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# *S*-Adenosylhomocysteinase from Yellow Lupin Seeds: Stoichiometry and Reactions of the Enzyme·Adenosine Complex†

Hieronim Jakubowski* and Andrzej Guranowski

ABSTRACT: Plant (*Lupinus luteus*) *S*-adenosylhomocysteinase, an $\alpha_2$ dimer, forms a 1:2 enzyme·adenosine complex. The binding sites for adenosine are not equivalent. Binding of the first molecule of adenosine is fast ($k > 7 \times 10^5$ $M^{-1}$ $s^{-1}$), whereas the second molecule of adenosine binds in a slow process with a half-life of 5 min. Adenosine in the 1:1 and 1:2 enzyme–substrate complexes reacts slowly ($k = 0.05$ $min^{-1}$) to give finally free enzyme, adenine, and ribose. The enzyme does not lose its ability to catalyze the synthesis of *S*-adenosylhomocysteine during the reactions. The relevance of the data to the catalytic functioning of the plant *S*-adenosylhomocysteinase is discussed.

*S*-Adenosylhomocysteinase (EC 3.3.1.1) catalyzes the following reaction (De la Haba & Cantoni, 1959):

*S*-adenosylhomocysteine + $H_2O$ ⇌ adenosine + homocysteine

This enzyme has not been found in procaryotes but occurs in all eucaryotes examined (Waler & Duerre, 1975) and has been purified to homogeneity from plant (Guranowski & Pawelkiewicz, 1977) and animal (Richards et al., 1978; Saebø & Ueland, 1978; Schatz et al., 1979; Palmer & Abeles, 1979) tissues.

Recently it has been shown that plant (*Lupinus luteus*) *S*-adenosylhomocysteinase, an $\alpha_2$ dimer, forms isolatable 1:1 complex with one of its substrates, adenosine (Jakubowski & Guranowski, 1978). In this report, the evidence is presented for the existence of a 1:2 *S*-adenosylhomocysteinase·adenosine complex. Reactions of both 1:1 and 1:2 enzyme·adenosine complexes leading finally to free fully active enzyme, adenine, and ribose are also described.

## Materials and Methods

*Materials.* Radiochemicals were purchased from The Radiochemical Centre (Amersham, England). Sephadex G-50 (fine) was from Pharmacia (Uppsala, Sweden). Nitrocellulose filters were from Schleicher & Schüll (Dassel, West Germany). Thin-layer chromatographic plates, aniline phthalate spray and ribose were obtained from Merck (Darmstadt, West Germany). Adenine, adenosine, dithiothreitol, Bicine[1] buffer, and *S*-adenosylhomocysteine were from Calbiochem (Los Angeles, CA). DL-Homocysteine was from Nutritional Biochemicals (Cleveland, OH).

*Enzymes.* Homogeneous *S*-adenosylhomocysteinase was prepared from yellow lupid seeds as described previously (Guranowski & Pawełkiewicz, 1977) and was stored at −20 °C as a 21 μM stock solution in 10 mM potassium phosphate buffer (pH 6.8) containing 5 mM 2-mercaptoethanol and 10% glycerol. Adenosine nucleosidase was purified from barley leaves as described previously (Guranowski & Schneider, 1977). Adenosine deaminase (specific activity 235 units/mg) was purchased from Sigma (St. Louis, MO).

Unless otherwise indicated, all experiments were performed at 25 °C in a medium (pH 8.3) containing 50 mM $K_2HPO_4$ and 2.5 mM 2-mercaptoethanol.

*Nitrocellulose Filter Assay.* Indicated concentrations of [$^{14}$C]adenosine (559 Ci/mol, 1 Ci = $3.7 \times 10^{10}$ Bq) and lupin *S*-adenosylhomocysteinase were mixed in the medium, and after appropriate time intervals, aliquots of the reaction mixture were applied on nitrocellulose disks presoaked in the medium. The filters were then washed 4 times with 1-mL portions of ice-cold medium, oven-dried, and counted in a scintillation counter. Blanks (without enzyme) were subtracted from all experimental values. Morever, since it has been demonstrated that nitrocellulose filters adsorb adenine (Schneider & Guranowski, 1975), blanks with adenine were also run, and the results were corrected accordingly. The reproducibility of the assay was ±10%.

*Gel Filtration.* Gel filtration was carried out at 0–1 °C. Aliquots (20 μL) of reaction mixtures were applied onto a small Sephadex G-50 (fine) column (0.4 × 6 cm) equilibrated with the medium. Fractions (50 μL) were collected and assayed for radioactivity by scintillation counting.

*Measurement of Adenine Formation.* Aliquots (5 μL) of the reaction mixtures containing *S*-adenosylhomocysteinase and [$^{14}$C]adenosine in the medium were spotted on the origin line of silica gel plates. The plates were developed with 2-

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[1] Abbreviations used: E, *S*-adenosylhomocysteine hydrolase (EC 3.3.1.1); Ado, adenosine; Ade, adenine; Rib, ribose; Bicine, *N*,*N*'-bis(2-hydroxyethyl)glycine.

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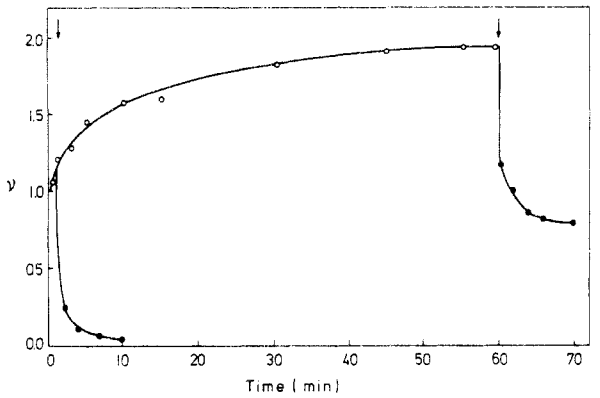



FIGURE 1: Time-dependent increase in the binding of adenosine to the *S*-adenosylhomocysteinase from yellow lupin. The enzyme (0.56 μM) was incubated with [U-$^{14}$C]adenosine (4.5 μM, 559 Ci/mol), and at indicated time intervals, aliquots were filtered through nitrocellulose disks (○). At the time indicated with an arrow unlabeled adenosine (final concentration 1 mM) was added, and the disappearance of the radioactivity was followed by nitrocellulose disk filtration (●). Stoichiometry ($\nu$), defined as the number of moles of [$^{14}$C]adenosine bound per mole of the enzyme, is plotted as a function of time.

propanol/ethyl acetate/ammonia/water (23:27:5:3 v/v). Adenine ($R_f$ 0.68) and adenosine ($R_f$ 0.57) were visualized under ultraviolet light, and ribose ($R_f$ 0.16) was localized after the plates were sprayed with aniline phthalate and heated at 120 °C. No radioactivity was found at the origin, i.e., in the region occupied by the ribose 1-phosphate marker. Alternatively, cellulose sheets developed in water were used ($R_f$ for adenosine was 0.5 and for adenine 0.3 and ribose migrated with the solvent front).

*Assay for S-Adenosylhomocysteinase Activity.* Standard incubation mixture contained 50 mM Bicine/KOH buffer (pH 8.3), 2 mM dithiothreitol, 20 mM DL-homocysteine, 1 mM [$^{14}$C]adenosine (2.5 Ci/mol), and enzyme. After appropriate time inverals, 5-μL aliquots were spotted onto the origin line of the silica gel plate. Unlabeled *S*-adenosylhomocysteine as a marker was applied onto the origin, and the chromatogram was developed with 2-propanol/ethyl acetate/ammonia/water (23:27:5:3 v/v). *S*-Adenosylhomocysteine spots ($R_f$ 0.07) were visualized under ultraviolet light, cut out, and counted.

## Results

From the work on aminoacyl-tRNA synthetases (Mulvey & Fersht, 1977; Jakubowski, 1978), it is known that the binding of a second molecule of substrate to enzyme, which is $\alpha_2$ dimer or monomer with an internal pseudo $\alpha_2$ structure, may be an extremely slow process compared to binding of the first molecule of substrate. This behavior has also been found with plant *S*-adenosylhomocysteinase. The enzyme forms a 1:1 complex with adenosine, which can be measured by trapping the $^{14}$C-labeled complex on a nitrocellulose filter (Jakubowski & Guranowski, 1978). Upon prolonged incubation of *S*-adenosylhomocysteinase with [$^{14}$C]adenosine before nitrocellulose filtration, the number of moles of [$^{14}$C]-adenosine bound per mol of the enzyme increases from 1 to plateau which is dependent on the concentration of [$^{14}$C]-adenosine (Figures 1 and 3). The half-life for this increase is 5 min.

The dissociation of [$^{14}$C]adenosine from the complex is apparently a biphasic process (Figure 1). About 80% of the label can be chased out with unlabeled adenosine within 0.5 min, and the remaining radioactivity dissociates with $k = 0.3$ min$^{-1}$ from the complex formed after 1.5-min preincubation of [$^{14}$C]adenosine with *S*-adenosylhomocysteinase. By use of

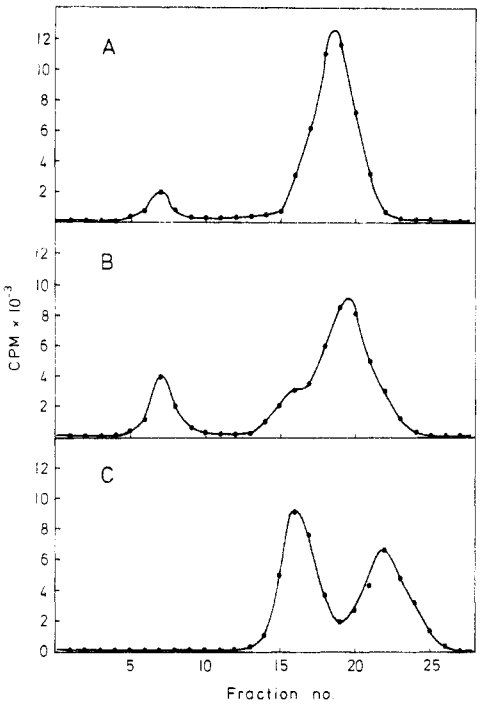



FIGURE 2: Gel filtration of the *S*-adenosylhomocysteinase·adenosine complexes. The enzyme (1.1 μM) was mixed with 4.5 μM [U-$^{14}$C]adenosine at 25 °C and passed through a Sephadex G-50 (fine) (0.4 × 6 cm) column at 0–1 °C. The flow rate was 50 μL (fraction volume) per 2 min. (A) Elution profile after 1-min preincubation at 25 °C; (B) elution profile after 1-h preincubation at 25 °C; (C) an equimolar mixture of [$^{14}$C]adenine and [$^{14}$C]ribose (prepared by digestion of 4.5 μM [U-$^{14}$C]adenosine with adenosine nucleosidase from barley leaves) was passed through the Sephadex column.

the value for the rate constant of dissociation estimated for the fast phase ($k > 3$ min$^{-1}$) and $K_{diss} = 5 \times 10^{-8}$ (Jakubowski & Guranowski, 1978), the second-order rate constant for formation of the enzyme·adenosine complex may be estimated as $> 7 \times 10^5$ M$^{-1}$ s$^{-1}$. The rates of dissociation of the 1:1 enzyme·adenosine complex (formed with $k > 7 \times 10^5$ M$^{-1}$ s$^{-1}$) and presumed 1:2 complex (observed after prolonged incubation) seem to be similar. However, only part of the radioactivity associated with the complex measured after 1-h preincubation can be chased with unlabeled adenosine whereas almost all radioactivity associated with the complex measured after 1.5-min preincubation was exchangeable with unlabeled adenosine. This unexchangeable radioactivity is associated with adenine and ribose (see below).

The *S*-adenosylhomocysteinase·[$^{14}$C]adenosine complex was separated from excess substrate by gel filtration (Figure 2) and analyzed for adenosine, adenine, and ribose by thin-layer chromatography. As expected from the nitrocellulose filter titration experiment described in Figure 1, the enzyme-bound radioactivity isolated after 1-h preincubation was twice as high as that recovered after 1-min preincubation. however, the 1-h-old complex contained, on a molar basis, only 33% adenosine, 33% adenine, and 33% ribose, whereas the 1-min-old complex contained 95% adenosine and traces of adenine and ribose. Thus, the data indicate that the complex formed within the first minute of preincubation of the enzyme with [$^{14}$C]adenosine is 1:1 enzyme·[$^{14}$C]adenosine complex and the complex assayed after 1 h is 1:1:1:1 enzyme·[$^{14}$C]adenosine·[$^{14}$C]adenine·[$^{14}$C]ribose complex.

The gel filtration data (Figure 2) indicate that both adenine and ribose can dissociate from the enzyme. Both adenine and ribose were detected in the peak containing low molecular weight substances when the 1-h-old complex was passed through the Sephadex G-50 column (Figure 2B). A faster

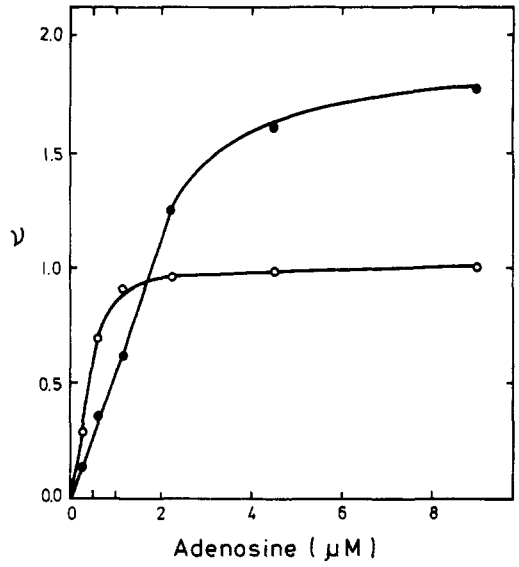



FIGURE 3: Titration of the *S*-adenosylhomocysteinase with adenosine. The enzyme (1.1 μM) was mixed with [U-$^{14}$C]adenosine (0.28–9 μM) and filtered through nitrocellulose disks after 1 (○) and 60 min (●).

migrating shoulder of the peak of low molecular weight compounds was observed when *S*-adenosylhomocysteinase was preincubated with uniformly labeled [$^{14}$C]adenosine for 1 h before gel filtration (Figure 2B). This has not been observed when [$^{14}$C]adenosine labeled only in adenine moiety was used (not shown). Thus, the shoulder could be attributed to ribose. Thin-layer chromatography of the corresponding fractions demonstrated that the shoulder fraction (fraction 16) contained in fact ribose in addition to adenosine whereas the descending part of the peak (fraction 22) contained adenine in addition to adenosine. Adenine and ribose were formed in equivalent amounts. A control experiment in which a mixture of authentic [$^{14}$C]adenine and [$^{14}$C]ribose was subjected to gel filtration confirmed the ability of Sephadex G-50 (fine) to retard adenine and to separate it from ribose (Figure 2C).

Titration of the *S*-adenosylhomocysteinase with [$^{14}$C]-adenosine measured by nitrocellulose filtration immediately and 1 h after the enzyme had been mixed with the ligand is depicted in Figure 3. At concentrations of adenosine lower than or equal to the concentration of the enzyme (the first three experimental points), the amount of radioactivity retained on the filters after 1-h incubation of the complex is smaller than that measured after 0.5-min incubation. At adenosine concentrations that saturate the *S*-adenosylhomocysteinase, the plateau observed after 1 h is approximately twice as high as that observed after 0.5 min.

The apparent increase in stoichiometry with time (Figures 1 and 3) could be the results of the following reactions:

$$\text{E·Ado} \rightleftharpoons \text{E·Ade·Rib} \overset{+\text{Ado}}{\rightleftharpoons} \text{E·Ado·Ade·Rib}^1 \qquad (1)$$

or

$$\text{E·Ado} \overset{+\text{Ado}}{\rightleftharpoons} \text{E·Ado}_2 \rightleftharpoons \text{E·Ado·Ade·Rib} \qquad (2)$$

In order to distinguish between reactions 1 and 2, we have compared the rate of adenine formation (Figure 4B) with the rate of increase in apparent stoichiometry (Figure 4A). The first-order rate constant of adenine formation calculated from the data shown in Figure 4 is 0.05 min$^{-1}$, and the pseudo-first-order rate constant for association of adenosine with *S*-adenosylhomocysteinase·adenosine complex is 0.14 min$^{-1}$. Thus, the formation of the E·Ado$_2$ complex is faster than the conversion of E·Ado into the E·Ade·Rib complex. The [$^{14}$C]adenosine from E·[$^{14}$C]adenosine$^2$ complex formed during

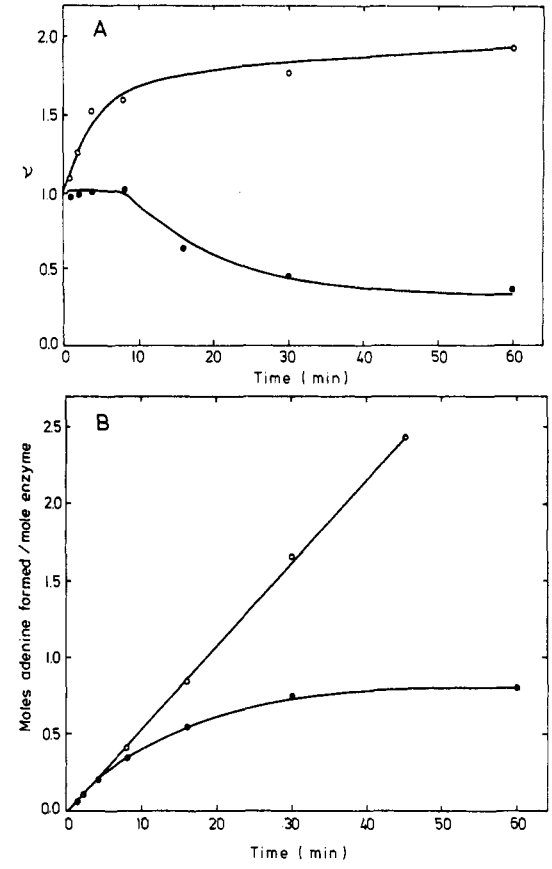



FIGURE 4: Comparison of the rates of increase and decrease in stoichiometry of the *S*-adenosylhomocysteinase·adenosine complexes (A) with the rate of formation of adenine (B). The enzyme (2.2 μM) was mixed with either 2.2 μM [U-$^{14}$C]adenosine (●) or 22 μM [U-$^{14}$C]adenosine (○) and assayed for the complexes by nitrocellulose disk filtration (A) and for adenine by thin-layer chromatography (B).

the first minutes of incubation can be fully exchanged with unlabeled adenosine. Again, the rate of increase in unexchangeable radioactivity is slower than the rate of increase in the stoichiometry of the complex (not shown). The E·Ado$_2$ complex transforms into the E·Ado·Ade·Rib complex at the same rate at which the E·Ado complex transforms into the E·Ade·Rib complex (Figure 4B). After prolonged incubation of the enzyme with an excess of [$^{14}$C]adenosine, the amount of adenine formed considerably exceeds the amount of the enzyme present in the reaction mixture (Figure 4A). This indicates that adenine dissociates from the enzyme during the reaction. Conversion of adenosine to adenine and its subsequent dissociation from the enzyme can be easily demonstrated with the 1:1 *S*-adenosylhomocysteinase·adenosine complex. The half-life of the complex measured by nitrocellulose filtration is 14 min (Figure 4A). The rate constants for disappearance of the 1:1 complex and for the formation of adenine are the same ($k$ = 0.05 min$^{-1}$). The disappearance of the complex is not due to enzyme inactivation. It has been verified that the prolonged incubations of the enzyme with adenosine at concentrations up to 0.25 mM do not affect its ability to catalyze the synthesis of *S*-adenosylhomocysteine.

The cleavage of adenosine to adenine and ribose can be in part reversed by removal of adenosine with adenosine deaminase (Table I). Only the enzyme-bound adenine is able to revert into adenosine. Exogenous adenine neither transforms into adenosine nor binds to the enzyme. The same has been found with mouse liver *S*-adenosylhomocysteinase (Ueland & Saebø, 1979; Saebø & Ueland, 1979). Additional evidence for reversibility came from gel filtration experiments. As it has been shown above, the radioactivity recovered in break-

Table I: Partial Reversibility of the *S*-Adenosylhomocysteinase-Catalyzed Hydrolysis of Adenosine to Adenine[a]

| | [$^{14}$C]adenine (cpm/5 μL) | [$^{14}$C]adenosine (cpm/5 μL) | [$^{14}$C]inosine (cpm/5 μL) |
|---|---|---|---|
| before[b] | 3120 | 2456 | 0 |
| after[c] | | | |
| 1 min | 2304 | 732 | 2490 |
| 2 min | 2208 | 636 | 2620 |
| 4 min | 2244 | 504 | 2946 |
| 8 min | 2244 | 492 | 2994 |

[a] The plant *S*-adenosylhomocysteinase (2.8 μM) was incubated with 2.8 μM [$^{14}$C]adenosine (labeled in the adenine moiety, 250 Ci/mol) in a total volume of 60 μL in a medium with 50 mM Bicine/KOH (pH 8.3) and 2 mM dithiothreitol for 30 min at 25 °C, and two 5-μL aliquots were analyzed for [$^{14}$C]adenosine and [$^{14}$C]adenine. After that time, 10 μL of a 1 mg/mL solution of adenosine deaminase (sufficient to convert all [$^{14}$C]adenosine to [$^{14}$C]inosine in less than 15 s in the absence of the *S*-adenosylhomocysteinase) was added, and aliquots were analyzed for [$^{14}$C]adenosine, [$^{14}$C]adenine, and [$^{14}$C]inosine after the indicated time intervals. [$^{14}$C]Adenosine ($R_f$ 0.57), [$^{14}$C]adenine ($R_f$ 0.68), and [$^{14}$C]inosine ($R_f$ 0.15) were separated by thin-layer chromatography on silica gel plates developed in ethyl acetate/2-propanol/ammonia/water (27:23:5:3 v/v). The results were corrected for dilution due to the addition of adenosine deaminase. [b] Before addition of adenosine deaminase. [c] After addition of adenosine deaminase.

through fractions after gel filtration of 1-h-old complex of the plant *S*-adenosylhomocysteinase with [$^{14}$C]adenosine (Figure 2B) contained [$^{14}$C]adenosine and [$^{14}$C]ribose. In a separate experiment, the [$^{14}$C]adenosine was chased from the complex with unlabeled adenosine (1 mM final concentration see Figure 1), and the mixture was subjected to gel filtration. As expected, the radioactivity recovered in the breakthrough fractions was about 2-fold lower than that in the experiment without chasing. However, the fractions still contained [$^{14}$C]adenosine in addition to [$^{14}$C]adenine and [$^{14}$C]ribose in a 1:1:1 molar ratio.

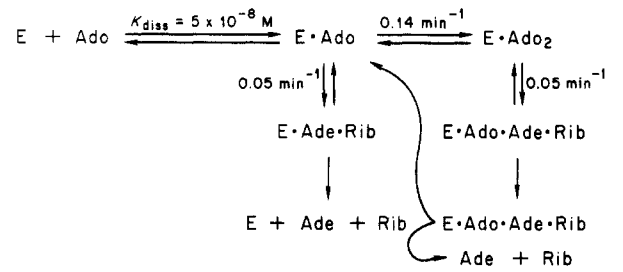
## Discussion

The major aim of this investigation was to resolve the apparent discrepancy between the subunit structure of the plant *S*-adenosylhomocysteinase and the stoichiometry of its complex with adenosine. The *S*-adenosylhomocysteinase, which is an $\alpha_2$ dimer (Guranowski & Pawełkiewicz, 1977), has previously been shown to form a 1:1 complex with adenosine (Jakubowski & Guranowski, 1978). Results of the experiments presented herein indicate that the enzyme binds a second molecule of adenosine to form a 1:2 enzyme·substrate complex. The binding of the second molecule of adenosine is probably too slow to have any physiological significance. However, it is good evidence for a second binding site.

Both 1:1 and 1:2 enzyme·adenosine complexes undergo further slow transformations to yield finally free enzyme, adenine, and ribose. In contrast with mammalian *S*-adenosylhomocysteinases (Chiang et al., 1979, 1981; Hershfield et al., 1979), which are irreversibly inactivated by adenosine, the plant enzyme does not loose any activity during those transformations. Formation of the *S*-adenosylhomocysteinase·adenosine complexes and their transformations are consistent with Scheme I.

On mixing equimolar amounts of *S*-adenosylhomocysteinase with adenosine, there is fast ($k > 7 \times 10^5$ M$^{-1}$ s$^{-1}$) and reversible formation of the 1:1 enzyme·adenosine complex. The 1:1 complex slowly reacts ($k = 0.05$ min$^{-1}$) to give a reversible E·Ade·Rib complex, which in turn dissociates irreversibly to give free active enzyme, adenine, and ribose. The possibility

Scheme I




of phosphorolytic cleavage of adenosine is excluded by finding that ribose separates from ribose 1-phosphate during thin-layer chromatography on silica gel plates, and no radioactivity was associated with the ribose 1-phosphate spot on the chromatograms. In addition, the *S*-adenosylhomocysteinase-catalyzed cleavage of adenosine proceeds in phosphate-free medium as well.

In the presence of excess adenosine, the 1:1 enzyme·adenosine complex binds reversibly a second molecule of adenosine. This is a slow process (pseudo-first-order rate constant is 0.14 min$^{-1}$), albeit it is 3-fold faster than the reactions of E·Ado and E·Ado$_2$ complexes to give the enzyme-bound adenine and ribose. The E·Ado$_2$ complex transforms slowly to reversible E·Ado·Ade·Rib complex, which then becomes irreversible. This mechanism is based on the observation that a part of the radioactivity from the $^{14}$C-labeled complex is exchangeable with unlabeled adenosine whereas all label is chased with unlabeled adenosine during the initial phase of incubation of the *S*·adenosylhomocysteinase with excess [$^{14}$C]adenosine.

The apparent hydrolysis of adenosine to adenine and ribose has been described earlier for mammalian *S*-adenosylhomocysteinases (Ueland & Saebø, 1979; Saebø & Ueland, 1979; Ueland & Helland, 1980; Chiang et al., 1981). The reaction described here for the plant *S*-adenosylhomocysteinase may be a manifestation of an oxidative catalytic mechanism with 3′-ketoadenosine intermediate, which has been shown to operate with mammalian *S*-adenosylhomocysteinase (Palmer & Abeles, 1979). This assertion is strengthened by the observation that the plant enzyme hydrolyzes 2′-deoxyadenosine to adenine but 3′-deoxyadenosine is not hydrolyzed (not shown). Irreversible inactivation of mammalian *S*-adenosylhomocysteinase by 2′-deoxyadenosine (Hershfield, 1979) *is* accompanied by hydrolysis of 2′-deoxyadenosine to adenine according to the oxidative catalytic mechanism (Abeles et al., 1980). However, according to the work of Hershfield (1979, 1980), 3′-deoxyadenosine is also an inactivator of the enzyme, but it seems to act by a different mechanism. Thus, the hydrolysis of adenosine by the plant *S*-adenosylhomocysteinase, although it is probably of no physiological significance due to its extreme slowness, is meaningful in mechanistic terms. It is remarkable that the breakdown of adenosine in the presence of mammalian *S*-adenosylhomocysteinase is about as fast [$k = 0.1$ min$^{-1}$ can be calculated from the data of Ueland & Saebø (1979)] as the reaction catalyzed by the plant enzyme ($k = 0.05$ min$^{-1}$).

Although the plant enzyme is able to bind two molecules of adenosine, only the binding of the first molecule of adenosine seems to be catalytically competent. The adenosine binding sites of the enzyme are not equivalent. The binding of one molecule of adenosine hinders the binding to the second site on the plant enzyme. In order to explain the apparent negative cooperativity in binding of adenosine, it is tempting to propose that the enzyme operates according to a flip-flop mechanism (Harada & Wolfe, 1968; Lazdunski et al., 1971) in which the subunits do not work simultaneously but rather consecutively.

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# Stereochemistry of *meso*-$\alpha,\epsilon$-Diaminopimelate Decarboxylase Reaction: The First Evidence for Pyridoxal 5′-Phosphate Dependent Decarboxylation with Inversion of Configuration†

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ABSTRACT: The stereochemistry of the decarboxylation of *meso*-$\alpha,\epsilon$-diaminopimelate catalyzed by *meso*-$\alpha,\epsilon$-diaminopimelate decarboxylase (EC 4.1.1.20) of *Bacillus sphaericus* was determined by stereochemical analyses of [6-$^2$H]-L-lysine produced by the reaction in $D_2O$. The product [6-$^2$H]-L-lysine was converted to levorotatory methyl 5-phthalimido[5-$^2$H]-valerate by the reactions not affecting the absolute configuration of the asymmetric carbon atom. By contrast, methyl 5-phthalimido[5-$^2$H]valerate derived from [2,6-$^2H_2$]-L-lysine, which was produced from [2,6-$^2H_2$]diaminopimelate by decarboxylation in $H_2O$, was dextrorotatory. The authentic methyl (*R*)-5-phthalimido[5-$^2$H]valerate prepared from L-glutamate with glutamate decarboxylase was levorotatory. These results indicate that the *meso*-$\alpha,\epsilon$-diaminopimelate decarboxylase reaction proceeds in an inversion mode. The deuterium label in [6-$^2$H]-L-lysine was fully conserved during the conversion into pelletierine through [1-$^2$H]cadaverine by the stereospecific diamine oxidase reaction. Thus, the enzymatic decarboxylation of *meso*-$\alpha,\epsilon$-diaminopimelate occurs with inversion of configuration in contrast to the other amino acid decarboxylases reported so far.

The reactions catalyzed by pyridoxal-P[1] dependent enzymes proceed through the reversible formation of a Schiff base between the 4′-aldehyde group of the cofactor and a primary amino group of a substrate (Snell & Di Mari, 1970). The following cleavage of one of the bonds to the substrate $\alpha$ carbon in the Schiff base yields a carbanionic quinoid intermediate. The specificity of this bond breaking as well as the nature of the reaction thereafter is determined by the structure of substrate and by the influence of the apoenzyme. Stereochemical analyses have provided a common feature of pyridoxal-P enzyme reactions as reviewed by Dunathan (1971). Thus, all bond-forming and bond-breaking reactions would occur on the same side of the plane formed with the Schiff base and the cofactor pyridine ring (Dunathan & Voet, 1974; Vederas & Floss, 1980). In support of this suggestion, all pyridoxal-P dependent decarboxylases studied so far catalyze

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[1] Abbreviations used: pyridoxal-P, pyridoxal 5′-phosphate; DAP, $\alpha,\epsilon$-diaminopimelate; NMR, nuclear magnetic resonance.

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