Characterization of Thyroidal Membrane-Bound Mg-Adenosinetriphosphatase Activated by Trypsin or Poly-l-lysine

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The Mg-adenosinetriphosphatase (ATPase) in the thyroidal NaI-treated microsome fraction was activated by treatment with basic polyamino acids or trypsin, but not with acidic polyamino acids and basic proteins such as lysozyme and ribonuclease.

The enzyme kinetics showed that the activation of trypsin or poly-l-lysine was due to an increase in the maximal velocity of the hydrolyzing reaction without a change in the affinity of the enzyme for its substrate.

A break at about 25°C was observed in the Arrehenius plots of Mg-ATPase in the trypsin- or poly-l-lysine treated preparations, but there was no break in the control preparation.

These results suggest that the activating effect of trypsin or poly-l-lysine on Mg-ATPase activity in the thyroidal NaI-treated microsome fraction is related to the lipid environment surrounding the enzyme molecule in the thyroid cell membrane.

Keywords Mg-ATPase; thyroidal membrane; trypsin; poly-l-lysine

Introduction

Mg-adenosinetriphosphatase (ATPase) activity has been reported in plasma membranes derived from a variety of eukaryotic cell types. This activity can be distinguished from Na, K-ATPase and Ca-ATPase by its sensitivity to Na, K, Ca and ouabain. Although it has not been demonstrated that this Mg-ATPase activity results from a single enzyme in all of these cell types, its ubiquitous presence at high levels, often constituting over 50% of the plasma membrane-bound ATPase activity, 2(a) suggests that it serves an important function.

In the preceding papers, 3(a) we have shown that the treatment of the NaI-treated microsome fraction and the plasma membrane fraction of the thyroid with trypsin or poly-l-lysine (PLL) results in the activation of Mg-ATPase and the reduction of Na, K-ATPase. This activation of Mg-ATPase by trypsin or PLL was specific in the thyroid, since no increase of Mg-ATPase activity was observed in the preparations obtained from other tissues. However, the exact mechanism for activation is not clear.

To approach this problem, we studied some characteristics of trypsin- or PLL-activated Mg-ATPase as well as some factors affecting the activation of the enzymes. These results are presented in this paper.

Materials and Methods

Hog thyroids were obtained from a local slaughterhouse.

The enzyme preparation was obtained from thyroid microsome fraction by treatment with deoxycholate and then NaI, as described previously. 2(a) Na, K-ATPase and Mg-ATPase activities were measured under conditions described elsewhere. 2(a) ATPase activity was expressed as μmol Pi liberated per mg of protein per hour or percent of the control value. Experimental points are the average of the three or more experiments performed in triplicate.

Protein was determined by the method of Lowry et al. 3(a)

Treatment of Enzyme Preparation with Trypsin or PLL

A prior incubation with trypsin or PLL was carried out as follows: enzyme preparation (10 mg as protein) was incubated with trypsin (1 mg) or PLL (10 mg) in 100 mM Tris-HCl (pH 7.4) containing 6 mM MgCl2 for 10 min at 37°C. After preincubation, trypsin inhibitor was added when trypsin was used, and the incubation mixture was centrifuged at 105000 × g for 60 min at 4°C. The supernatant solution was discarded. The pellet was resuspended in a 0.25 M sucrose solution, and then centrifuged at 105000 × g for 60 min at 4°C. The washed pellet was finally resuspended in a 0.25 M sucrose solution and used for enzyme assay and other experiments.

To determine the washing effect of poly-L-arginine (PLLAsp), a PLL-activated preparation was resuspended in preincubation medium containing 10 mg of PLL (PLL, 240000) and centrifuged at 105000 × g for 60 min at 4°C. This washing procedure was repeated twice. The control sample was subjected to the same conditions without trypsin or PLL.

Trypsin, trypsin inhibitor, ribonuclease, histones, polyamino acids and adenosine triphosphate (ATP) were purchased from Sigma Chemical Co. Lysozyme was obtained from Seikagaku Kogyo (Tokyo). All other chemicals were of an analytical grade.

Results

As shown in Table I, three basic polyamino acids, PLL, poly-L-ornithine and poly-L-arginine, strongly reduced Na, K-ATPase activity in the thyroidal NaI-treated microsome fraction, while Mg-ATPase was activated by these polyamino acids. However, acidic polyamino acids did not affect either of the ATPase activities. This result seemed to

<table>
<thead>
<tr>
<th>Additions</th>
<th>Na,K-ATPase activity</th>
<th>Mg-ATPase activity</th>
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<tbody>
<tr>
<td>Control</td>
<td>7.14 ± 0.22 (100)</td>
<td>2.01 ± 0.12 (100)</td>
</tr>
<tr>
<td>PLL</td>
<td>5.00 ± 0.11 (71)</td>
<td>3.48 ± 0.13 (173)</td>
</tr>
<tr>
<td>Poly-L-ornithine</td>
<td>3.64 ± 0.15 (51)</td>
<td>3.64 ± 0.14 (171)</td>
</tr>
<tr>
<td>Poly-L-glutamic acid</td>
<td>7.10 ± 0.10 (99)</td>
<td>2.00 ± 0.08 (99)</td>
</tr>
<tr>
<td>Control</td>
<td>10.95 ± 0.24 (100)</td>
<td>2.59 ± 0.11 (100)</td>
</tr>
<tr>
<td>PLL</td>
<td>6.13 ± 0.15 (56)</td>
<td>3.63 ± 0.14 (140)</td>
</tr>
<tr>
<td>PLXp</td>
<td>10.85 ± 0.19 (99)</td>
<td>2.58 ± 0.09 (109)</td>
</tr>
<tr>
<td>Basic proteins</td>
<td>7.14 ± 0.22 (100)</td>
<td>2.01 ± 0.12 (100)</td>
</tr>
<tr>
<td>Histone</td>
<td>5.93 ± 0.02 (83)</td>
<td>2.83 ± 0.05 (141)</td>
</tr>
<tr>
<td>Control</td>
<td>17.00 ± 0.39 (100)</td>
<td>3.00 ± 0.13 (100)</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>16.90 ± 0.27 (99)</td>
<td>2.98 ± 0.10 (99)</td>
</tr>
<tr>
<td>Cytochrome c</td>
<td>17.01 ± 0.32 (100)</td>
<td>2.89 ± 0.09 (96)</td>
</tr>
<tr>
<td>Ribonuclease</td>
<td>16.80 ± 0.40 (99)</td>
<td>2.81 ± 0.14 (94)</td>
</tr>
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</table>

(a) Enzyme preparation was incubated with equal amounts of polyamino acids or basic proteins for 10 min at 37°C. b) All values are the mean of at least five observations. c) Percent from control value is shown in parentheses. d) Molecular weight of polyamino acids: PLL (240000), poly-L-ornithine (120000), poly-L-arginine (170000), poly-L-glutamic acid (100000), PLL (90000).
Table II. Effect of PLAsp on the Thyroidal ATPase Activities in Pretreated Enzyme Preparation with PLL

<table>
<thead>
<tr>
<th></th>
<th>Na,K-ATPase activity</th>
<th>Mg-ATPase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( P_i \mu mol/mg of protein·h ) (% of control)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>12.06±0.12 (100)</td>
<td>1.16±0.08 (100)</td>
</tr>
<tr>
<td>PLL</td>
<td>8.81±0.13 (73)</td>
<td>3.25±0.18 (280)</td>
</tr>
<tr>
<td>PLAsp</td>
<td>11.43±0.17 (95)</td>
<td>1.11±0.10 (96)</td>
</tr>
<tr>
<td>PLL ( \rightarrow ) PLAsp</td>
<td>11.93±0.11 (99)</td>
<td>1.05±0.09 (92)</td>
</tr>
</tbody>
</table>

\( ^a \) For experimental details see Materials and Methods. \( ^b \) All values are the mean of at least three observations. \( ^c \) Percent from control value is shown in parentheses. \( ^d \) PLL and PLAsp were of average molecular weight, 24000 and 9000, respectively.

![Fig. 1. pH Profiles of Mg-ATPase of Control, Trypsin- and PLL-Treated Preparations](image)

Each point shows the mean of three observations. Buffers used were sodium acetate for pH 4.0-7.4, Tris-maleate for pH 7.4-9.5. PLL is of an average molecular weight of 240000. --- ○ ---, control; --- ● ---, trypsin; --- △ ---, PLL.

indicate that the alteration of both ATPase activities was dependent on the basicity of the polyamino acid.

Therefore, naturally occurring basic polypeptides were examined instead of synthetic polyamino acids. None of the basic polypeptides, such as ribonuclease, cytochrome c or lysozyme had any effect on the activities, except for histones, whose effect was less than the synthetic basic polyamino acids. These results suggest that the basicity of a polyamino acid or a poly-peptide alone is not enough to cause the activation of Mg-ATPase.

The effect of PLAsp on PLL-pretreated preparation is shown in Table II. The elevated activity (280%) of Mg-ATPase and decreased Na, K-ATPase activity (73%) were all restored to the control level by washing with a PLAsp solution.

Figure 1 shows the effects of pH on the Mg-ATPase activities of the control, trypsin- and PLL-treated preparations. In the control preparation, the activity of Mg-ATPase was less dependent on the change in pH of the assay medium. A significant increase in Mg-ATPase activity was observed in both trypsin- and PLL-treated preparations over a wide range of pH. With an acidic pH, the change in activity was more pronounced than with neutral or alkaline pH.

We have already shown that the increase of Mg-ATPase after trypsin digestion was due to an increase in \( V_{\text{max}} \), while \( K_m \) for ATP was unchanged.\(^{ab}\) In this study, we obtained double reciprocal plots of Mg-ATPase in the PLL-treated preparation, indicating that the activation of Mg-ATPase by PLL is also due to an increase in \( V_{\text{max}} \) of the enzyme reaction (Fig. 2).

![Fig. 2. Lineweaver-Burk Plots of Mg-ATPase of Control, Trypsin- and PLL-Treated Preparations](image)

\( ^{ab} \) MgCl\(_2\) was present at 6 mm. The straight lines drawn were obtained by two variable linear regression analyses of the points indicated. PLL is of an average molecular weight of 240000. --- ○ ---, control; --- ● ---, trypsin; --- △ ---, PLL.

![Fig. 3. Effect of NaCl Concentrations on Activation of Mg-ATPase by PLL](image)

PLL (100 μg) was added in the preincubation medium after addition of NaCl solution. Each point shows the mean of three observations. PLL is of an average molecular weight of 240000. --- ○ ---, control; --- ● ---, PLL.

double reciprocal plots of Mg-ATPase in the PLL-treated preparation, indicating that the activation of Mg-ATPase by PLL is also due to an increase in \( V_{\text{max}} \) of the enzyme reaction (Fig. 2).

Figure 3 shows the effect of PLL on the activation of Mg-ATPase as a function of various concentrations of NaCl. A progressive increase in NaCl concentration eliminated the effect of PLL on Mg-ATPase activity; at 400 mM NaCl, the effect was completely abolished. However, the enzyme activity in the control was constant over a wide range of salt concentration as far as our experimental conditions were concerned.

When the Mg-ATPase activities of control, trypsin- and PLL-treated enzyme preparations were determined at various temperatures, the increase of Mg-ATPase activity was increased in both trypsin- and PLL-treated enzyme preparations at any temperature examined. A discontinuity was found at about 25 °C in Arrehnius plots of trypsin- and PLL-treated preparations, but there was no break in the control preparation (Fig. 4).
Fig. 4. Arrhenius Plots of Mg-ATPase Activities in Control, Trypsin-, and PLL-Treated Preparations

Enzyme preparation was preincubated with trypsin or PLL for 10 min at 37°C. Enzyme assay was performed under each temperature indicated after removal of reagents by centrifugation. Each point shows the mean of three observations. PLL is of an average molecular weight of 240000. —○—, control; —●—, trypsin; —△—, PLL.

Discussion

It is evident from Table I that the activation of Mg-ATPase in thyroidal NaI-treated microsome fraction is dependent on the basicity of polyamino acids, since the activation of Mg-ATPase could not be observed by treatment with acidic polyamino acids. However, the results obtained with basic proteins, such as ribonuclease, cytochrome c and lysozyme, except for histones, indicate that the activation of Mg-ATPase occurs as a result of neither basicity nor structure. PLL forms a flexible random coil structure, suggesting a large number of amino groups of PLL simultaneously attached to the negatively charged surface of the cell membrane. In fact, PLL binding to erythrocyte membrane is thought to be planar by the observation of Katchalsky et al. If multiple attachments to a cell membrane are required for the activation of Mg-ATPase, the activation of Mg-ATPase by treatment with PLL may be explained as follows: in the condition of our experiments, PLL exists in the form of a flexible random coil, so it is feasible that a number of basic groups reach the plane of the cell surface. If the notion of multiple attachments is acceptable, the activity of PLL should decrease when the spacing of ε-amino groups is increased in the primary structure and/or when the molecules as a whole become more rigid because of its tertiary structure. These structural factors may explain why histone is considerably less active than PLL. Since only one-quarter to one-third of the amino acid residues in the histone molecule are basic, the average distance between NH₂-groups would be relatively longer than in PLL. Furthermore, parts of the histone molecule are considered to be in the ε-helical form, which contributes to a rigidity of the histone structure which is lacking in the PLL molecule. Such an explanation could be applied to other basic proteins. Therefore, it is possible to deduce that PLL causes, through interactions with negatively charged sites in the plasma membrane, a conformational change of membrane proteins which results in activation of the enzyme.

When the PLL-treated preparation was washed with a PLAsp solution, both Mg-ATPase and Na, K-ATPase activities were recovered to the original level (Table II). This observation suggests that PLL is rapidly removed from the aggregates by an oppositely charged substance.

It was suggested by Wolff et al. that the stimulation of thyroidal adenylate cyclase activity by PLL was caused by charge interaction, since the activation of the enzyme was abolished by the addition of a high concentration of salt to the enzyme assay system. If the effect of PLL on the thyroidal Mg-ATPase activity is induced by the same mechanism, no activation of Mg-ATPase by treatment with PLL should be observed under a high concentration of NaCl. The results shown in Fig. 3 clearly prove such a prediction, suggesting the importance of the charge-to-charge interaction for activation of Mg-ATPase.

It has been reported by Cook et al. that the negative charge of the membrane surface is due to N-acetyleneuraminic acid residues. In addition, the amounts of PLL binding to these glycoproteins were reduced when the partially purified glycoproteins from erythrocyte membranes were treated with neuraminidase. We tested the effect of PLL on the Mg-ATPase activity according to the method of Emmelot and Bos using the neuraminidase treated thyroidal plasma membrane fraction. However, the degree of activation of Mg-ATPase did not significantly differ from the neuraminidase untreated plasma membrane (data not shown). Other possibilities, such as PLL-binding to other negatively charged component(s) of plasma membrane or binding to the Mg-ATPase molecules, which are reported to be glycoprotein, are not excluded.

As shown in Fig. 2, the double reciprocal plots clearly indicated an increase in the Vₘₐₓ without change in Kₘₐₓ value after trypsin or PLL treatment. The increase in the Vₘₐₓ of Mg-ATPase could be the result of an increase in the number of Mg-ATPase molecules or an increase in the catalytic constant of Mg-ATPase. However, the former possibility is probably due to the following facts. It is well known that limited protease action on the cell surface can promote the exposure of cryptic binding sites for lectins and that the increase in the specific binding of insulin to fat cells or fat cell membranes after phospholipase digestion reflects an increase in the total quantity of receptors available for interaction with this hormone.

The appearance of a discontinuous point in the Arrhenius plots after treatments with trypsin or PLL reflects a change in lipid environment around the enzyme molecule in the membrane. There is considerable evidence to show that the activities of a number of membrane-bound enzymes are regulated by the lipid environment of membranes, and the fluidity of membrane phospholipids is changed by treatment with protease by the binding of lectins. These facts enable us to explain that the modulation of Mg-ATPase activity might result from a conformational change induced by altered membrane lipid fluidity.

More investigations will be necessary to elucidate the mechanism by which treatment with PLL or trypsin activates Mg-ATPase activity in the thyroidal microsome fraction.

References


