The pH Optimum of Tissue ATP-ases

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Introduction

Recently, the specificity of histochemical demonstration of ATP-ase was investigated by many workers¹⁻¹⁰⁾. As a result, boundless uncertainties in the histochemical demonstration of ATP-ase were more distinctly delineated. However, it was by the use of acid ATP-ase medium,^{9,10)} which enabled histochemical demonstration of cytoplasmic or mitochondrial ATP-ase, that most of the uncertainties which had been common to all of them were elucidated.

It is generally accepted that adenosine triphosphate (ATP) is a universal constituent of the organism, and with its terminal high-energy phosphate radical, it plays a part of fundamental importance in biological exchanges of energy. Therefore, it seems likely that every tissue component can utilize ATP at a certain pH optimum which is specific to each tissue component, specific to the coupled enzyme-catalyzed reactions in complex biochemical systems taking place in different fashions in each tissue component. However, in spite of biochemically substantiated presence of ATP-ase in the nucleus,^{11,12)} ATP-dephosphorylating activity of the nucleus was low in the substrate mixtures of both acid and alkaline pH employed. Besides there were some tissue components which showed neither acid nor alkaline ATP-ase activity.

The present study was designed to investigate the presence of ATP-dephosphorylating enzyme (ATP-ase), of which the pH optimum is other than pH 5 or pH 9.

Materials and Methods

The animals used were albino rats and rabbits, and the sections employed were paraffin sections. For a detailed description of preparing paraffin sections and incubating media, readers are referred to Maekawa et al.⁹⁾ or Nakamoto.¹⁰⁾ In this paper, only the characters and compositions of incubating media of various pH with different PO_4 -binding cations, which is one of the subjects of this investigation, will be given in detail.

As is well known, in the histochemistry of dephosphorylating enzymes with lead phosphate or calcium phosphate method, one PO_4 -binding cation cannot be employed in all the media of pH 3 to pH 11, because quantitative precipitation of lead phosphate or lead calcium occurs only in a certain limited pH range.

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shown in Table 3, the intensity of ATP dephosphorylation was highest in the media containing Pb alone between pH 4.0 and pH 6.0, in the media containing Pb and Ca between pH 6.0 and pH 8.0, and in the media containing Ca alone between pH 8.0 and pH 10.0. And if the media of pH 4 to pH 10 was divided into 3 groups, acid, neutral and alkaline, the difference in the final concentrations of ATP and PO_4 -binding cations can be minimized in each group which consists of the same PO_4 -binding cation or cations and ATP of nearly the same concentration. The compositions of the media finally employed were shown in Table 4. Thus, at pH 6 and pH 8, ATP-ase was demonstrated with 2 or 3 media containing different PO_4 -binding cations in order to see the difference in the effect of each PO_4 -binding cation on tissue ATP-ases.

Results

I. Effects of Pb and Ca upon different tissue ATP-ases at the same pH. As shown in Table 5, Ca markedly inhibited cytoplasmic ATP-ase of rat

Table 3 Intensity of Reactions in the Media of the Same pH with Different PO_4 -binding Cations

pH	3	4	5	6	7	8	9	10	11
Pb ⁺⁺	±	†	‡	†	+	+			
Pb ⁺⁺ +Ca ⁺⁺	—	±	±	†	‡	†	‡	‡	‡
Ca ⁺⁺						‡	‡	‡	‡

Table 4 Compositions of Incubating Media Finally Employed

Acid Group (from pH 4.0 to pH 6.0)		Neutral Group (from pH 6.0 to pH 8.0)		Alkaline Group (from pH 8.0 to pH 10.0)	
M/5 Acetate buf.	: 2.0cc	M/5 Tris buf.	: 2.0cc	M/10 Veronal buf.	: 4.0cc
2% Pb(NO ₃) ₂	: 0.6cc	2% Pb(NO ₃) ₂	: 0.3cc		
		3% CaCl ₂	: 0.5cc	3% CaCl ₂	: 1.0cc
ATP-2Na	: 10mg	ATP-2Na	: 10mg	ATP-2Na	: 10mg
Distilled water	: 7.4cc	Distilled water	: 7.2cc	Distilled water	: 5.0cc

To see the effect of Mg, 0.1cc of 1% MgCl₂ was added to the above respectively.

Table 5 Rat Liver ATP-ase at pH 5.0 in the Media with Different Cations

Cations	Pb	Pb+Mg	Pb+Ca
Hepatic Cells	‡	‡	±
Bile Canaliculi	—	—	—

Table 6 Rat Liver ATP-ase at pH 7.0 in the Media with Different Cations

Cations	Pb	Pb+Mg	Pb+Ca
Hepatic Cells	—	—	—
Bile Canaliculi	—	†	‡

Table 7 Ret Heart ATP-ase at pH 8.0 in the Media with Different Cations

Cations	Pb	Pb + Ca	Ca
Cardiac Muscles	++	—	++
Capillaries	—	—	##

Table 8 Rat Liver ATP-ase at Various pH

pH	4	5	6	7	8	9	10
Hepatic Cells	++	##	++	±	—	—	±
Bile Canaliculi	—	—	++	##	±	++	##
Endothelia of Central Veins	—	—	—	±	++	±	—

Table 9 Rat Pancreas ATP-ase at Various pH

pH	4	5	6	7	8	9	10
Acinar Cells	+	##	+	±	—	—	±
Islets Cells	—	±	—	+	++	##	++
Intralobular Ducts	—	—	—	++	##	+	±
Capillaries	—	—	—	—	±	##	++

Table 10 Rat Kidney ATP-ase at Various pH

pH	4	5	6	7	8	9	10
Glomeruli	—	±	—	—	+	++	++
Proximal Convolution {	—	—	+	++	##	##	##
Brush Borders							
Cytoplasm	++	##	++	+	±	±	±
Distal Convolution: Cytoplasm	+	++	+	±	—	—	—
Collecting Tubules: Cytoplasm	+	++	+	±	—	—	—
Capillaries	—	—	—	—	+	++	++

Table 11 Rabbit Brain ATP-ase at Various pH

pH	4	5	6	7	8	9	10
Nuclei of Nerve Cells	—	—	±	##	++	+	±
Axons	—	—	+	##	++	+	—

Table 12 Rabbit Adrenal ATP-ase at Various pH

pH	4	5	6	7	8	9	10
Cortical Cells	++	##	++	—	—	—	—
Nuclei of Cortical Cells	—	—	+	##	##	##	±
Capillary Sinuses	—	—	—	—	—	+	##
Erythrocytes	##	##	++	±	—	—	—

liver cell at pH 5. While, Pb markedly inhibited bile canaliculus ATP-ase at pH 7.0 and capillary ATP-ase of rat heart at pH 8.0 as shown in Tables 6 and 7.

II. Effect of the pH of incubating media upon ATP-ases of various tissue components.

As shown in Tables 8, 9, 10, 11, and 12, each tissue component had its own pH optimum specific to it. Histochemical scores at pH 6.0 and 8.0 given in the tables were those obtained in the media of the neutral group.

a) Rat liver

The cytoplasm of the liver cells showed the highest activity at pH 5.0, the bile canaliculus at pH 7.0 without zonal difference in the hepatic lobule, the stellate cells and endothelial cells of the central veins at pH 8.0 and again the bile canaliculus at pH 9.0 with a stronger reaction in the periportal area. The nucleus showed the highest activity at pH 7.0.

Fig. 1 rat liver, pH 4.0, Pb alone, 180 min, $\times 100$.

Fig. 4 rat liver, pH 7.0, Pb & Ca, 150 min, $\times 100$.

Fig. 2 rat liver, pH 5.0, Pb alone, 180 min, $\times 100$.

Fig. 5 rat liver, pH 8.0, Pb & Ca, 120 min, $\times 100$.

Fig. 3 rat liver, pH 6.0, Pb & Ca, 150 min, $\times 100$.

Fig. 6 rat liver, pH 9.0, Ca alone, 120 min, $\times 100$.

b) Rat pancreas

As shown in Table 9, the cytoplasm of acinar cells showed the highest activity at pH 5.0; the nuclei at pH 7.0, the intralobular ducts at pH 8.0 and the capillaries and islet cells at pH 9.0.

c) Rat kidney

The cytoplasm of epithelial cells of the proximal and distal convoluted tubules, thick limbs of Henle and collecting tubules showed the highest activity at pH 5.0, the nuclei at pH 7.0 and the brush borders of the proximal convoluted tubules at pH 9.0.

d) Rabbit brain

As shown in Table 11, the axons and nuclei of nerve cells showed the highest activity at pH 7.0, the neuroglia at pH 5.0 or at pH 9.0 depending on the layers at which it located, and the capillaries at pH 9.0.

Fig. 7 rat pancreas, pH 8.0, Pb & Ca
120 min $\times 100$.

Fig. 8 rat pancreas, pH 9.0, Ca
alone, 120min, $\times 100$.

Fig. 9 rat heart, pH 8.0, Ca alone,
120 min, $\times 100$.

Fig. 10 rat heart, pH 8.0, Pb alone,
120 min, $\times 100$.

Fig. 11 rabbit brain, pH 7.0, Pb &
Mg, $\times 200$.

Fig. 12 rabbit adrenal gland, pH 7.0,
Pb & Mg, $\times 200$.

e) Rabbit adrenal gland

The cytoplasm of cortical cells and erythrocyte showed the highest activity at pH 5.0, the nucleus of cortical cells at pH 7.0 and the capillary sinuses at pH 9.0. The behaviour of the nucleus was the same in every organ and in both rabbits and albino rats. The intensity of nuclear staining was greater in non-deparaffinized sections than in deparaffinized sections.

Discussion

I. Mg-Activated and Ca-inhibited ATP-ase in the cytoplasm

In 1948 Kielley and Meyerhof¹⁸⁾ reported the presence of a new ATP-ase in the muscle which is activated by Mg and inhibited strongly by Ca. The pH-activity curve appeared in their report shows that the pH optimum of this ATP-ase is present between pH 6.0 and 7.0, and activity is nearly zero at pH 9.5. In 1953 Kielly and Kielly reported an ATP-dephosphorylating enzyme in the liver mitochondria which is also Mg-activated and Ca-inhibited. Observing an increase in the ATP-ase activity at pH values acid to the pH optimum (pH 8.5) with an increase in Mg concentration, they stated that Mg functions in the hydrolytic reaction by increasing the negative charge on the terminal phosphate of ATP forming a ATP-Mg complex which may be a true substrate of ATP-ase. Since, as is generally accepted, enzyme proteins are multivalent dipolar ions, their dissociation depends on the pH of the medium. The acid ATP-ase medium we employed contains 0.003M Pb as PO_4 -binding cation. This Pb may act as Mg in Kielley and Kielley's biochemical medium forming enzyme-substrate compounds by way of ATP-Pb complex. In 1952 Novikoff et al.¹⁹⁾ reported that the degree of stimulation of ATP dephosphorylation by Mg and Ca was strikingly different in the nuclear and mitochondrial fractions: the two ions were equally effective stimulators for the nuclear fraction, with the mitochondrial fraction, however, Mg stimulated but Ca did not. Basing on these observations, they stated that it is possible that the nuclei possess an entirely different enzyme or enzymes from the mitochondrial ATP-ase. Nakamoto¹⁰⁾ found histochemically that Mg activates cytoplasmic acid ATP-ase and is nearly indifferent to alkaline ATP-ase.

In the present experiment, cytoplasmic or mitochondrial acid ATP-ase was strongly inhibited by Ca, and the nuclei and axons reacted more strongly in the medium containing Pb and Mg at pH 7.0 and in the medium containing Pb and Ca at pH 8.0 than in the medium containing Pb alone, respectively.

III. Degree of ATP dephosphorylation

Novikoff et al.¹⁹⁾ stated that during periods of incubation in an enzyme-limited system, at a Mg concentration optimal for ATP dephosphorylation, very little A-5'-P dephosphorylation occurs, and alkaline phosphatase, although it splits ATP even at neutral pH, is of little quantitative significance since it occurs in very small quantities in normal rat liver. They further stated that although there are many enzyme-catalyzed reactions participated in by ATP and its derivatives, it would appear that under conditions similar to those of Dubois and Potter the rate of orthophosphate liberation for rat liver homogenate

and its mitochondrial fraction is chiefly a measure of ATP-ase, and the high level of adenylate kinase activity makes it likely that any ADP formed by ATP-ase action would quickly be converted to A-5'-P and ATP. While, Kielley and Kielley¹⁴⁾ stated that the apparent apyrase activity of mitochondria may be the combined activity of a true ATP-ase and the adenylate kinase. Employing the sections in which acid phosphatase was inactivated and acid ATP-ase remained active, and the sections in which alkaline phosphatase remained active and alkaline ATP-ase was inactivated by heated water pretreatment, we previously demonstrated that with short incubation the reaction products in the acid ATP-ase medium is due to acid ATP-ase activity and those in the alkaline ATP-ase medium due to alkaline ATP-ase, although ATP is dephosphorylated in small quantities by alkaline phosphatase in the alkaline ATP-ase medium with a longer incubation. For a detailed description of the procedures of this heated water pretreatment, readers are referred to Maekawa et al⁹⁾. or Nakamoto¹⁰⁾.

Summary

The pH optimum of tissue ATP-ases was investigated by the use of incubating media of various pH containing Pb and/or Ca as PO_4 -binding cations. 1) Ca inhibited cytoplasmic ATP-ase at pH 5.0, and Pb inhibited capillary and bile canaliculus ATP-ases at pH 7.0. 2) The cytoplasmic ATP-ase showed the highest activity at pH 5.0, the nuclei, axons and bile canaliculi at pH 7.0, the intralobular pancreatic ducts and endothelial cells of the cells of the central veins of the liver at 8.0, and the capillaries, periportal bile canaliculi, brush borders of the proximal convoluted tubules and sinusoid walls of the adrenal gland at pH 9.0.

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- Dr. Takeuchi: Which do you mean by "ATP-ase reaction", the summerized reactions by use of ATP as substrate or the reaction for specific ATP-ase activity? This is an important matter from the stand point of histochemical specificity. The dissociation of phosphor from ATP may occur by the action of not only specific ATP-ase but of alkaline phosphatase, acid phosphatase, 5-nucleotidase and apyrase. The present study does not differentiate between the specific and nonspecific reactions.
- Dr. Ogawa: It seems to me that the present study is incomplete to appreciate the specificity of ATP-ase.
- Dr. Nagata: It is fairly difficult to estimate quantitatively histochemical reactions by means of such as the present studies.
- Dr. Nakamoto: In regard to the specificity of the ATP-ase reactions, we have already investigated and reported at the first meeting of this association. We can recognize the specificity from 1) distribution, 2) resistance to water and heat, 3) influence of phenothiazine. I wish to be informed any other more appropriate method for the differentiation.
- Dr. Takamatsu: I have ever had the identical doubt with those of Dr. Takeuchi and Dr. Ogawa to the works of Dr. Nakamoto, and have had the chance to observe his specimens. At the last meeting of this association, I have discussed to him as follows; "If we can sufficiently differentiate the specificity through a simple procedure such as heat treatment it may be a "powerful and excellent method." Now, I wish to repeat the same discussion.

Techniques in Semi-Quantitative ATP-ase Histochemistry With Paraffin Sections

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Introduction

In spite of vigorous efforts to convert a qualitative histochemistry to a quantitative one, quantitative estimation of histochemical results has been much criticized because enzyme histochemistry is subjected to many uncontrollable factors in the processes. Nakamoto¹⁾ studied various factors affecting the amount of reaction products on paraffin sections and discussed the problems in semi-quantitative phosphatase histochemistry. Basing on his conclusions, semi-quantitative histochemical techniques with paraffin sections were further systematized and employed in ATP-ase histochemistry of experimental atheroma in rabbits and that of blood vessels in albino rats.

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