BIOCHEMICAL INVESTIGATION OF THE MOLECULAR MECHANISM OF ATP SYNTHESIS

by

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Thesis submitted
in fulfilment of the requirements of the degree of Doctor of Philosophy
to the

INDIAN INSTITUTE OF TECHNOLOGY DELHI

APRIL, 2004

CERTIFICATE

This is to certify that the thesis entitled "**Biochemical Investigation of the Molecular Mechanism of ATP Synthesis**" being submitted by **Ritu Mehta** to the Indian Institute of Technology Delhi, for the award of degree of Doctor of Philosophy, has been prepared under my supervision and guidance in conformity with the rules and regulations of Indian Institute of Technology Delhi. The research report and results presented in this thesis have not been submitted to any other University or Institute for the award of any degree or diploma.

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ACKNOWLEDGEMENTS

I deem it a great privilege to express my deep sense of gratitude and reverence to my research supervisor Dr Sunil Nath, for his expert guidance and support. His encouraging attitude and perpetual inspiration has helped me to sail through difficult phases of this work with a positive attitude. His gamut of knowledge of the field helped me in taking directed approach towards the topic and in enhancing my research skills to a great extent.

I would like to thank my SRC chairman Prof. Subhash Chand for his keen interest and valuable suggestions at all stages of the work. I am highly thankful to my SRC members, Dr. Nalin Pant and Dr. Prashant Misra. I am grateful to Prof. Saroj Misra, Head, Department of Biochemical Engineering and Biotechnology for providing me the laboratory facilities and the requisites for the work.

I am extremely thankful to Dr. Vandana Gupta for her unconditional support and affection. It was because of her positive attitude that I could remain composed and keep faith in myself during the most crucial phases of this work especially in the initial stages of phosphorylation experiments. I take this opportunity to thank Ms. Ruchi Shukla for being a constant support and a wonderful friend. Her caring attitude and ever-willing cooperation helped me overcome all the hurdles for which I owe her more than I can express. A thanks is reserved for Ms. Reetu Jindal for all the warmth and support she has given me during this tenure that kept me motivated.

I would like to thank Ms. Mili Prabhakar and Ms. Roopali for their extreme affection and cooperation. I would find them by my side whenever I needed them. I am thankful to Mr. Ashwini Mathur for his timely help. I am thankful to all the students of the department for their help during various stages of the work specially Ms. Salony, Ms. Pranita, Dr. Preeti Srivastva and Ms. Nidhi for keeping my spirits high. I thank the members of the research lab for bearing with me when I would switch off the lights for certain experiments.

I would like to thank Ms. Renu Sethi for her help at the critical junctions during the various phases of the work and for making the lab atmosphere pleasant to work in. I am thankful to all other staff members of the department especially Ms. Neera Verma, Ms. Meena Mathur, Mr. Tarzan, Mr. Mukesh, Mr. Bhagwan Singh, Mr. D. V. Sharma, Mr. S. P. Rana, Mr. J. A. Khan. I am thankful to Mr. Rajkumar and Mr. Pitambur for providing me with acid washed tubes that made my work easier.

I express my sincere thanks to my husband Mr. Sanjeev Verma for his tremendous support, constant faith and eternal confidence in me. His utmost caring attitude and encouragement helped me to work with great zeal and overcome all the difficulties patiently. A special thanks is for my daughter Ms. Saachi for her encouraging smiles that would reenergize me to work harder. I take this opportunity to thank my parents and parents in law for their affection, constant encouragement and moral support that has helped me tremendously in successful completion of this work and in writing this thesis patiently.

Finally, I am thankful to all my friends for their support and encouragement during all these years.

(Ritu Mehta)

ABSTRACT

Adenosine triphosphate, the universal currency of the cell is synthesized by the enzyme F_1F_o ATP synthase present in the coupling membranes of mitochondria, chloroplasts and bacteria. According to the chemiosmotic theory, the transmembrane protonmotive force generated only by protons imparts the energy for the phosphorylation of ADP. The requirement of anions in the process of ATP synthesis was noticed by scientists, but no biological role has been attributed to their presence and requirement. The present study explored the functional significance of anions in driving phosphorylation. The phosphorylation was performed in freshly isolated thylakoids from spinach by the technique of 'acid-base' transition. The rate of phosphorylation and the amount of ATP produced were found to be a strong function of the concentration of succinic and hydrochloric acids. For both these anions, the rates and yields increased with decrease in the concentration of the anions. A number of mono, di and tricarboxylic acid and other anions were tested. Chloride was suggested to be the physiological anion involved in photophosphorylation based on these studies. The increase in the time of exposure of thylakoids with acid stage time was found to cause tremendous increase in the rates and yields because the established concentration gradients of anions and protons were of high magnitude. High magnitude concentration gradients led to greater number of translocations of anions and protons across the membrane in the vicinity of F_o and hence stimulated the rates and yields, as these translocations imparted energy to F_1 for ATP synthesis. As the phosphorylation time (base stage time) was increased, the substrates and the concentration gradients of anions and protons depleted with successive increments

leading to the observed reduction in the rates. The yields till each interval increased with increment in the phosphorylation time. These results have been shown to be consistent with Nath's torsional mechanism of energy transduction and ATP synthesis. The increase in number of ATP synthase complexes led to the increase in the available channels for the passage of both the ions, hence causing significant stimulation in the rates and amount of ATP produced. The blockage of chloride channel with DIDS led to considerable reduction in the rate and yield obtained while performing the phosphorylation with hydrochloric acid. The addition of DIDS to the BS solution also reduced the rate of phosphorylation and the amount of ATP produced to a significant extent. This further strengthened the correlation between anion transport and the process of phosphorylation. DIDS-treated thylakoid membranes displayed reduced extent of proton uptake as compared to untreated membranes. The results obtained have been shown to be readily explained by the torsional mechanism. In fact, the unifying framework of the torsional mechanism can be fruitfully used as a guide for further experimentation and research in bioenergetics.

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