生物化学与分子生物学前沿研讨班

Workshop on New Trends in Biochemistry and Molecular Biology

中国生物化学与分子生物学会
北京生物化学与分子生物学会

1994 年 9 月

Workshop on New Trends in Biochemistry and Molecular Biology

中国生物化学与分子生物学学会

北京生化学会

The Workshop was Organized by
Chinese Society of Biochemistry and Molecular Biology
Beijing Society of Biochemistry and Molecular Biology

The Workshop was sponsored by
The International Union of Biochemistry and Molecular Biology
Federation of Asian and Oceanian Biochemists and Molecular Biologists Inc.

前　言

“生物化学与分子生物学进展研讨班”是由我会与北京生物化学与分子生物学会联合主办的一次涉及本领域发展前沿的研讨班。在“八五”计划即将结束，“九五”计划行将开始之际主办这个研讨班，其目的是想使现在从事科学研究和教学的高、中级人员能了解生物化学与分子生物学各主要领域的最新进展和新技术、方法，开阔思路，提高知识水平，改进科研和教学质量，为“九五”期间更好创新做出贡献。

这此研讨班得到了国际生物化学与分子生物学联合会和亚太地区生物化学家与分子生物学家联合会的支持和赞助。两个联合会组织了五名国外著名专家在研讨班上介绍他们工作的最新结果和国际最新进展。

这此研讨班也得到国内专家们的支持，许多国内院士、教授等知名学者都在班上讲授和介绍他们的工作和有关领域的发展。

这此研讨班是由我会农业生化专业委员会和教育委员会承办的，在研讨班的筹备和主办期间他们和北京大学生命学院等都做了大量工作，使研讨班的以顺利进行。

在此，我们向所有支持、赞助和为研讨班工作的人员表示由衷的感谢。

中国生物化学与分子生物学会

1994年9月

目　录

CONTENTS

THE CONCEPT OF ENERGY-RICH PHOSPHATE COMPOUNDS: WATER TRANSPORT ATPases AND ENTROPIC ENERGY

Leopoldo de Meis

Instituto de ciencias Biomedicas, Dept. Bioquimica, Universidade Federal do Rio de Janeiro, Rio de Janeiro, CEP 21941- 590, Brazil. FAX int +55 21 270-8647.

The concept of "energy- rich" phosphate compounds was formalized by Lipmann (1) in a review published in 1941 which determined the way bioenergoties was viewed during the following three decades. The notion prevailing at that time were:

1) The energy derived from the hydrolysis of a phosphate compound would be determined solely by the chemical nature of the bond which links the phosphate moiety to the rest of the molecule.

2) The energy of hydrolysis of "energy-rich phosphate" compounds such us ATP. PP. aspartyl phosphate, phosphoenolpyruvate and creatine phosphate would be the same regardless of whether they were bound to the enzyme or free in solution. In water the G° for the hydrolysis of these compounds is in the range of -8 to - 12 kcal/mol. phosphoesters such as glucose 6-phosphate and glycerol phosphate were not considered "energy-rich" phosphate compounds. The G° for the hydrolysis of phosophoester varies between -1.5 and -2.5 kcal/mol.

3)The transfer of phosphate from one molecule to another was thought to be determined exclusively by the energy of hydrolysis of the bonds involved. The -phosphate of ATP could be transferred to a molecule of glucose forming glucose 6-phosphate, but the reverse reaction could not occurs without an extra imput of energy. Thus, the ATP hydrolyzed in the cell could only be regenerated starting from phosphate compounds having the same, or a higher energy of hydrolysis than ATP itself such as creatine phosphate. On the basis of the formulations described above , the sequence of events during the process of energy transduction in enzymes was thought to be :

(A) The enzyme binds ATP; (B) ATP is hydrolyzed and energy is released at the catalytic site at the precise moment of hydrolysis of the phosphate bond; (C) The energy is immediately absorbed by the enzyme and used to perform work. For the synthesis of ATP from ADP and Pi, the sequence of events would be similar, but in the reverse order:

(A) The enzyme now would bind ADP and Pi; (B) An energy imput at the catalytic site would drive the synthesis of ATP; (C) Once formed, the ATP molecule would dissociate form the enzyme and diffuse into the cytosol without any further need of energy. From these sequences, it was inferred that the work would be performed in a part of the enzyme molecule where energy is released, i. e. in the immediate vicinity of the catalytic site. During the past two decades it has been found that the energy of hydrolysis of ditterent phosphate compounds varies greatly depending on whether they are in solution or bound to the enzyme. Reactions that were thought to be practically irreversible in aqueous solution occur spontaneously when the reactants are bound to the enzyme (for reviews, see 2 to8). The energy for work does not become available to the enzyme at the moment of the phosphate compound cleavage and the sequence of events for the transduction of energy in transport ATPases seems to be:

(A) The enzyme binds ATP or other phosphate compounds; (B) the enzyme performs work without the phosphate compound being hydrolyzed. This is accompanied by a decrease in the energy level of the phosphate compound (C) then the phosphate compound is cleaved and the products of hydrolysis dissociate from the enzyme in a process which involves relatively small energy change. In the reverse process, phosphate compounds such as ATP, PPi and acyl phosphate residue are synthesized on the catalytic site of the enzyme without the need of energy. Energy is then needed for the conversion of the phosphate compound from " low energy" into "high energy". This occurs at the catalytic site of the enzyme before the phosphate compound synthesized is released into the cytosol. The information now available on the structure of transport ATPases indicate that work is performed in a region of the tertiary structure of the protein distant form the region where the catalytic site is located and that conformational changes of the protein syneronize the sequence of events occuring in these two regions.

The events responsible for the change in the energy level of phosphate compound at the catalytic site of the different enzymes are not clearly understood at present. For transport ATPases, there is experimental evidence suggesting that one important factor is a change of water activity in the environment of the catalytic site (7,8).

The notion that work is performed by the enzyme before or after cleavage of the phosphate compound is related to the contribution of enthalpy (Ho) and entropy (So) to the free energy of hydrolysis according to the equation:

$$Go=Ho-T\ So$$

At the time of Lipmann review (1), the contribution of entropy in processes of energy transduction was thought to be minimal, thus free energy and enthalpy would be practically the same. Possible interactions of the phosphate compound with solvent, proteins and physiological ions were not taken into account. We know now that these interactions play an important role in determining the entropy of some, but not all hydrolytic reactions. For instance, the Go for the hydrolysis of PPi varies greatly depending on the water activity, pH and divalent cation concentration of the medium (9-11). These changes are related mainly to changes of the entropy of the reaction. On the other hand, water activity, pH and divalent cation have only a small effect on the G° of phosphoesters such as glucose 6- phosphate and phosphoserine (12). As a results, in different enzymatic systems glucose 6-phosphate and hexokinase can be used as an ATP regenerating system (13-15), a condition that in earlier works (1) was thought to be impossible.

REFERENCES

1. Lipmann, F. (1941) Advancesin Enzymology 1, 99-162.
2. George, P., Witonsky, R. J., Trachtman, M., Wu, C., Dorwatr, W.., Richman, L., Richman, W., Shuray, F. and Lentz, B. (1970) Biochim. Biophys. Acta 223, 1-15.
3. Hayes, M. D., Kenyon, L. G., and kollman, A. P. (1978) J. Am. Chem. Soc. 100, 4331-4340.
4. De Meis, L. and Vianna, A. L. (1979) Ann. Rev. Biochem. 48, 275-292.
5. Guldman, Y. E. and Brenner, B. (1987) Ann. Rev. Physiol. 49, 629-636.
6. Goldman, Y. E. (1987) Ann. Rev. Physiol. 49, 637-654.
7. De Meis, L. (1989) Biochim. Biophys. Acta (Reviews) 973, 333-349.
8. De Meis, L. (1994) Arch. Biochem. Biophys.
9. De Meis, L. (1984) J. Biol. Chem. 259, 6090-6097.
10. De Meis, L., Behrens, M. I., Petretsky, J. H. and Politi, M. J. (1985) Biochemistry 24, 7783-7789.
11. Wolfenden, R. and Williams, R. (1985) J. Am. Che. Soc. 107, 4345-4346.
12. Romero, P. and de Meis, L. (1989) J. Biol. Chem. 264, 7869-7873.
13. Montero-Lomeli, M., and de Meis, L. (1992) J. Biol. Chem. 267, 1829-1833
14. De Meis, L. Grieco, M. A., and Galina, A. (1992) FEBS Lett. 308, 197-201.
15. De Meis, L., Monteiro-Lomeli, M., Grieco, M. A., and Galina, A. (1992) Ann. N. Y. Acad. Sci. 671, 19-31.

THE CHALLENGE OF THE EDUCATION OF BIOCHEMISTRY IN DEVELOPING COUNTRIES.

L. de Meis

Inst. Ciencias Biomedicas, Dept. Bioquimica,
Universidade Federal Rio de Janeiro, Rio de Janeiro, Brazil.

Science in an institutionalized form began in the midninteen century. This led to a massive increase of new knowledge creating a complete change of the "way of life" of men in the planet. The production of knowledge that lay behind these changes originated in few countries: the USA, Britain, Germany, Japan, France, Canada and Russia. At present the scientists of these countries are responsible for 75% of the scientific papers published in indexed journals each year. These countries have been referred to as " producers of knowledge". The rest of the planet, representing 83% of the word population, consume this knowledge and their social and economic development are closely dependent on those countries that generate knowledge. There is a large difference in the young population aged 0 to 24 years between the populations of developed and developing countries, a difference that has increased over the last decade. The challenge faced by education is that countries which did not participate of the scientific revolution of the past 150 years are responsible for the education of the largest young population of the world. Furthermore, if there is an attempt to keep education atualized, this situation is aggravated by the overwhelming amount of new information produced each year in the different fields of knowledge.

REFERENCES

1. The stereotyped Image of the Scientist Among Students of Different Countries: Evoking the ALchemist? L. de Meis, R. C. P. Machado, P. Lustosa, V. R. T. Soares, M. T. Caldeira and L. Fonseca. Biochemical Education (1993) 21, 75-81.
2. The learning process in science: A study among brazilian biochemists . L. de Meis, P. H. Longo and E. B. M. Falcao Biochemical Education (1989) 17, 127-132.
3. Science and Industry in developed and developing countries. L. de Meis , R. Machado, F. Lessa and V. M. Rumjanek. Ciencia e cultura. (1991) 43, 278-281.
4. Cienciametria Y Evaluaciones por los propios investigadores. L. de Meis, R. Machado, L. Fonseac, P. Lustose and M. T. Caldeira. Interciencia. (1992) 17, 40-43.
5. Science and Art: Concepts and Misconceptions. L. de Meis, A. Braga, V . M. Rumjanek and F. Barrai. Biochemical Education (1993) 21, 195-196

THE USE OF MOLECULAR BIOLOGY AND PROTEIN CHEMISTRY IN THE CHARACTERIZATION OF THE P-TYPE CATION TRANSPORT ATPases.

Giuseppe Inesi.

Department of Biological chemistry, University of Maryland school of Medicine,

Baltimore, Maryland 21201, U. S. A.

Cation transport ATPases are a widely represented class of enzymes which are responsible for maintaining cation and osmotic gradients across plasma and intracellular membranes. The p-type cation transport ATPases share the common feature of forming a covalent phosphorylated intermediate (E-P) upon utilization of ATP. A main polypeptide chain of approximately 110,000 MW is the sole or principal operator of both catalytic and transport functions, although additional subunits or dimerization of the main chain occur in some cases. Early sequencing efforts on accessible peptide segments were followed by cloning and sequencing of full length cDNAs encoding various cation ATPases. For the same type of cation transport ATPase (e. g. , intracellular Ca^{2+} ATPase, plasma membrane Ca^{2+} ATPase, Na^{+}, K^{+} ATPase) various isoforms resulting from alternate splicing of the primary RNA transcript, have been described. The main chain of each enzyme includes a relatively large extramembranous domain and a number (eight or ten) of transmembrane helical segments which penetrate the membrane bilayer and return to the extramembranous domain in the form of (four or five) hairpins. Another common structural feature is the presence of an aspartyl residue which is the specific acceptor of ATP terminal phosphate for formation of E-P during the catalytic cycle. Alignment of amino acid sequences reveals extensive homology in the isoforms of the same cation ATPases, but relatively little homology in different cation ATPases. On the other hand, all cation ATPases retain a consensus sequence of high homology, spanning the distance between the phosphorylation site and the preceding transmembrane helix. Chemical and mutational analysis reveal that the catalytic (ATP binding and phosphorylation) domain resides within the globular head formed by the folded extramembranous segment of the ATPase chain. Information regarding the location of the cation binding domain is avaliable only for the intracellular Ca^{2+} ATPases. Mutational and recombinant analysis of these enzymes indicate that Ca^{2+} complexation occurs within the membrane bound ATPase region, with the cooperative intervention of four transmembrane helices which are clustered to form a tight channel. This region appears to be the target of thapsigargin, a highly specific inhibitor of intracellular Ca^{2+} ATPases. As the catalytic and cation binding domains are separated by a distance of approximately 50 A, a long range functional linkage is required to couple ATP utilization (i. e., enzyme phosphorylation) to

vectorial displacement of bound Ca^{2+}. Mutational analysis suggests that the homologous sequence intervening between catalytic and cation binding domains plays an important role in the long range functional linkage. Additional sequences at the carboxyl terminal provide regulatory domains in certain ATPases.

REFERENCES

1. Clarke, D. M., Loo, T. W., Inesi, G. & MacLennan, D. H. (1989) Nature 339, 476-478
2. De Meis, L. & Vianna, A. (1979) Annu. Rev. Biochem. 48, 275-292
3. Glynn, I. M. & Karlish, S. J. D. (1990) Annu. Rev. Biochem. 59, 171-205
4. Glynn, I. M. & Karlish, S. J. D. (1975) Annu. Rev. Physiol. 37, 13-55
5. Greeb, J. & Shull, G. E. (1989) J. Biol. Chem. 264, 18569-18576
6. Gryczynski, I., Wiczk, W., Inesi, G., Squier, T. C. & Lakowicz, J. R. (1989) Biochemistry 28, 3490-3498
7. Gunteski-Hamblin, A.-M., Greeb, J. & Shull, G. E. (1988) J. Biol. Chem. 263, 15032-15040
8. Inesi, G. & Kirtley, M. E. (1990) J. Membr. Biol. 116, 1-8
9. Inesi, G., Lewis, D., Nikic, D., Hussain, A., & Kirtley, M. E. (1992) Adv. in Enz. In press.
10. Inesi, G., Sumbilla, C. & Kirtley, M. E. (1990) Physiol. Rev. 70, 749-760
11. MacLennan, D. H., Brandl, C. J., Korczak, B. & Green, N. M. (1985) Nature 316, 696-700
12. Sagara, Y. & Inesi, G. (1991) J. Biol. Chem. 266, 13503-13506
13. Serrano, R., Kielland-Brandt, M. C. & Fink, G. R. (1986) Nature 319, 689-693
14. Shull, G. E., schwartz, A. & Lingrel, J. B.(1985) Nature 316, 691-695
15. Strehler, E. E., Strehler-page, M.-A., Vogel, G. & Carafoli, E. (1989) Proc. Natl. Acad. Sci. USA 86, 6908-6912
16. Verma, A. K., Filoteo, A. G., Stanford, D. R., Wieben, E. D., Penniston, J. T., Strehler, E. E., Fischer, R., Heim, R., Vogel, G., Mathews, S., Strehler-Page, M.-A., James, P., Vorherr, T., Krebs, J. & Carafoli, E. (1988) J. Biol. Chem. 263, 14152-14159

PLANT AND FUNGAL VACUOLES: BIOCHEMICAL AND PHYSILOGICAL ASPECTS IN CELL GROWTH AND IN CELLULAR ION HOMEOSTASIS

Yasuhiro Anraku

Department of Plant Sciences, Graduate School of Science,

University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

The fungal vacuole, like the plant vacuoles, is an acidic compartment and plays indispensable roles in metabolic storage and in cytosolic ion and pH homeostasis. In addition, it functions in endolytic macromolecular degradation in a manner similar to that ocurring in phagocytotic animal lysosomes (Figure 1). The acidification of these organelles is accomplished by proton-translocating ATPase belonging to the V-type H^+ - ATPase superfamily by a mechanism of chemiosmotic coupling of H^+ and Cl^- transports (Anraku et al., 1992).

In 1981, we discovered the yeast vacuolar proton- translocating ATPase(V-ATPase)as the first member of the V- ATPases, which are now known to be ubiquitously distributed in eukaryotic vacuo- lysosomal organelles and archaebacteria. The V-ATPases are also present in plasma membranes of vertebrate renal tissues, osteoclasts and insect gastrointestinal and sensory epithelia. The enzyme is bafilomycin A1-and nitrate-sensitive, and is inhibited by DCCD under steady- state conditions. V-ATPases in most fungal, plant and animal cells are large multimeric enzymes with a functional molecular mass of about 500 kDa and composed of at least nine subunits. The peripheral and catalytic complex of the enzyme contains subunits with Mr of 69, 60, 54, 42, 36, 32, 27kDa (see Figure 2) whereas the integral and proton- translocating sector is composed of at least 2 or 3 hydrophobic subunits (100 and two 17kDa polypeptides). The yeast V-ATPase catalyses ATP hydrolysis by a mechanism similar to that mitochondrial and bacterial F_0F_1-ATPases do, thus it be called a big sister of F_0F_1.

Up to now, nine VMA genes(for Vacuolar Membrane ATPase) that are indispensable for the expression of V- ATPase activity have been identified and cloned in the yeast Saccharomyces cerevisiae. We found that null vma mutants show Pet-cls phenotypes: The vma1 and other vma disruption mutants can grow well in YPD medium(2% Bacto- yeast extract -2% polypeptone and 2% glucose), indicating that each VMA gene is not essential for growth. However, they all are unable to grow on YPD plate containing 100 mM $CaCl_2$ and on Yp plate (2% Bactoyeast extract and 2% polypeptone)_ containing nonfermentable carbon source such as glycerol. Most VMA genes have been

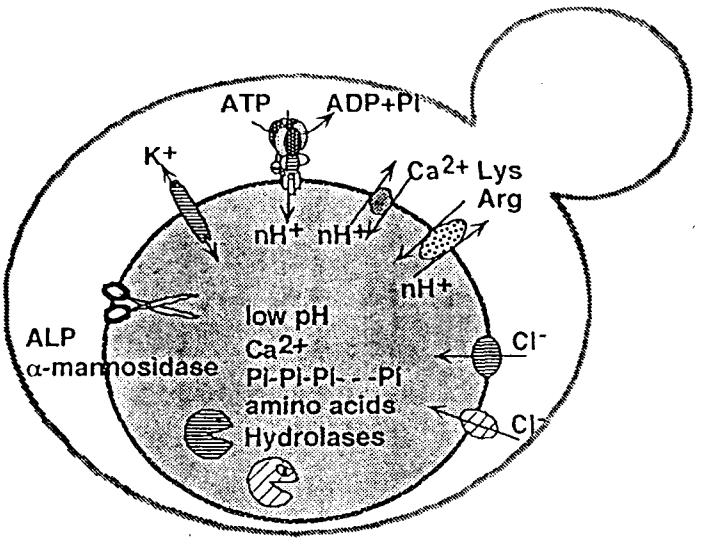
identified by screening for the Pet- cls phenotype. Current list of the VMA genes and the functions of their products will be given in details.

We have also demonstrated that the yeast vacuole is the center for regulation of ionic homeostasis in the cytosol (Anraku et al. 1992) . Even if the family of the VMA genes is all present and normal, the large volume of a central vacuole is needed physiologically to confer on the organelle a high capacity for maintenance of homeostatic levels of cytosolic free calcium and other ions. We have developed several genetic methods for isolating yeast mutants defective in vacuolar morphogenesis and identified VAM genes (for vacuolar morphology) involved in acquisition of large vacuoles. The function of the VAM genes in vacuolar morphogenesis will be summarized and discussed (Wada et al. 1992).

References

1. Anraku, Y, Umemoto, N, Hirata, R. & Ohya, Y(1992) J. Bioenerg. Biomembr., 24:395
2. Wada, Y, Ohsumi, Y, & Anraku, Y(1992) J. Biol. Chem., 267:18665

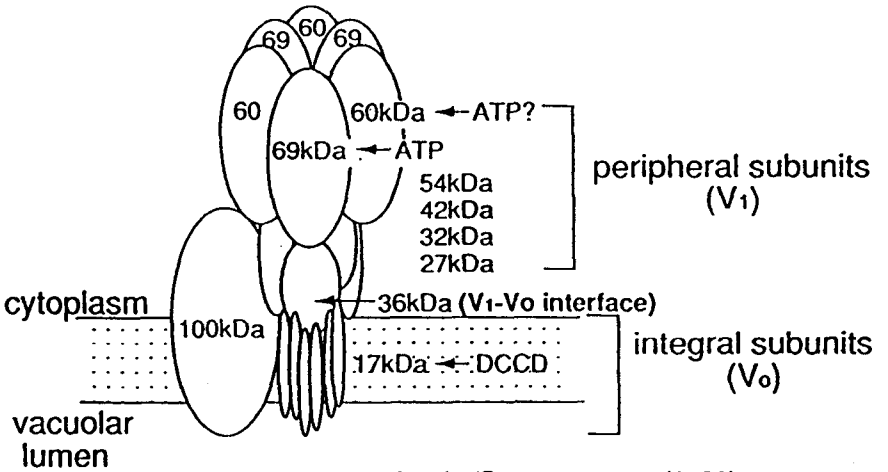
Figure 1



Chemiosmotic and Metabolic Functions in Yeast Vacuole

Figure 2

Structural model for the yeast vacuolar H^+-ATPase



Anraku/Stevens groups (1993)

MEMBRANCES AND DISEASE

C. A. Pasternak, Director of OIBC

Current address: Dept of Cellular and Molecular Sciences, St George's Hospital Medical School

(University of London), London SW17 ORE (UK)

Many diseases are associated with altered membrane function: some can be ascribed to changes in membrane lipids, others to changes in membrane proteins.

We have examincd changes in two basis functions of the surface membrane. The first concerns the uptake of glucose, which is increased by stress-like situations such as a virus infection or hyperthermia: in each case glucose uptake is increased by a mechanism similar to that by which insulin promotes glucose uptake.

The second situation concerns the induction of an increased permeability to ions and metabolites by clinically important pore -forming compounds as varied as bacterial toxins and immune molecules. In each case the effect can be ameliorated by simple ions like zinc. This study has led to the recognition of novel properties of flow through any narrow pore.

Control of membrane permeability by divalent cations: from biophysics to medicine

C. A. Pasternak

The action of pore - forming agents as diverse as clinically- important bacterial or animal toxins and immune molecules on susceptible cells is inhibited by divalent cations and protons(1, 2) . Elucidation of the mechanism by which this protective effect is exerted has led to the realisation -that flow through any narrow pore. , including endogenous ion channels, is affected by divalent cations in the same manner (3).

1. Bashford, C. L., Alder, G. M., Menestrina, G. Micklem, K. J., Murphy, J. J. & Pasternak, C. A. (1986) Membrane damage by hemolytic viruses, toxins, complement and other cytotoxic agents: a common mechanism blocked by divalent cations. J. Biol. Chem. 261:9300-9308
2. Pasternak, C. A. (1987) Virus, toxin, complement: common actions and their prevention by Ca^{++} or Zn^{2+}. BioEssays 6:14-18