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A STUDY ON THE SEQUESTRATION OF ADENOSINE AND ITS CONVERSION TO ADENINE BY THE CYCLIC AMP-ADENOSINE BINDING PROTEIN/*S*-ADENOSYLHOMOCYSTEINASE FROM MOUSE LIVER

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Summary

A fraction of adenosine bound to the cyclic AMP-adenosine binding protein/*S*-adenosylhomocysteinase from mouse liver is almost instantaneously converted to adenine. The conversion ratio, defined as the concentration of adenine divided by the sum of the concentrations of adenine and adenosine, and the sequestration of adenosine, determined as the amount of adenosine not deaminated in the presence of high concentration of adenosine deaminase, were determined under various conditions.

The fraction of adenosine not available for deamination increased in a time-dependent manner by preincubating adenosine in the presence of the protein before the addition of adenosine deaminase. The reversion, i.e., the decrease in the conversion ratio (in the absence of adenosine deaminase), showed the same time dependency. The sequestration of adenosine, the reversion and the initial conversion ratio were promoted by 2-mercaptoethanol, alkaline pH, potassium chloride and high temperature. NaCl was inefficient.

Adenine, AMP and ADP inhibited the reversion in a concentration-dependent manner with decreasing efficiency in the order mentioned. These purines were also shown to increase the amount of adenosine available to the enzyme, adenosine deaminase, in a manner which parallelled their inhibitory power towards the binding of [^{3}H]adenosine. ATP (3 mM) and cyclic AMP (10 μM) were without effect.

A rapid reversion was observed at low concentrations of adenosine (less than

Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Mes, 2-(*N*-morpholino)-ethanesulfonic acid.

5 μM) whereas the conversion ratio was nearly constant at higher concentrations than 5 μM. Under the same conditions, the amount of adenosine sequestered as a function of time of preincubation in the absence of adenosine deaminase, increased by increasing the concentration of the nucleoside up to 5—10 μM. No further increase was observed beyond this concentration.

Introduction

A protein which binds adenosine, cyclic AMP and other adenine nucleotides has been purified to apparent homogeneity from mouse liver [1—5]. This protein was eventually identified as the enzyme, *S*-adenosylhomocysteinase (EC 3.3.1.1) [6,7] which catalyzes the reversible thioether bond formation between L-homocysteine and adenosine [8] and is widely distributed in mammalian tissues [9—13].

A fraction of adenosine bound to this enzyme seems to be converted to adenine [14]. Adenine formed from adenosine is only slightly liberated from the protein and the conversion is reversed under certain conditions. Adenosine is protected against deamination in the presence of this protein and the protection seems more efficient under conditions where a large fraction of adenosine is converted to adenine [14]. In light of these results a more detailed study was undertaken to investigate the relation between the sequestration of adenosine and its conversion to adenine.

Materials and Methods

Chemicals. The sources of most of the reagents used have been given in a preceding paper [5]. Adenosine deaminase (type I from calf intestinal mucosa) was obtained from Sigma Chemical Co., St. Louis, U.S.A., and 2-mercaptoethanol from Merck, Darmstadt. Polyethyleneimine 400 was purchased from Serva, Heidelberg and cellulose powder (MN 300) from Macherey Nagel, Co., F.R.G. Polyethyleneimine-impregnated cellulose thin-layer sheets (0.25 mm) on glass plates (20 X 20 cm) were prepared as described by Randerath and Randerath [15]. The thin-layer plates were developed in distilled water before use.

Purification of the cyclic AMP-adenosine binding protein/S-adenosylhomocysteinase. The protein was purified to apparent homogeneity as described previously [2,5].

Assay for metabolism of adenosine. [^{3}H]Adenosine was incubated in the presence of the binding protein and adenosine deaminase under the conditions described in the legends to the separate figures. Samples of 25 μl from the incubation mixture were mixed with 25 μl of 0.8 N perchloric acid, the acid neutralized, and adenine, adenosine and inosine separated by thin-layer chromatography as described [14].

Protein determination. Protein was determined by measuring the absorbance at 280 nm using a specific extinction coefficient ($E^{1\%}_{280nm}$) of 13.0 for the protein [3].

Definitions. Conversion ratio is defined as ([adenine]/[adenine] + [adeno-

sine]). Reversion is referred to as a decrease in conversion ratio as a function of time in the absence of adenosine deaminase. Sequestration of adenosine is used synonymously with protection of the nucleoside against deamination to inosine by the enzyme adenosine deaminase throughout this paper.

Results

Effect of time of preincubation

Adenosine (1 μM) was incubated in the presence of the binding protein. After various periods of time, adenosine deaminase was added to the incubation mixture. The experiment was conducted in the absence (Fig. 1A) and presence of 2-mercaptoethanol (Fig. 1B).

After 15 s of incubation, about 60% of adenosine present is converted to

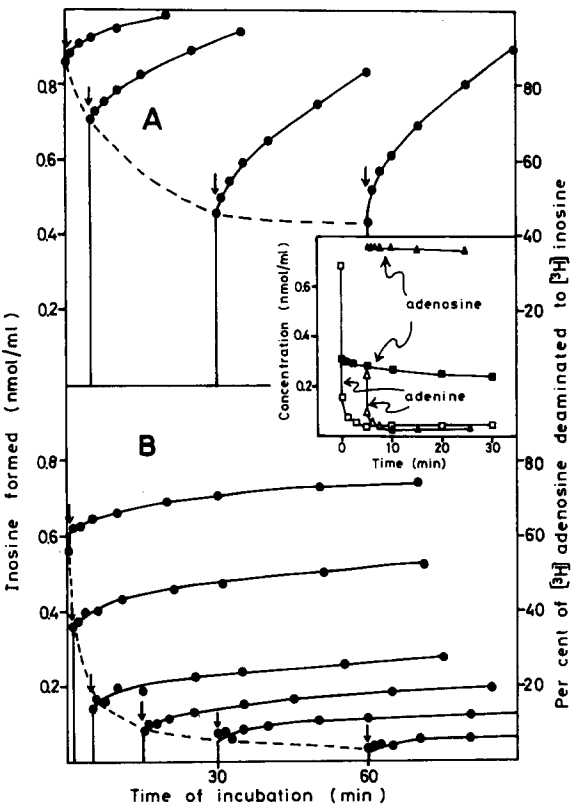

Fig. 1. The time course of the sequestration of adenosine in the absence (A) and presence (B) of 2-mercaptoethanol. Adenosine (1 μM) was incubated in the presence of purified cyclic AMP-adenosine binding protein/*S*-adenosylcysteinase (150 μg/ml) at 30°C for the time indicated. The incubation buffer was 15 mM Hepes (pH 7.0) containing 150 mM KCl, 10 mM magnesium acetate and either no (A) or 5 mM 2-mercaptoethanol (B). After various periods of time the incubation mixture was supplemented (arrow) with sufficiently high concentration (50 units/ml) of adenosine deaminase to deaminate adenosine to inosine in less than 10 s. Inosine formed is plotted versus time of incubation. Inset, shows the concentration of adenosine (■, ▲) and adenine (□, △) immediately before (first symbols) and after the addition of adenosine deaminase at time 15 s (■, □) and 5 min (▲, △) of incubation. The experiment was performed in the presence of 2-mercaptoethanol.

adenine in the absence of the reducing agent (data not shown) and about 65% in the presence of 2-mercaptoethanol (Fig. 1, inset). At this time the incubation mixture is supplemented with high concentration of adenosine deaminase. The conversion of adenosine is rapidly reversed (as shown in the presence of 2-mercaptoethanol in the inset of Fig. 1) and the main fraction of adenosine is deaminated to inosine. After this initial phase of inosine formation, the concentration of inosine increases slowly (Fig. 1). The fraction of adenosine available for deamination decreases progressively as a function of time of preincubation in the absence of adenosine deaminase. The sequestration of adenosine is promoted by 2-mercaptoethanol (Fig. 1).

Effect of reducing agent

The initial conversion ratio (conversion ratio at time 5 s of incubation), the reversion and the sequestration of adenosine are promoted by 2-mercaptoethanol in a concentration-dependent manner (Fig. 2A and B).

The protein was preincubated for increasing periods of time (0—90 min) in the presence of 2-mercaptoethanol (5 mM) before the addition of [^{3}H]-adenosine (1 μM). The experiment was otherwise performed as described in the legend to Fig. 2A and B. Neither the reversion nor the sequestration is affected by this treatment (data not shown). Thus, the effect of 2-mercaptoethanol is dependent on the presence of adenosine which perhaps should be related to altered reactivity of SH-groups in the presence of adenosine [3].

Effect of pH, cations, temperature and concentration of cyclic AMP-adenosine binding protein/S-adenosylhomocysteinase

The decline in the conversion ratio as a function of time (reversion) was

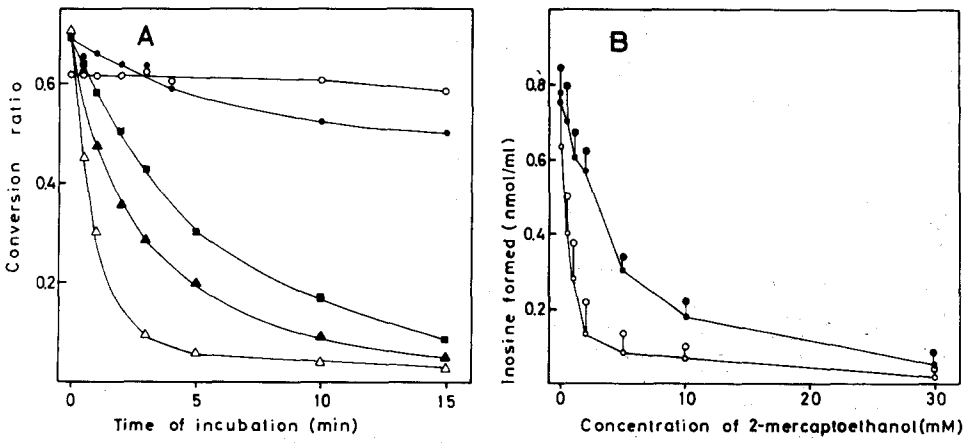

Fig. 2. Effect of 2-mercaptoethanol on the reversion (A) and sequestration (B). (A) Adenosine (1 μM) was incubated in the presence of cyclic AMP-adenosine binding protein/*S*-adenosylhomocysteinase (150 μg/ml) at 30°C for the time indicated. The incubation buffer was 15 mM Hepes (pH 7.0) containing 150 mM KCl, 10 mM magnesium acetate and 0 mM (○), 0.5 mM (●), 2.5 mM (■), 5 mM (▲) or 20 mM (△) 2-mercaptoethanol. The conversion ratio is plotted versus the time of incubation. (B) Adenosine (1 μM) was incubated under the conditions described under (A) in the presence of 2-mercaptoethanol at concentrations indicated. After 2.5 (●) and 15 min (○) of preincubation, adenosine deaminase was added to the incubation mixture to a final concentration of 50 units/ml. Inosine formed after 1 min (small symbols) and 10 min (big symbols) is plotted against the concentration of 2-mercaptoethanol.

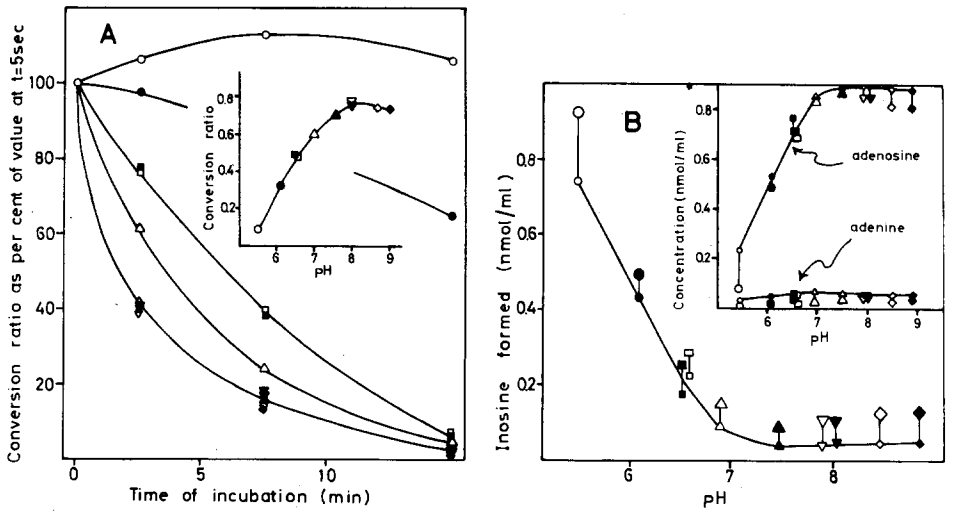

Fig. 3. Reversion (A) and sequestration (B) at various pH. (A) Adenosine (1 μM) was incubated in the presence of cyclic AMP-adenosine binding protein/*S*-adenosylhomocysteinase (150 μg/ml) at 30°C for the time indicated. The incubation buffers were 30 mM Mes buffer (○, ●, □), 30 mM Hepes buffer (■, △, ▲, ▽) or 30 mM Tris/maleate buffer (▼, ◇, ◆) adjusted to the pH value indicated. All buffers contained 150 mM KCl, 10 mM magnesium acetate and 5 mM 2-mercaptoethanol. The conversion ratio as percent of the ratio determined at short time of incubation (5 s) is plotted against time of incubation. Inset, shows the conversion ratio at 5 s of incubation versus pH. (B) Adenosine (1 μM) was incubated at 30°C in the presence of cyclic AMP-adenosine binding protein/*S*-adenosylhomocysteinase (150 μg/ml). The incubation buffers and the symbols are as in (A). After 5 min of preincubation, adenosine deaminase (50 units/ml) was added to the incubation mixture. Inosine formed after 1 min (small symbols) and 10 min (big symbols) of incubation in the presence of adenosine deaminase is plotted against pH. Inset, shows the distribution between adenine and adenosine of the fraction of adenosine not deaminated to inosine. The same symbols are used as in (A).

determined at various pH values and was shown to proceed rapidly at alkaline pH (Fig. 3A). The initial conversion ratio is high (Fig. 3A, inset) and the sequestration of adenosine is stimulated (Fig. 3B) at high pH. These parameters (initial conversion ratio, the reversion and the sequestration of adenosine) also showed the same requirement for monovalent cation: they are increased in a concentration dependent manner by KCl up to about 100 mM where the stimulation is two-fold. NaCl is without effect (data not shown). The sequestration of adenosine and the reversion increases in parallel as a function of the temperature (0—35°C). The amount of adenosine sequestered increased almost linearly with respect to the concentration of cyclic AMP-adenosine binding protein/*S*-adenosylhomocysteinase (data not shown).

The effect of adenine and adenine nucleotides on the reversion, the initial conversion ratio and the sequestration of adenosine

The reversion was determined in the presence of 2-mercaptoethanol and various concentrations of adenine, AMP, ADP, ATP and cyclic AMP and was shown to decrease in the presence of adenine, AMP and ADP. The efficiency decreases in the order mentioned (Fig. 4). ATP (3 mM) and cyclic AMP (10 μM) are without effect (data not shown).

The initial conversion ratio is decreased in the presence of adenine, AMP

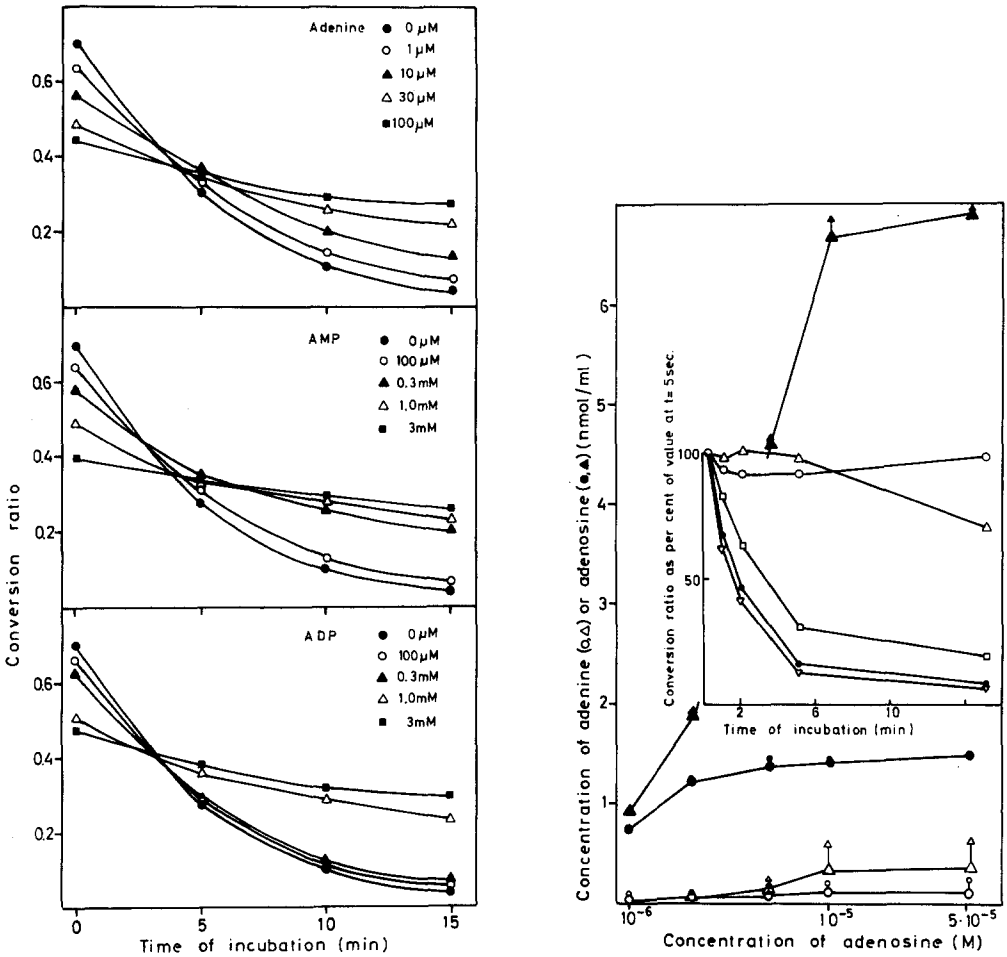

Fig. 4. The reversion in the presence of increasing concentrations of adenine, AMP and ADP. Adenosine (1 μM) was incubated at 30°C in the presence of cyclic AMP-adenosine binding protein/*S*-adenosylhomocysteine (150 μg/ml) and adenine or AMP or ADP at concentrations indicated. The incubation buffer was 15 mM Hepes (pH 7.0) containing 5 mM magnesium acetate, 150 mM KCl and 5 mM 2-mercaptoethanol. The conversion ratio is plotted against time of incubation.

Fig. 5. The sequestration of adenosine and the reversion at various concentrations of adenosine. Adenosine at concentration indicated on the figure was incubated at 30°C in the presence of cyclic AMP-adenosine binding protein/*S*-adenosylhomocysteine (150 μg/ml). The incubation buffer was 15 mM Hepes (pH 7.0) containing 5 mM magnesium acetate, 150 mM KCl and 5 mM 2-mercaptoethanol. After 2.5 min (●, ○) and 15 min (△, ▲), the incubation mixture was made 50 units/ml in adenosine deaminase. The concentration of adenine (○, △) and adenosine (●, ▲) after 2 min (small symbols) and after 10 min (big symbols) of incubation in the presence of adenosine deaminase is plotted against the concentration of adenosine. Inset, shows the decrease of the conversion ratio (reversion) at 1 μM (▽), 2 μM (●), 5 μM (□), 10 μM (△) and 50 μM (○) of adenosine. The incubation conditions were the same as above except that adenosine deaminase was not added to the incubation mixture.

and ADP (Fig. 4). The sequestration of adenosine (1 μM) is inhibited by adenine, AMP and ADP with decreasing efficiency in the order mentioned and in a manner which closely parallels the displacement of [^{3}H]adenosine binding by these purines [5]. At 1 mM adenine, the sequestration of adenosine is

almost completely inhibited. Cyclic AMP and ATP are without effect (data not shown).

The sequestration of adenosine and the reversion at various concentrations of adenosine

The time course of the reversion was determined at various concentrations (1—50 μM) of adenosine. At low concentrations (less than 5 μM) of adenosine a rapid reversion was observed whereas at high concentrations (higher than 5 μM) the conversion ratio is nearly constant or decreased slowly (Fig. 5, inset). The amount of adenosine sequestered after preincubation for 2 and 10 min in the absence of adenosine deaminase was determined at the same concentrations of adenosine (Fig. 5). The amount of adenosine not available for deamination after 2 min of preincubation increased as a function of the concentration of adenosine up to about 5 μM (Fig. 5). These data are consistent with the interpretation that the velocity of the sequestration process increases up to this concentration of adenosine. The plateau obtained is not a result of saturation of adenosine binding capacity as the amount of adenosine sequestered is further increased by increasing the preincubation time to 10 min (Fig. 5).

Discussion

It has previously been shown that adenine formed from adenosine is mainly bound to the protein and slightly liberated [14]. The conversion of adenosine to adenine, the reversion and the sequestration of adenosine are promoted and inhibited in a parallel manner by the same factors and thus seem to be closely related processes. These observations are consistent with the interpretation that conversion to adenine may be an intermediate step in the formation of tight binding of adenosine to the protein.

Oxidation of adenosine to 3′-ketoadenosine has recently been suggested as a step in the catalytic mechanism of *S*-adenosylhomocysteinase from beef liver [16]. The intermediate could not be isolated because it spontaneously eliminates adenine [16]. This may offer an explanation to the formation of adenine. The biological relevance of the conversion of adenosine to adenine is indicated by the observation that at high (cellular) level of *S*-adenosylhomocysteinase a fraction of adenosine is not available for the synthesis of *S*-adenosylhomocysteine. This fraction was identified as adenine [14].

Adenine and adenine nucleotides both inhibits the reversion and the sequestration process. Both processes seem to proceed at a high rate at low concentrations of adenosine (Fig. 5). Thus the sequestration of adenosine may be regulated by the cellular level of adenine, adenine nucleotides and adenosine itself. Furthermore, efficient protection of adenosine at low level of the nucleoside may be an important factor in the cellular economy of this purine.

The observation that adenosine is protected against deamination in crude extract when the concentration of soluble proteins approaches that existing in the cell [17], suggests that the sequestration process may operate in vivo.

Acknowledgements

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