THERMODYNAMIC INVESTIGATIONS OF SOME BIOCHEMICALLY IMPORTANT SYSTEMS

by

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. . . . *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.

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Swarita
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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 ΔH_d, and entropy of denaturation ΔS_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 > β-alanine >γ-aminobutyric acid > α-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 ΔH_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 creeped in due to oversight.

GLOSSARY OF SYMBOLS AND ABBREVIATIONS

aq.	aqueous
BSA	Bovine Serum Albumin
CD	Circular Dichroism
$\bar{C}^{o}_{P2}$	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
ΔC^{o}_{p}	limiting heat capacity of dissolution
ΔC_{Pd}	heat capacity of denaturation of protein
ΔC_{PL}	heat capacity of binding.
°C	degree celcius
D	denatured state of protein
DSC	differential scanning calorimeter
f.s.d.	full scale deflection
GABA	γ-amino butyric acid
GlcNAc	N-acetyl-D-glucosamine
Gdn.HCl	guanidinium hydrochloride
Gly-HCl	glycine hydrochloride
ΔG^{o}	standard Gibbs free energy of transition
ΔG_{d}	free energy of denaturation
ΔH^{o}	standard enthalpy of transition

ΔH_d	enthalpy of denaturation
ΔH_L	enthalpy of binding
ΔH_{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
$K_L(T_o)$	binding constant at denaturation temperature T_o (zero ligand concentration)
$K_L(298)$	binding constant at 298K
m	molality
M	mol l^{-1}
MW	molecular weight
μV	microvolt
η	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
$\Delta S°$	standard entropy of transition

ΔS_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
ΔT_d	change in denaturation temperature
θ	extent of reaction in the protein transition
UV	ultraviolet
ΔV	change in volume
W	watt

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