Molecular Mechanisms of Transport

Editors:
E. Quagliariello
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Developments in Biochemistry Volume 29

ELSEVIER

MOLECULAR MECHANISMS OF TRANSPORT

Proceedings of the 22nd Bari Meeting on Bioenergetics, International Symposium on Molecular Mechanisms of Transport, Bari, Italy, 29 September – 1 October 1991

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1992

ELSEVIER SCIENCE PUBLISHERS
AMSTERDAM · NEW YORK · LONDON · TOKYO

ISSN 0165-1714
ISBN 0 444 89408 X (Vol. 29)

This book is printed on acid-free paper.

Published by:
Elsevier Science Publishers B.V.
(Biomedical Division)
P.O. Box 211
1000 AE Amsterdam
The Netherlands

Sole distributors for the USA and Canada:
Elsevier Science Publishing Company Inc.
655 Avenue of the Americas
New York, NY 10010
USA

Library of Congress Cataloging in Publication Data:

Printed in The Netherlands

MOLECULAR MECHANISMS OF TRANSPORT

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PREFACE

This volume comprises the proceedings of the International Symposium on 'Molecular Mechanisms of Transport' which was held at Selva di Fasano near Bari, September 29 – October 1, 1991. This symposium is the 22nd of the 'Bari Meetings' that began in Bari in 1965 with the symposium 'Regulation of Mitochondrial Processes in Mitochondria'. The organizers feel that the old-style 'Bari-meetings' are still very stimulating scientific events with, not too broad topics, limited numbers of participants, and with emphasis on the presentation of papers by young members of the biochemical community. This volume contains the lectures presented at the symposium.

The topic of this meeting represents a particularly timely and, at present, exciting area. The vast field of biomembrane transport has, in recent years, made great strides towards a better understanding of its mechanisms by the molecular approach. More transport systems are being defined in terms of their catalysts, pumping systems and carriers. Also the description of transport has advanced to a better understanding of its regulation, control and the mechanism by which energy drives transport.

It is felt that the present proceedings represents a comprehensive and up-to-date record of the biochemical aspects of biomembrane transport. Major achievements as well as new openings in the field have been stressed in many of the contributions to this book. Thus, it represents a valuable source and reference book, comprising the most recent results in this area. Since transport systems in a variety of membranes such as cell membranes, mitochondria and bacteria are discussed, it should attract the attention of scientists from various fields, who are interested in biological transport.

The volume has been published as quickly as possible thanks to the efforts of the publishers whose co-operation is gratefully acknowledged.

The Editors

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SPECTROSCOPIC STUDIES OF MEMBRANE PROTEINS AND ASSOCIATED POLYPEPTIDES

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INTRODUCTION

Elucidation of the structure of membrane proteins remains an important challenge in biochemistry. Many important biological processes such as the transport of ions and molecules are mediated by membrane spanning proteins. Rapid advances in molecular biology are providing a wealth of information about the amino acid sequence of these proteins. However, as detailed structural data on these proteins are scarce, the molecular mechanism of such biological processes are as yet little understood. Application of X-ray diffraction and nuclear magnetic resonance (NMR) spectroscopy to the structural analysis of membrane proteins are being made but at a slow pace. We are exploring the technique of Fourier Transform Infrared (FT-IR) spectroscopy for studying proteins and polypeptides in a membrane environment.

Infrared spectroscopy is one of the earliest techniques to be applied to the study of protein structure. The potential of the technique was first demonstrated by the pioneering work of Elliott and Ambrose [1]. Initially, the problems encountered in the study of biomolecules included the low sensitivity of infrared spectrometers, absorption of liquid water over much of the infrared spectrum as well as difficulties in extracting information from the broad overlapping infrared bands. The advent of computerised FT-IR instrumentation has largely overcome most of these problems. It is now possible to obtain high signal-to-noise ratio spectra of dilute (1mM) protein solutions in H_2O. The background H_2O absorption can be digitally subtracted from the spectrum of the

protein solution. Furthermore, the broad overlapping bands can be examined in detail by the use of several mathematical procedures including deconvolution and second-derivative methods [2,3,4].

MEMBRANE PROTEINS

FT-IR spectroscopic studies of a large number of membrane proteins have been performed. These include, bacteriorhodopsin, rhodopsin, Ca^{2+}-ATPase, Na^+/K^+-ATPase, H^+/K^+-ATPase, photosynthetic reaction centres, cytochrome c oxidase, acetylcholine receptor and porin [3,4,5]. A particular feature of these membrane systems is the remarkable consistency in the frequency of the main amide I band associated with the α-helical structure present, except for the membrane proteins bacteriorhodopsin and porin. The latter is known to differ from other membrane proteins in having a ß-barrel structure [2,3,5]. Bacteriorhodpsin is an integral membrane protein found in the purple membrane of *Halobacterium halobium*. It is a light driven proton pump consisting of 248 amino acid residues with an *all-trans* retinal chromophore. FT-IR spectroscopic studies conducted in our laboratory as well as other laboratories have shown that the amide I frequency of this membrane protein is unusually high compared with what is expected for normal α-helical structure [6,7,8]. This high frequency band for bacteriorhodopsin has been attempted to be explained in terms of α_{II}-helices [7] or the presence of short 3_{10}-helical regions in addition to normal α-helices [8].

Hydrogen-deuterium exchange of membrane proteins studied using FT-IR spectroscopy has also been valuable for understanding membrane protein structure. A large number of proteins have been examined [2,3]. These include bacteriorhodopsin, rhodopsin, glucose transporter etc. The glucose transporter protein is found to have a remarkably high rate of hydrogen-deuterium exchange [9]. Almost 90% of its amide protons are exchanged within one hour. This was explained in terms of an aqueous pore present in this protein [9], an interpretation supported by other techniques [10].

Studies have also been performed to investigate the effect of ligands, lipid composition, pH and temperature as well as drugs on the structure of membrane proteins [2,3,4]. In a recent study for example we demonstrated dramatic changes in the infrared spectra of photosystem II reaction centre of higher plants as a function of light and temperature [11].

Information obtained using derivative and deconvolution methods applied to FT-IR spectra of proteins have been mainly of a qualitative nature. Quantitative studies have been made by curve-fitting analysis of the amide I band [2,3,4]. However, this approach has some difficulties and recent studies in our laboratory have led to the development of a more satisfactory procedure [12]. This method involves factor analysis of the infrared spectra of 17 proteins whose crystal structures are known from X-ray studies. A good correlation is observed between the infrared estimates and those calculated from X-ray data. This method gives standard errors of prediction 3.9% for α-helix, 8.3 % for ß-sheet, and 6.6% for turns. No preliminary treatment of the bands such as deconvolution is required. More recently we have applied the partial least squares method [PLS, see ref. 13] to our calibration set of 17 proteins and also extended the method to the FT-IR spectra of membrane proteins (see Table I). The accuracies of the FT-IR method compares very well with other spectroscopic

TABLE 1 Secondary Structure Prediction for Membrane Proteins using PLS

Membrane Protein	% α-helix	% ß-sheet	% turn
Ca^{+2}-ATPase	65.1	15.7	6.8
H^+/K^+-ATPase	47.6	33.0	21.5
Photosystem II Reaction Centre	60.0	17.6	22.2
Bacteriorhodopsin	71.5	5.8	-11.2

techniques such as Circular Dichroism (CD) and Raman spectroscopy. The results obtained for the membrane proteins (Table I) are consistent with previous estimations based on predictions from sequence data, electron diffraction and CD and Raman spectroscopic data.

MEMBRANE ASSOCIATED POLYPEPTIDES

The large size and complexity of membrane proteins makes it difficult to apply many of the techniques normally used in protein structural studies. Thus there is a need to find ways to simplify these systems so they can be made amenable for structural studies. Using solid-phase methods, polypeptide segments of large membrane proteins can be synthesised. We have began to use this approach to study polypeptide segments of a number of membrane proteins.

Mitochondrial transit peptide: Many proteins are synthesised in the cytoplasm of eukaryotic cells and then are translocated to their final destinations in subcellular organelles, for example the mitochondria, crossing one or more membranes in the process [14]. The information which a protein needs to select one intracellular membrane from several is contained in the initially-translated protein. The preprotein consists of the mature protein plus an N-terminal extension,referred to as a signal or transit sequence, of between 17 and 60 residues in length. In recent studies we have been using a combination of FT-IR, CD and NMR spectroscopy to understand the structural features of some of these systems. We have used these techniques to determine the structure of a mitochondrial transit peptide in trifluroethanol. The peptide MLSALARPVGAALRRSFSTSAQNNAK, is the N-terminal sequence of the precursor of rat malate dehydrogenase and two residues of the mature protein.

The primary structure of mitochondrial transit peptides are not conserved; however, they do have secondary and tertiary structural features in common. They are relatively rich in positively-charged (basic) amino acids (mainly Arg), they lack acidic residues, have a high content of hydroxylated residues and small groups of adjacent hydrophobic residues. Hydropathicity analyses have suggested that these peptides may form amphiphilic helices, with a hydrophobic

face and a positively charged face, and also the possibility of a two domain structure, consisting of helical segments connected by a turn or bend.

Our FT-IR and CD results (see Figure 1) indicate that the mitochondrial transit peptide (sequence shown above) adopts a predominantly α-helical conformation in trifluroethanol solution. This observation is supported by 2D NMR results which shows nuclear Overhauser enhancement (NOE) patterns characteristic of

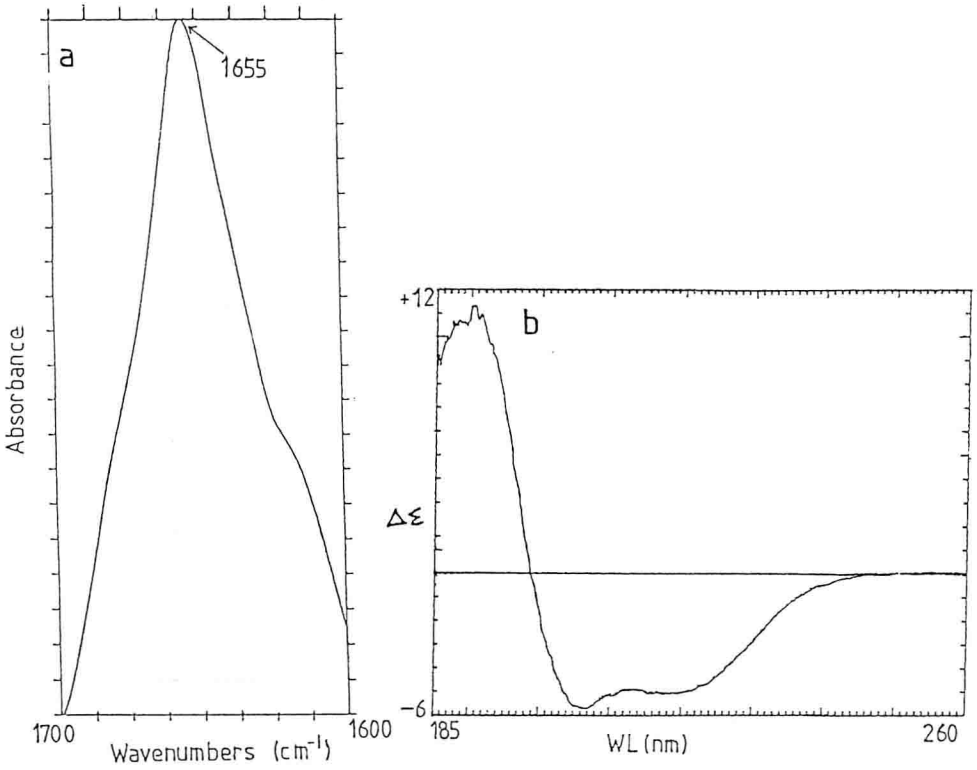

Fig. 1: a) FT-IR spectrum of the mitochondrial transit peptide. The amide I maximum at 1655cm^{-1} is consistent with a predominantly α-helical peptide conformation.
b) CD spectrum of the mitochondrial transit peptide. The minimum at 207nm and shoulder around 220nm are indicative of the presence of α-helical structure in the peptide.