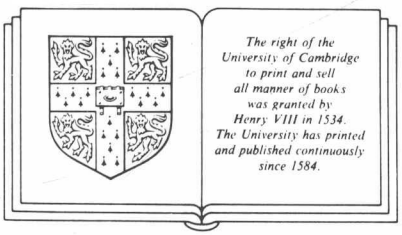
Yuji
Tonomura

Energy-transducing ATPases – structure and kinetics

Energy-transducing ATPases – structure and kinetics

Yuji Tonomura

Faculty of Science, Osaka University, Toyonaka, Osaka 560



Cambridge University Press

Cambridge
London New York New Rochelle
Melbourne Sydney

Published by the Press Syndicate of the University of Cambridge
The Pitt Building, Trumpington Street, Cambridge CB2 1RP
32 East 57th Street, New York, NY 10022, USA
10 Stamford Road, Oakleigh, Melbourne 3166, Australia

First published 1986

Printed in Great Britain at The Bath Press, Avon

British Library cataloguing in publication data
Tonomura, Yuji
Energy-transducing ATPases – structure and kinetics.
1. Muscles 2. Adenosine triphosphate
I. Title
612'.744 QP321

Library of Congress cataloging in publication data
Tonomura, Yuji.
Energy-transducing ATPases – structure and kinetics.
Includes index.
1. Adenosine triphosphatase. 2. Actomyosin.
3. Energy metabolism. I. Title.
QP609.A3T66 1986 599'.0133 85-26951

ISBN 0 521 30479 2

BS

Energy-transducing ATPases – structure and kinetics

Foreword

In 1972 Professor Yuji Tonomura published a voluminous monograph *Muscle Proteins, Muscle Contraction and Cation Transport* (University of Tokyo Press). This book summarizes Tonomura's famous discovery of M–P–ADP complex, the intermediate of the myosin ATPase reaction. Although this volume was highly valued by the specialists, general readers claimed that it was hard to understand.

When Tonomura was invited to write a book by the Cambridge University Press, he seriously considered this criticism and decided to make the new book as readable and accessible as possible, even for undergraduate students. For this purpose he asked his young associates and graduate students (see below) to write respective chapters in Japanese. He wanted to know how the younger generation comprehended the matter. Tonomura then scrutinized the manuscripts carefully, spending several years. He did not want to make the book too bulky, so he was trying hard to shorten the length of the manuscripts to half. Unfortunately, however, Professor Tonomura succumbed to a sudden heart attack on November 28, 1982.

At that time he was preparing his lecture, entitled 'The evolution of energy-transducing ATPases' to be delivered in my laboratory at the University of Tokyo on the next day. This problem was his last adventure and, in a sense, a conclusion to his long-lasting scientific pursuit. All Japanese scientists concerned with bioenergetics were keenly looking forward to this talk. If he had lived, the last chapter of this book might have contained the essence of this lecture.

After his death, I immediately started to talk about the manuscript with Professors Koichi Yagi, Tonomura's successor at Hokkaido University, and Koscak Maruyama, Tonomura's long-time friend (Chiba University). All of us wanted Tonomura's posthumous book to be published. So we requested his colleagues responsible for each chapter to

translate the Japanese version into English. Drs. Fumi Morita (Hokkaido University), Tohru Kanazawa (Asahikawa Medical College), and Taibo Yamamoto (Osaka University) cooperated with us in reviewing the English manuscripts. Thus the friendship and admiration felt for Tonomura by his friends and colleagues have made this book possible. Generous cooperation of the Cambridge University Press is also greatly appreciated, without which this book would not have been realized.

Finally, I should like to briefly refer to the late Professor Tonomura's career. He was born on February 9, 1923, in Nara, the capital of Japan in the eighteenth century. After graduation from the Department of Botany, University of Tokyo, in 1946, he worked on the kinetics of catalase under the supervision of the late Professor Hiroshi Tamiya, who profoundly influenced Tonomura personally as well as scientifically. In 1951, Tonomura started his life-long research on the actomyosin–ATP system together with Dr. Shizuo Watanabe at the Research Institute for Catalysis, Hokkaido University; in 1958 he became a Professor of Biochemistry there. He moved to Osaka University in 1963 as a Professor of Biology. He published 25 reviews and about 200 original papers (in English) in the field of contractile proteins, especially myosin ATPase, sarcoplasmic reticulum Ca^{2+}, Mg^{2+}-ATPase, Na^{+}, K^{+}-ATPase and other subjects.

I personally feel confident that the late Professor Yuji Tonomura's monumental book can now be presented to those who are interested in biological sciences. I wish to express my sincere gratitude to all those who have shared the task to publish this book. I am particularly grateful to those who have acted as moderators: Chapters 2 and 3, Akio Inoue and Toshiaki Arata; Chapter 4, Satoshi Ogihara; Chapter 5, Masami Takahashi; Chapter 6, Ichiro Matsuoka; Chapter 7, Taibo Yamamoto and Haruhiko Takisawa; Chapter 8, Motomori Yamaguchi and Junshi Sakamoto.

February 1985 at Okazaki —Setsuro Ebashi

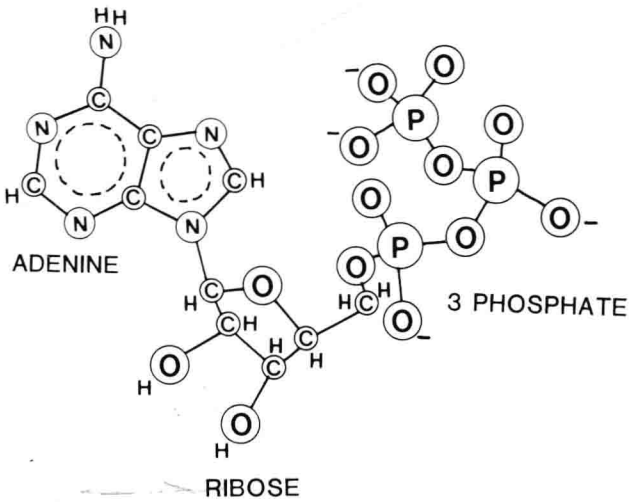
Contents

1 Prologue

Living cells synthesize adenosine 5′-triphosphate (ATP) from adenosine 5′-diphosphate (ADP) and inorganic phosphate (Pi) through glycolysis and oxidation–reduction reactions. By using the energy derived from ATP hydrolysis, cells carry out their fundamental physiological functions such as mechanical work and transport of various substances as well as biosynthesis of various materials.

Progress in research to elucidate the basic principle of biological energy transduction may be divided into three major phases. It was found by Lipmann (1941) that a high-energy phosphate compound, ATP (Fig. 1.1), plays key roles in a number of cell activities. ATP is relatively stable under physiological conditions; when hydrolyzed to ADP and Pi, however, it releases enough free energy for cells to carry out one

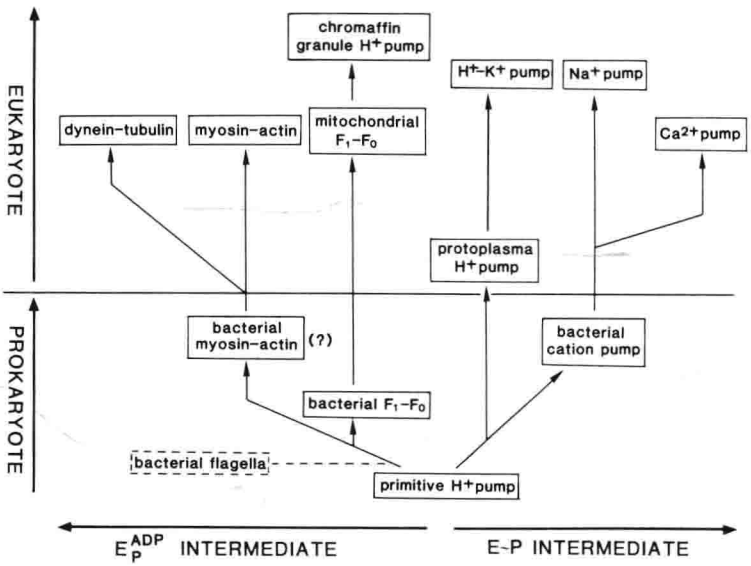
Fig. 1.1 Structure of the ATP molecule, after Kennard *et al.* (1971).



step of a chemical reaction. The free-energy change of ATP hydrolysis inside the living cell, $\Delta G'$ has been estimated to be between -12 and -13 kcal/mol (Curtin *et al.*, 1974).

The second major phase was the finding that each energy-transducing system has its own specific ATPase. In 1939, Engelhardt & Ljubimova found that the contractile protein of muscle (myosin) has an ATPase activity. Gibbons (1963) found another ATPase (dynein) involved in cell motility in cilia. In 1957, Skou found a Na^+,K^+-ATPase in the cell membrane, which is involved in the active transport of cations. This was followed by the discovery of the Ca^{2+},Mg^{2+}-ATPase of sarcoplasmic reticulum by Hasselbach & Makinose (1961). Furthermore, Racker's group identified mitochondrial membrane ATPase that plays a key role in ATP synthesis as a coupling factor (F_1). They also showed that a complex of F_1 with an oligomycin-sensitive factor (F_0) is actually the H^+-translocating ATPase (Penefsky *et al.*, 1960). Subsequently, this type of ATPase was found in chloroplasts (Avron, 1963), and also in bacteria (Abrams, McNamara & Johnson, 1960). The evolutionary relationships among the energy transducing ATPases discussed in this book are summarized in Fig. 1.2.

Fig. 1.2 The relationship among the various ATPases that appear in this book. The relations among these ATPases are discussed in Chapter 9.



The third major phase of progress in research on biological energy transduction is characterized by a deepened understanding of the molecular aspects of energy transduction. This was achieved through studies on the dynamic and energetic properties of the elementary steps in the ATPase reaction and on the coupling mechanism between the movement of the ATPase molecule and the elementary steps in the reaction.

The object of this book is to document in detail current knowledge of the energy-transducing mechanisms in the living cell, in particular those of the contractile and transport ATPases. Accordingly, we first describe the structural aspects of these ATPases. Secondly, the dynamic properties of the elementary steps of the ATPase will be discussed in detail. Thirdly, functional states of ATPase molecules within the cell will be considered as a basis for understanding the coupling of the elementary steps of the ATPase with the movement of the enzyme molecule.

It is thought that all biological energy-transducing systems have many similarities in the mechanism of ATP hydrolysis and the molecular movement during the ATPase reaction. Therefore, the studies of one energy-transducing system at the molecular level can assist the investigation of other energy-transducing systems. The relationships among the molecular mechanisms of the various energy-transducing systems will be summarized in the final chapter and discussed from an evolutionary point of view.

This account is not intended to give an overall view of the energy-transducing systems. Although very important to living cells, the properties of various nucleotide triphosphatases (NTPases) involved in protein syntheses and structural changes of DNA are left out, since these NTPases function by mechanisms different from those of the contractile and transport ATPases discussed here. These NTPases have already been described in detail by Kornberg (1980) in his superbly written book, and their reaction mechanisms have been described lucidly by Kaziro (1978).

References

Abrams, A., McNamara, P. & Johnson, F. B. (1960). Adenosine triphosphate in isolated bacterial cell membranes. *J. Biol. Chem.*, **235**, 3659–62.

Avron, M. (1963). A coupling factor in photophosphorylation. *Biochim. Biophys. Acta*, **77**, 699–702.

Curtin, N. A., Gilbert, C., Kretzschmar, K. M. & Wilkie, D. R. (1974). The

effect of the performance of work on total energy output and metabolism during muscle contraction. *J. Physiol.*, **238,** 455–72.

Engelhardt, W. A. & Ljubimova, M. N. (1939). Myosin and adenosine triphosphatase. *Nature*, **144,** 668–9.

Gibbons, I. R. (1963). The protein components of cilia from *Tetrahymena pyriformis*. *Proc. Natl. Acad. Sci. (USA)*, **50,** 1002–6.

Hasselbach, W. & Makinose, M. (1961). The calcium pump of the granules of the muscle and their dependence on adenosine triphosphate hydrolysis. *Biochem. Z.*, **333,** 518—26.

Kaziro, Y. (1978). The role of guanosine 5′triphosphate in polypeptide chain elongation. *Biochim. Biophys. Acta*, **505,** 95–127.

Kennard, O., Isacs, N. W., Motherwell, W. D. S., Coppola, J. C., Wampler, D. L., Larson, A. C. & Watson, D. G. (1971). The crystal and molecular structure of adenosine triphosphate. *Proc. R. Soc. A.*, **325,** 401—36.

Kornberg, A. (1980). *DNA Replication*, 2nd edn. San Francisco: W. H. Freeman and Company.

Lipmann, F. (1941). Metabolic generation and utilization of phosphate bond energy. *Adv. Enzym.*, **1,** 99–162.

Penefsky, H. S., Pullman, H. E., Datle, A. & Racker, E. (1960). Partial resolution of the enzymes catalyzing oxidative phosphorylation. II. Participation of a soluble adenosine triphosphatase in oxidative phosphorylation. *J. Biol. Chem.*, **235,** 3330–6.

Skou, J. C. (1957). Influence of some cations on an adenosine triphosphatase from peripheral nerves. *Biochim. Biophys. Acta*, **23,** 394–402.

2 Myosin ATPase

Muscle contraction has characteristics different from those of machines. Muscle cells can transduce chemical energy directly to mechanical work with high efficiency. Also, metabolic activity in the cell increases several hundred- to several thousandfold within 100 ms after excitation. Szent-Györgyi (1949) showed that muscle contraction with these characteristics is caused by a reaction between ATP and actomyosin, a complex of two proteins, actin and myosin. ATP is hydrolyzed by myosin, and this ATPase reaction of myosin is highly activated by F-actin. This highly activated ATPase, actomyosin ATPase, is coupled with muscle contraction.

In this chapter, we introduce first the structure of the muscle cell and the sliding filament theory, the basic theory of muscle contraction, proposed from the study of the structure of muscle cell. We then discuss the structure of myosin and the elementary steps of the ATPase reaction. Many books have been published on the mechanism of muscle contraction, notably the *Cold Spring Harbor Symposium on Quantitative Biology* (1973) and the book edited by Bourne (1973) cover various fields of study on muscle contraction. Needham (1971) wrote a history of the research in this field, and Tonomura (1972) wrote a monograph on the subject.

Muscle cells and structural proteins

Structure of muscle cells

The structure of muscle cells varies greatly with type. In the case of vertebrate skeletal muscle, the most popular and well studied, the muscle cell, called a muscle fiber, is 20–100 μm in diameter and

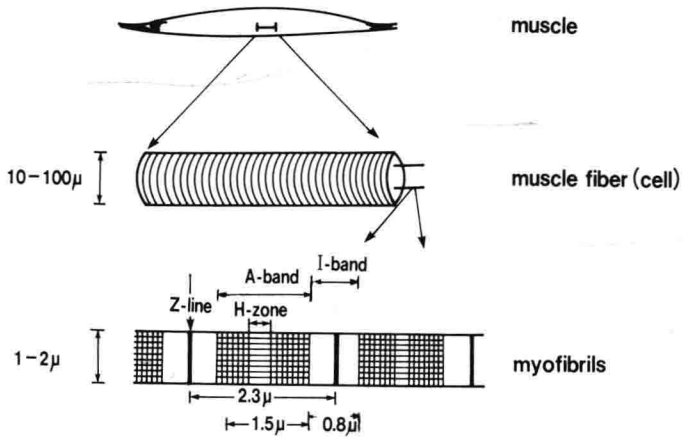
several centimeters in length. This huge multinucleate cell is formed by unidirectional fusing of the mononucleate myoblast during the process of development.

In living muscle fiber, components in the intracellular fluid are held stationary by the action of the plasma membrane, the sarcolemma. For the physiological study of muscle contraction, skinned fibers were prepared by mechanically removing the plasma membrane (Natori, 1954), or the membrane was made leaky by treatment with glycerol or other reagents (Szent-Györgyi, 1949).

Muscle cells have a special membrane system: the transverse (T)-system and the sarcoplasmic reticulum (SR; Porter & Palade, 1957). The T-system is a tubular structure which extends from the plasma membrane into the interior of the cell, where it joins with the SR which surrounds the contractile organ, the myofibrils. The merging of the T-system with the SR forms a structure called the triad. Excitation of the plasma membrane is transmitted to the SR through the T-system and induces the release of Ca^{2+} from the SR, thus causing the contraction of myofibrils.

Muscle fibers characteristically have many myofibrils lying parallel to the main axis of the fiber. Myofibrils are about 1–2 μ in diameter and contract with the addition of Mg^{2+}-ATP in the presence of a low concentration of Ca^{2+}. As shown in Fig. 2.1, the contractile materials in the myofibrils are organized within a repeating unit called the sarcomere. A sarcomere is divided by Z-lines and is 2.3 μm in length when

Fig. 2.1 The structure of striated muscle at three levels of organization. Dimensions shown are for rabbit psoas muscle.



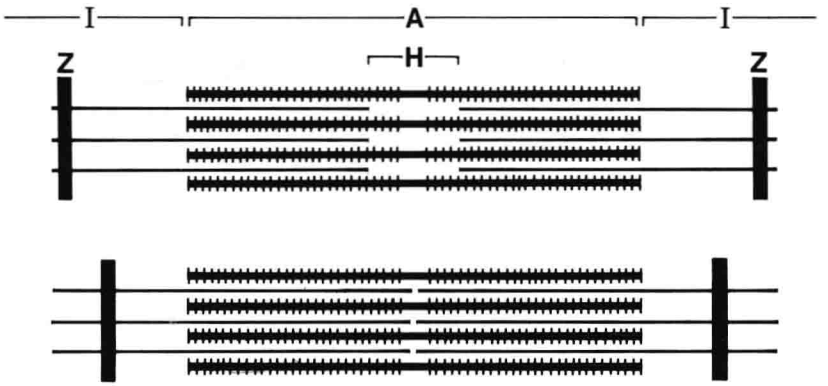
the muscle is at resting state. The central part of the sarcomere is dark under the light microscope and is called the A-band (anisotropic band). On either side of the Z-line are regions which appear light under the microscope; these together are called the I-band (isotropic band). The central part of the A-band, about 0.3 μm in width, has lower optical density than the rest of the A-band and is called the H-zone (see review by H. E. Huxley, 1960).

Structure of the sarcomere and the sliding filament theory of muscle contraction

A. F. Huxley & R. Niedergerke (1954) using living muscle and H. E. Huxley & J. Hanson (1954) using isolated myofibrils found independently that during physiological shortening both the length of A-band and the length from the Z-line to the end of the H-zone did not change, although the overall sarcomere length decreased. As shown in Fig. 2.2, they explained these results by proposing that two different protein filaments with constant lengths are distributed in the A-band and between the Z-line and the end of H-zone, respectively. Shortening is brought about by the sliding of these two filaments relative to each other. This model is generally called the 'sliding filament theory' of muscle contraction.

The two kinds of filaments proposed by these investigators were later identified by electron microscopy. The thick filaments, 1.5 μm in length

Fig. 2.2 Schematic view of the extent of overlapping of the thin filaments with the bridges from the thick filaments, based on the sliding filament theory of muscle contraction.



and 10 nm in width, are present in the A-band, and the thin filaments, 1.0 μm in length and 5–6 nm in width, are present running from the Z-line to the end of the H-zone (Fig. 2.2). This structure was confirmed by electron microscopic investigations of transverse sections of muscle fiber. Only thick filaments are observed in the H-zone and only thin filaments in the I-band. In the A-band, except for the H-zone, both thick and thin filaments are observed in a hexagonal arrangement (H. E. Huxley, 1957).

The A-band disappears after KCl extraction of myosin from myofibrils; the optical density of the I-band decreases markedly after extraction of actin with KI. On the basis of quantitative comparisons between the decrease in optical density and the quantity of the extracted proteins, it was demonstrated that the main component of thick filaments is myosin, and that of thin filaments is actin (Huxley, 1960). These findings agree well with findings from biochemical studies that the complex of actin and myosin (actomyosin) hydrolyzes ATP, and that muscle contraction is coupled with this ATPase reaction.

Within the framework of the sliding filament theory, the fundamental problem in the investigation of the molecular mechanism of muscle contraction is the clarification of the structure and function of the overlap region of the two filaments during the development of tension. The structure of the overlap region was investigated by Huxley and coworkers (Huxley, 1957; Huxley & Brown, 1967) using electron microscopy and X-ray diffraction. They found projections along the backbone of the thick filaments and thought that crossbridges formed between these projections and the thin filaments (Fig. 2.2). Consequently, it was proposed that muscle contraction includes at least the following three processes that are coupled with ATP hydrolysis: (1) the formation of crossbridges; (2) the movement of crossbridges; and (3) the dissociation of crossbridges (A. F. Huxley, 1957; Huxley, 1969).

The sliding theory has been widely accepted for two reasons. First, it was actually shown that the two kinds of filaments have the polarity that would be expected with this model. Second, it was found that the tension developed during isometric contraction at various sarcomere lengths was proportional to the length of the overlap region between the part of the thick filament with projections and the thin filament (Gordon, Huxley & Julian, 1966).

The sliding filament theory of muscle contraction provides a framework for the understanding of the molecular mechanism of muscle contraction. Further progress in this field was made with the discovery of

the Ca^{2+}-regulatory system of muscle contraction. Ca^{2+} is released from the sarcoplasmic reticulum as described above. Ca^{2+} receptors differ according to muscle type, as will be described in the next chapter. In the case of vertebrate skeletal muscle, the thin filaments are composed of F-actin and the regulatory proteins, tropomyosin and troponin, and muscle contraction is induced by the binding of Ca^{2+} to troponin.

Structure of myosin

'Myosin', a structural protein of muscle, was discovered and named by Kuhne in 1859. Studies of energy transformation were initiated by the discovery by Engelhardt & Ljubimova (1939) that myosin has an ATPase activity. Subsequently, Banga & Szent-Györgyi (1941) discovered that the substance previously called 'myosin' is a complex of myosin and actin, another important structural protein of muscle. This complex is also called actomyosin or myosin B.

Myosin is located in the A-band of myofibrils where it forms thick filaments. It constitutes 54% of the total protein of myofibrils. Since myosin is soluble at high ionic strength (more than 0.3 M KCl) and forms the thick filaments, and precipitates at low ionic strength, it can be purified easily.

Myosin plays a central role in muscle contraction and has the following three important functions: (1) formation of thick filaments at low ionic strength; (2) hydrolysis of ATP into ADP + Pi; and (3) binding with F-actin to form crossbridges. This section describes the structure of myosin emphasizing these functions. For further information on the structure of myosin see reviews by Perry (1967), Tonomura (1972), Yagi (1975) and Margossian & Lowey (1982).

Size and shape of the myosin molecule

Myosin has a molecular weight (m.w.) of about 480 000. It decomposes into peptides (subunits) in a solution of guanidine HCl, urea, high alkali or sodium dodecyl sulfate (SDS). The myosin molecule consists of two heavy chains (HC) of molecular weight 200 000 and four light chains (LC) of molecular weights from 15 000 to 25 000.

The size and shape of the myosin molecule have been studied by hydrodynamic methods, light scattering and precisely by electron

microscopy (Slayter & Lowey, 1967; Elliott & Offer, 1978; Takahashi, 1978). As shown in Fig. 2.3, myosin is a rodlike protein with two globular tips. The total length of the myosin molecule is about 160 nm. Two heads about 10 nm in length and 5 nm in diameter are connected to a tail 140 nm in length and 3 nm in diameter. A bend is often observed around 63–73 nm from the end of the tail.

Structure of the myosin filament

At low ionic strength myosin forms a filament essentially the same as that of the thick filament in myofibrils (Huxley, 1963). As shown

Fig. 2.3 Electron micrograph of native thick filament from rabbit psoas muscle, freeze dried and shadowed with platinum. (From Trinick & Elliott, 1979.)