hormone
Seventeenth Annual Lorne Conference on Protein Structure and Function, Feb. 1992.

Bastiras, S. (1992)
Mutational Effects on the Conformational Stability and Folding of Porcine Growth Hormone.
Workshop on "Understanding Protein Folding", NOVUM Research Park, Stockholm, August 1992.

ABBREVIATIONS

A_{xxx}	absorbance at xxx nm
AUFS	absorbance units of full scale
bGH	bovine growth hormone
brGH	bream growth hormone
C D	circular dichroism
DTT	dithiothreitol
F_{app}	the apparent fraction of unfolded protein
GH	growth hormone
GuHCl	guanidine hydrochloride
hGH	human growth hormone
HPLC	high performance liquid chromatography
K_a	association constant
λ_{max}	fluorescence emission maximum
pGH	porcine growth hormone
pGH_{wt}	wild type porcine growth hormone
PL	placental lactogen
pRL	prolactin
$\Delta\alpha_{app}$	apparent average difference in degree of exposure to solvent
ΔG_{app}	apparent free energy of unfolding
$\Delta G(H_2O)$	ΔG in the absence of denaturant (conformational stability)
m	measure of the dependence of ΔG on denaturant concentration