Biochimica et Biophysica Acta, 659 (1981) 31—37
Elsevier/North-Holland Biomedical Press

BBA 69259

REGULATION OF CYTOSOL 5'-NUCLEOTIDASE BY ADENYLATE ENERGY CHARGE

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(Received November 21st, 1980)

Key words Adenylate energy charge, 5'-Nucleotidase regulation, (Rat liver)

Summary

In the physiological range of the adenylate energy charge in liver (0.7—0.9), the rate of AMP-hydrolysis catalysed by rat liver cytosol 5'-nucleotidase (5'-ribonucleotide phosphohydrolase, EC 3.1.3.5) increased sharply with decreasing energy charge. In addition, a decrease in the concentration of P_i caused marked acceleration of the AMP-hydrolysing activity over the physiological range of adenylate energy charge. These responses seem to serve to protect the cells against a metabolic stress which could result from sudden utilization of ATP by removal of AMP. The AMP-hydrolysing activity of this enzyme decreased sharply as the size of the adenine nucleotide pool decreased in the physiological range. This effect may be a self-limiting response to prevent excess depletion of the pool.

IMP-hydrolysing activity of this enzyme increased with increasing adenylate energy charge. But no marked response to its variation within the physiological range was observed. On the basis of the data obtained in this study, the IMP-hydrolysing activity of the cytosol 5'-nucleotidase in rat liver cells seems to be comparable to that of AMP deaminase reaction, but the AMP-hydrolysing activity was estimated to be less than 10% of AMP deaminase reaction at energy charge value of about 0.7. This strongly suggests that the AMP → IMP → inosine pathway is more significant than the AMP → adenosine → inosine pathway in rat liver.

Introduction

In most tissues and organisms studied, the adenylate energy charge (ATP + 0.5 ADP/ATP + ADP + AMP) [1] has been shown to be maintained at a value of about 0.9 [2—4]. The energy charge is stabilized by removing excess AMP

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that might otherwise accumulate as a consequence of a sudden utilization of ATP.

In the liver, two mechanisms can convert AMP to inosine and ultimately into uric acid or allantoin the amino group could be removed by AMP deaminase (AMP aminohydrolase, EC 3.5.4.6), producing IMP, or the phosphate group could be removed by a cytosol 5′-nucleotidase (5′-ribonucleotide phosphohydrolase, EC 3.1.3 5), producing adenosine. Inosine is formed through the dephosphorylation of IMP catalysed by the cytosol 5′-nucleotidase or through the deamination of adenosine catalysed by adenosine deaminase. Conversion of AMP to inosine has been suggested to occur mainly through the AMP deaminase reaction in higher animals [5—8]. The regulatory properties of AMP deaminase from mammalian cells show that the reaction catalysed by this enzyme serves to protect the cells against sharp decrease in adenylate energy charge [9,10].

Some investigators have suggested that the cytosol 5′-nucleotidase is important in degradation of IMP rather than AMP [7,8,11—13] This enzyme purified from chicken liver or rat liver has the highest affinity for IMP among the various 5′-mononucleotides tested, but is also potentially active with AMP as substrate [14,15].

In the present experiments, responses of the cytosol 5′-nucleotidase to variation of adenylate energy charge were studied with AMP or IMP as substrate. The purpose of the work is to evaluate the contribution of this enzyme to maintenance of the adenylate energy charge in liver cells under the conditions of a sudden increase in the rate of utilization of ATP

Materials and Methods

Chemicals. AMP and IMP (free acids) were purchased from Yamasa Shoyu Co. and used as Tris salts. ADP and ATP were also obtained as sodium salts from Yamasa Shoyu Co. Concentrations of all nucleotide solutions were determined spectrophotometrically. [8-^{14}C]AMP (spec act. 59 Ci/mol) and [8-^{14}C]-IMP (60 Ci/mol) were purchased from The Radiochemical Center, Amersham. All other chemicals were of reagent grade or the highest quality available.

Cytosol 5′-nucleotidase preparation Cytosol 5′-nucleotidase was purified from rat liver as described previously [15] The specific activity of the preparation used was 22 μmol P_i liberated from IMP/min per mg protein. The preparation was homogeneous on the criteria of disc-gel electrophoresis [15].

Assay of 5′-nucleotidase activity. The activity of the cytosol 5′-nucleotidase was assayed as described previously [14]. The reaction mixture contained 100 mM imidazol-HCl buffer, pH 6.5 or 7.4/100 mM KCl/0 1% bovine serum albumin/appropriate amount of adenine nucleotides/$MgCl_2$. [8-^{14}C]Nucleoside 5′-monophosphates were used as substrates.

Calculation of total Mg^{2+} concentration The total Mg^{2+} concentration corresponding to 1 mM free Mg^{2+} at pH 6.5 was calculated by the method described by Chapman et al. [10].

Results

The response of 5'-nucleotidase activity to variations in adenylate energy charge The response of the activity of rat liver cytosol 5'-nucleotidase to variations in adenylate energy charge was studied. The total adenine nucleotide concentration was held constant at 4 mM and the energy charge was varied between 0 (all AMP) and 1.0 (all ATP). AMP-hydrolysing activity was low at normal physiological energy charge value (0.9), and it was greatly activated when the energy charge decreased within the range of surviving mammalian cells (0.7—0.9) (Fig. 1a).

Since the substrate for the reaction, AMP, is one of the components of the adenine nucleotide pool, it is expected that the rate will increase as the energy charge decreases and AMP increases. This is reflected in the bottom control curve which shows the response of this enzyme to increasing levels of AMP alone over the same range of AMP concentration as is found in the upper curve. It is apparent that, in the absence of modifiers, the reaction proceeds at a very low rate.

A similar experiment was repeated at pH 7.4, which would correspond to an intracellular pH of 7.0—7.2 [16]. Although the rate of AMP hydrolysis decreased, the energy charge response was essentially the same as that at pH 6.5 (Fig. 1b).

When IMP is used as substrate, the cytosol 5'-nucleotidase activity is stimulated by adenine nucleotides, including a substrate AMP [14,15]. Response of IMP-hydrolysing activity to variation in adenylate energy charge is shown in Fig. 2. In this experiment, total adenine nucleotide concentration was also held constant at 4 mM and IMP concentrations were varied from 0.25 to 1.0 mM. These concentrations of IMP are within the range reported by Woods et al. [17] for perfused rat liver after sudden utilization of ATP resulting from fructose loading. As expected from the fact that IMP-hydrolysing activity of the cytosol 5'-nucleotidase is effectively activated by ATP, it decreased with decreasing energy charge. But no marked change in the IMP-hydrolysing activity was observed, when adenylate energy charge decreased within the range observed in surviving mammalian cells.

Inhibition of the cytosol 5'-nucleotidase activity by inorganic phosphate The cytosol 5'-nucleotidase is inhibited by P_i [14,15]. Fig. 3 shows the energy charge response in the presence of P_i at concentrations ranging from 0 to 5 mM. Total adenine nucleotide concentration was held constant at 4 mM and adenylate energy charge was varied from 0.6 to 1.0. A decrease in concentration of P_i effectively enhanced AMP-hydrolysing activity over the range of the energy charge tested.

IMP-hydrolysing activity was also inhibited by P_i (Fig. 4). When the concentration of IMP was fixed at 0.5 mM, the IMP-hydrolysing activity was more effectively inhibited in the range of low energy charge.

Effect of total adenine nucleotide concentration on the response of 5'-nucleotidase activity to variation in adenylate energy charge The energy charge response of AMP-hydrolysing activity at various total concentrations of adenine nucleotides ranging from 2 to 8 mM is shown in Fig. 5 In these experiments,

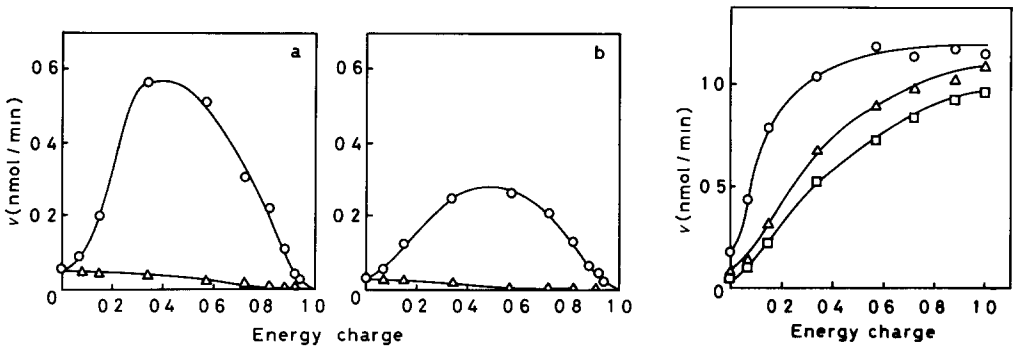

Fig 1 Response of AMP-hydrolysing activity of rat liver cytosol 5′-nucleotidase to variation in the adenylate energy charge at a total adenine nucleotide concentration of 4 mM The upper curve (○———○) shows the energy charge response of the enzyme with 4 mM adenylate pool, and the lower curve (△———△) shows the rate of the reaction at concentrations of AMP alone, corresponding to the AMP concentration in a 4 mM adenylate pool at the energy charge value indicated in the figure The relative amounts of ATP, ADP and [^{14}C]AMP at different energy charge values were calculated from a value of 0 8 for the equilibrium constant of the adenylate kinase reaction The reaction mixture contained 100 mM imidazol-HCl, pH 6 5 (a) or 7 4 (b)/100 mM K^+/0 1% bovine serum albumin/8 mM Mg^{2+}/4 mM adenine nucleotides (upper curve) or 0—4 mM [^{14}C]AMP (lower curve)/appropriate amount of purified 5′-nucleotidase Incubation was for 30 min

Fig 2 Response of IMP-hydrolysing activity of rat liver cytosol 5′-nucleotidase to variation in the adenylate energy charge at a total adenylate concentration of 4 mM The reaction mixture contained 100 mM imidazol-HCl, pH 6 5/100 mM K^+/0 1% bovine serum albumin/8 mM Mg^{2+}/[^{14}C]IMP at a concentration of 0 25 (□———□), 0 5 (△———△) or 1 mM (○———○), appropriate amount of purified 5′-nucleotidase and adenine nucleotides at a total concentration of 4 mM at energy charge value indicated on the abscissa Incubation was for 5 min

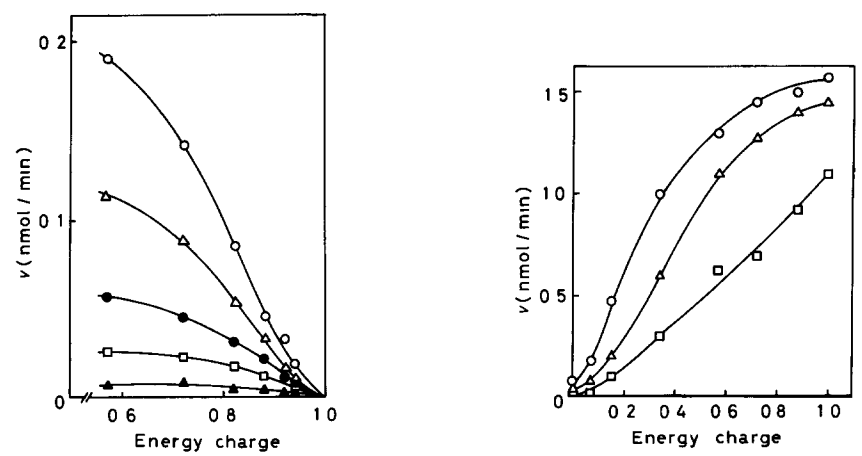

Fig 3 Inhibition of AMP-hydrolysing activity of rat liver cytosol 5′-nucleotidase by inorganic phosphate at different energy charge values The reaction mixture contained 100 mM imidazol-HCl, pH 6 5/100 mM K^+/0 1% bovine serum albumin/8 mM Mg^{2+}/inorganic phosphate at a concentration of 0 (○———○), 0 5 (△———△), 1 0 (●———●), 2 0 (□———□— or 5 mM (▲———▲), appropriate amount of purified 5′-nucleotidase, ATP, ADP and [^{14}C]AMP at a total concentration of 4 mM at energy charge value indicated on the abscissa Incubation was for 30 min

Fig 4 Inhibition of IMP-hydrolysing activity of rat liver cytosol 5′-nucleotidase by inorganic phosphate at different energy charge values The reaction mixture contained 100 mM imidazol-HCl, pH 6 5/100 mM K^+/0 1% bovine serum albumin/8 mM Mg^{2+}/inorganic phosphate at a concentration of 0 (○———○), 2 (△———△) or 5 mM (□———□), 0 5 mM [^{14}C]IMP, appropriate amount of purified 5′-nucleotidase and adenine nucleotides at a total concentration of 4 mM at energy charge value indicated on the abscissa Incubation was for 5 min

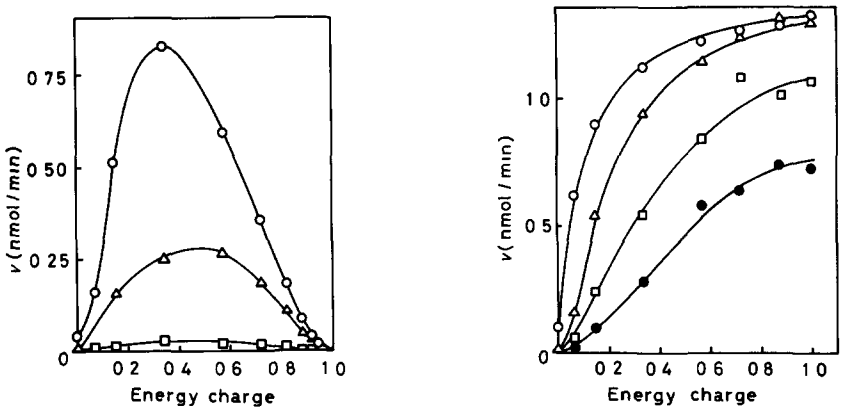

Fig 5 Response of AMP-hydrolysing activity of rat liver cytosol 5′-nucleotidase to variation in the energy charge at different adenine nucleotide pool sizes. The reaction mixture contained 100 mM imidazol-HCl, pH 6 5/100 mM K^+/0 1% bovine serum albumin/1 mM free Mg^{2+}/appropriate amount of purified 5′-nucleotidase, ATP, ADP and [^{14}C]AMP at a total concentration of 8 (○———○), 4 (△———△) or 2 mM (□———□) at energy charge value indicated on the abscissa Incubation was for 30 min

Fig 6 Response of IMP-hydrolysing activity of rat liver cytosol 5′-nucleotidase to variation in the energy charge at different adenine nucleotide pool sizes The reaction mixture contained 100 mM imidazol-HCl, pH 6 5/100 mM K^+/0 1% bovine serum albumin/1 mM free Mg^{2+}/appropriate amount of purified 5′-nucleotidase/0 5 mM [^{14}C]IMP, ATP, ADP and AMP at a total concentration of 8 (○———○), 4 (△———△), 2 (□———□) or 1 mM (●———●) at energy charge value indicated on the abscissa Incubation was for 5 min

the concentration of free Mg^{2+} (not complexed with adenylate) was fixed at 1 mM. This is a value estimated to be the concentration of free Mg^{2+} in the liver [18]. When total adenine nucleotide concentration was held at 4 mM, the response of the activity to variation in energy charge was almost the same as the results of the experiments presented in Fig. 1. As the total adenine nucleotide concentration decreased, the rate of the hydrolysis of AMP sharply decreased even at the range of low energy charge.

Fig. 6 shows the response of IMP-hydrolysing activity to variation in energy charge. The decrease in the rate of the hydrolysis of IMP due to the decrease in total adenine nucleotide concentration was less marked than that of AMP. Even at the lowest concentration of total adenine nucleotide tested, no significant change in the IMP-hydrolysing activity was observed, when adenylate energy charge decreased from 0.9 to 0.7.

Estimation of the cytosol 5′-nucleotidase activity in vivo Considering the absolute dependence on Mg^{2+}, the high affinity for IMP and the pH optimum at 6.5 of the cytosol 5′-nucleotidase, the difference between IMP-hydrolysing activity in the presence of Mg^{2+} and that in its absence at pH 6.5 is a good estimate of the activity of the cytosol 5′-nucleotidase in crude extract of the liver. Using optimal assay conditions [15], we estimated the activity of the cytosol 5′-nucleotidase with IMP to be 0.47 ± 0.09 μmol/min per g wet weight of liver (mean ± S.D. of the values obtained with five animals). Under the same assay conditions, the activity of the purified 5′-nucleotidase with AMP was 20% of that with IMP. To estimate the activity in vivo, hydrolysis of AMP by the purified preparation of the cytosol 5′-nucleotidase was assayed under the fol-

lowing reaction conditions total adenine nucleotide concentration, 4 mM, adenylate energy charge, 0.72, IMP, 0.1 mM, P_i, 1.5 mM, free Mg^{2+}, 1 mM, KCl, 100 mM, pH, 6.5 The AMP-hydrolysing activity under these conditions was 20% of that under the optimal assay conditions From these results the rate of about 0.02 μmol adenosine formed/min per g wet weight of liver was calculated. Under the same assay conditions that were adopted to estimate the AMP-hydrolysing activity in vivo, IMP-hydrolysing activity was 35% of that under the optimal assay conditions and was estimated to be 0.16 μmol inosine formed/min per g wet weight of liver.

Discussion

Results of the experiments described in this paper on the regulatory properties of a cytosol 5′-nucleotidase from rat liver, suggested a potential role of this enzyme to serve to protect the cells against sudden metabolic stress which could result from sudden utilization of ATP by removal of AMP.

Some investigators have reported a rapid decrease in adenylate energy charge and a depletion of adenine nucleotides followed by intravenous administration of D-fructose (for a review see Ref. 19). According to the results reported by Raivio et al. [20], rats injected with fructose showed a decrease in adenylate energy charge from 0.9 to 0.7 We found that AMP-hydrolysing activity of the cytosol 5′-nucleotidase increased sharply, when adenylate energy charge fell from 1.0 to 0 5. This suggests a contribution of the cytosol 5′-nucleotidase in maintaining adenylate energy charge by removal of AMP

The inhibition of the cytosol 5′-nucleotidase by P_i may also be of physiological significance. Following fructose injection, P_i concentration was observed to undergo a transient decrease, coincident with the drop in energy charge [20]. This would release the inhibition of this enzyme by P_i and further increase the rate of AMP removal.

After injection of fructose, total concentration of adenine nucleotides rapidly decreases and the depletion of the adenylate ceases when the pool size reaches 1.9 mM [20]. Our results showed that at an energy charge value of 0 7 and pool size of 2 mM, the rate of AMP-hydrolysis was low and about equal to that observed under the physiological conditions of 0.9 energy charge and a total pool size of 4 mM The dependence of the AMP-hydrolysing activity of the cytosol 5′-nucleotidase on the concentration of ATP (the major constituent of the pool) provides a built-in limit on how far the pool size will be reduced

Chapman et al [9,10] have already reported the similar regulatory properties of AMP deaminase. These two enzymes that participate in the reactions of the first steps of degradation of AMP share common regulatory properties This seems to provide an effective mechanism for maintaining adenylate energy charge and the size of adenine nucleotide pool during conditions of sudden increase in the rate of utilization of ATP

Although IMP-hydrolysing activity increased with increasing adenylate energy charge, no marked response to its variation with the range of surviving mammalian cells was observed. Woods et al. [17] have reported that concentrations of IMP increase from 0 165 to 1.14 μmol/g wet weight within 10 min, in perfused rat liver after fructose loading. When IMP was used as substrate,

the K_m value of the purified cytosol 5'-nucleotidase from rat liver was estimated to be less than 1 mM [15]. Thus, the IMP-hydrolysing activity of this enzyme seems to be mainly regulated through the variation of the concentration of the substrate in rat liver cells.

Hydrolysis of IMP in vivo is possibly regulated by P_i and total adenine nucleotide concentration to some extent. When substrate concentration was fixed at 0.5 mM and total adenine nucleotide concentration at 4 mM, the IMP-hydrolysing activity increased 1.8-fold with a decreasing P_i concentration from 5 to 2 mM, at an energy charge value of 0 7 With decreasing total adenine nucleotide concentration from 4 to 1 mM, the IMP-hydrolysing activity showed a 50% decrease at an energy charge value of 0.7. During a metabolic stress with depletion of ATP, the IMP-hydrolysing activity seems to increase with the accumulation of substrate and the decrease in P_i concentration. The decrease in the IMP-hydrolysing activity with the decreasing size of the adenine nucleotide pool may also be a self-limiting response to prevent excess depletion of purine nucleotides

Some investigators have suggested that the conversion of AMP to inosine occurs mainly via AMP deaminase reaction [5—8]. Chapman and Atkinson [9] calculated the maximal rate of deamination of AMP in rat liver to be 0.2 μmol/min per g wet weight at an energy charge value of about 0.7. According to our estimation and considering the intracellular pH, a contribution of the cytosol 5'-nucleotidase activity in the AMP removal at this value of energy charge should be less than 10% that of AMP deaminase in rat liver. On the contrary, IMP-hydrolysing activity was estimated to be comparable to that of AMP deaminase This strongly suggests that the AMP → IMP → inosine pathway is more significant than the AMP → adenosine → inosine pathway in rat liver.

It seems likely that a main role of the cytosol 5'-nucleotidase in rat liver is the removal of excess IMP produced by de novo purine nucleotide synthesis or by AMP deaminase reaction which is effectively regulated by adenylate energy charge.

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