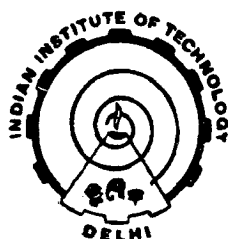


# THERMODYNAMIC INVESTIGATIONS OF SOME BIOCHEMICALLY IMPORTANT SYSTEMS

*by*

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**THESIS SUBMITTED  
IN FULFILMENT OF THE REQUIREMENTS  
FOR THE DEGREE OF  
DOCTOR OF PHILOSOPHY**



to the  
**INDIAN INSTITUTE OF TECHNOLOGY, DELHI**  
INDIA  
May, 1992

..... for Jitendra

*"No goal that I reached was a goal,  
every path was a detour,  
every rest gave birth to a new longing."*

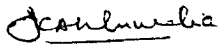
- Hermann Hesse

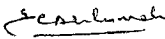
## CERTIFICATE

This is to certify that the thesis entitled "THERMODYNAMIC INVESTIGATIONS OF SOME BIOCHEMICALLY IMPORTANT SYSTEMS" being submitted by Ms. Swarita Gopal to the Indian Institute of Technology, Delhi for the award of the degree of Doctor of Philosophy in Chemistry is a record of bonafide research work carried out by her. Ms. Swarita Gopal has worked under my guidance and supervision, and has fulfilled the requirements for the submission of this thesis which to my knowledge, has reached requisite standard.

The results contained in this dissertation have not been submitted in part or in full, to any other University or Institute for the award of any degree or diploma.

I.I.T., Delhi  
4th, May 1992

  
(J.C. Ahluwalia)  
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22.9.92

## ACKNOWLEDGEMENTS

I would like to express my profound gratitude to Prof. J.C. Ahluwalia for his inspiring guidance and for ongoing exchange of ideas which has been my richest source of knowledge and inspiration.

My sincere thanks to Prof. A.S.N. Murthy for generously sharing his knowledge and resources with me, and for providing necessary facilities in the department.

It is my privilege and pleasure to acknowledge the help and advice of my seniors Dr. Rajiv Bhat and Dr. Rameshwar Jha, and other Laboratory colleagues - Dr. Tarlok Singh, Ms. Sandhya Jain, Mr. Sunil Kumar, Dr. Anita Saxena and Mr. Satish Bhardwaj, who had been particularly helpful in later stage of my work.

Help and support of my family members is thankfully acknowledged, with an affectionate mention of my elder sister Mrs. Vinita Pal for her constant encouragement throughout my academic career.

I offer my profound thankfulness to Ms. Latika Singh and Dr. (Mrs) Tanuja R. Kumar who gave me patient hearing during the stressful days and infused me with enthusiasm; they also helped me in numerous other ways. I am also thankful to Dr. G.S. Kapur and Ms. Sunita.

I would like to take this opportunity to thank all the faculty and staff members of the Department of Chemistry for their support, encouragement and for allowing me discuss many tentative ideas in our informal setting.

I am thankful to Mr. Jagdish K. Lohia and Mr. Rajeev Dua for neat typing and turning a huge manuscript into a well proportioned thesis. Also a word of thanks to Mr. Sushil Kumar, who helped me at various stages of my work.

A very special mention and immense gratitude is expressed to National Institute of Standards and Technology (NIST), USA and IIT Delhi for financial support provided throughout my research work.

*Swarita*  
(SWARITA GOPAL)

## ABSTRACT

The present work describes the investigations carried out using ultra-high sensitive Micro-DSC (Differential Scanning Calorimeter) to determine (a) the effect of osmolytes which include amino acids, methylamines and urea, on the thermodynamic parameters : denaturation temperature  $T_d$ , enthalpy of denaturation  $\Delta H_d$ , and entropy of denaturation  $\Delta S_d$ , of proteins -Ribonuclease A and Lysozyme, and (b) to estimate the binding constant for Lysozyme and N-acetyl-D-glucosamine.

Osmolytes are the solutes accumulated by marine organisms to keep the hyperosmotic or isosmotic condition to water. Amino acids, urea and methylamines are important classes of such osmoregulatory solutes. Transition temperature  $T_d$ , for Ribonuclease A is observed to increase in the presence of amino acids. The effectiveness of an amino acid in stabilizing the protein decreases with alkyl chain length: glycine >  $\beta$ -alanine >  $\gamma$ -aminobutyric acid >  $\alpha$ -alanine > L-valine > L-leucine. This reflects that an increase in hydrophobic character of the amino acid added leads to a decrease in the thermal stability of the protein. This observation has been explained on the basis of hydration cosphere overlap model. A similar denaturation study was carried out on Ribonuclease A and Lysozyme in the presence of methylamines and urea. We observe that while urea, as expected, destabilizes proteins, methylamines, on the other hand, stabilize proteins in the order : trimethylamine-N-oxide > sarcosine > betaine. Trimethylamine-N-oxide is found to be most stabilizing of all methylamines and amino acids studied

in this work. The maximum stabilizing effect observed for TMAO inspite of the destabilizing influence of its three hydrophobic methyl groups appears to be due to the favourable interaction of polar or ionic groups on the protein surface with TMAO.

Binding constant  $K_L$ , and enthalpy of binding  $\Delta H_L$ , for the weak binding of lysozyme with N-acetyl-D-glucosamine have been estimated from DSC data. The appropriate sets of equations derived by Brandts and Lin have been used for the calculation. The values so obtained agree very well with the experimentally determined binding constant and enthalpy of binding, from equilibrium studies (e.g. titration calorimetry, NMR, UV and CD). Binding constant has also been estimated by using the simulation method. The simulated DSC curves of lysozyme in GlcNAc, obtained by varying  $K_L(T_o)$  are compared with the experimental curves. This gives a good estimate of the binding constant.

Due acknowledgement has been made to other investigators wherever the work described is based on their findings. The author apologizes for any omission or mistake which might have crept in due to oversight.

## GLOSSARY OF SYMBOLS AND ABBREVIATIONS

aq.	aqueous
BSA	Bovine Serum Albumin
CD	Circular Dichroism
$\bar{C}_{p2}^{\circ}$	partial molar heat capacity of solute at infinite dilution
$C_{pN}$	heat capacity of native state of protein
$C_{pD}$	heat capacity of denatured state of protein
$\Delta C_p^{\circ}$	limiting heat capacity of dissolution
$\Delta C_{pD}$	heat capacity of denaturation of protein
$\Delta C_{pL}$	heat capacity of binding.
$^{\circ}\text{C}$	degree celcius
D	denatured state of protein
DSC	differential scanning calorimeter
f.s.d.	full scale deflection
GABA	$\gamma$ -amino butyric acid
GlcNAc	N-acetyl-D-glucosamine
Gdn.HCl	guanidinium hydrochloride
Gly-HCl	glycine hydrochloride
$\Delta G^{\circ}$	standard Gibbs free energy of transition
$\Delta G_d$	free energy of denaturation
$\Delta H^{\circ}$	standard enthalpy of transition

$\Delta H_d$	enthalpy of denaturation
$\Delta H_L$	enthalpy of binding
$\Delta H_{vH}$	van't Hoff enthalpy
J	joule
K	(i) Kelvin, or (ii) equilibrium constant
k	kilo = $10^3$ , when used as a prefix
$K_L(T_d)$	binding constant at denaturation temperature $T_d$
$K_L(T_0)$	binding constant at denaturation temperature $T_0$ (zero ligand concentration)
$K_L(298)$	binding constant at 298K
m	molality
M	$\text{mol l}^{-1}$
MW	molecular weight
$\mu\text{V}$	microvolt
$\eta$	cooperativity index = $\Delta H_d / \Delta H_{vH}$
N	native state of protein
nm	nanometer, unit of wavelength
ORD	optical rotatory dispersion
$Q_d$	heat of denaturation
$Q(T)$	heat absorbed at temperature, T
RNase A	Ribonuclease A
$\Delta S^\circ$	standard entropy of transition

$\Delta S_d$	entropy of denaturation
t	time, in seconds
T	the temperature
$T_d$	denaturation temperature of protein
$T_o$	denaturation temperature of protein in the absence of substrate
$\Delta T_d$	change in denaturation temperature
$\theta$	extent of reaction in the protein transition
UV	ultraviolet
$\Delta V$	change in volume
W	watt

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