Tritium Isotope Effects in Adenosylcobalamin-Dependent Methylmalonyl-CoA Mutase†

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ABSTRACT: Methylmalonyl-CoA mutase from *Propionibacterium shermanii* is an adenosylcobalamin-dependent enzyme which catalyzes the reversible isomerization of methylmalonyl-CoA and succinyl-CoA. The rate of tritium loss from 5′-[^{3}H]adenosylcobalamin during the enzymic reaction and the relative rates of tritium appearance in substrate and product were examined. Upon the addition of methylmalonyl-CoA to a solution of holoenzyme, tritium was completely released from the cofactor within about 500 ms. No tritium was found either bound to the enzyme or released into the water. The radioactivity was found in methylmalonyl-CoA and succinyl-CoA in a constant ratio of 1 to 3, which did not change during the first 300 ms of the reaction. Upon the addition of succinyl-CoA to a solution of holoenzyme, tritium was released at essentially the same rate, and the radioactivity was found in methylmalonyl-CoA and succinyl-CoA in the identical constant ratio of 1 to 3. The tritium isotope effect on the enzyme-catalyzed hydrogen transfer, measured using ^{14}C-labeled methylmalonyl-CoA as substrate, was $k_H/k_T = 4.9$. This low value shows that hydrogen transfer is only partly rate limiting and that at least one subsequent slow step, such as product release, contributes substantially to the overall reaction velocity. The identical partitioning of tritium, regardless of the substrate used, shows that the rearrangement of the substrate radical into the product radical is not rate limiting. The very low tritium isotope effect and the fact that all the tritium is found bound either to the CoA esters or to the cofactor make it very unlikely that a protein radical is an intermediate in the methylmalonyl-CoA mutase-catalyzed rearrangement.

Methylmalonyl-CoA mutase catalyzes the reversible isomerization of (2*R*)-methylmalonyl-CoA and succinyl-CoA (Eggerer et al., 1960; Sprecher et al., 1966). The enzyme belongs to a group of AdoCbl[1]-dependent enzymes which catalyze unusual 1,2 rearrangements and is the only one found in both bacterial and human cells [for reviews, see Rétey (1979, 1990)]. Methylmalonyl-CoA mutase from *Propionibacterium shermanii* has been expressed in *Escherichia coli* and purified to homogeneity (McKie et al., 1990). The X-ray crystal structure of the mutase from *P. shermanii* has recently been solved at 2.0 Å resolution, which has provided valuable insights into the mode of substrate and cofactor binding (Mancia et al., 1996). However, we still lack detailed information on the kinetics of the rearrangement and on key features of the mechanistic pathway.

The first step in the mutase-catalyzed reaction is the homolytic cleavage of the Co−C bond of the cofactor to generate a Co(II)alamin radical and a carbon-centered deoxyadenosyl radical (Zhao et al., 1992, 1994; Keep et al., 1993; Padmakumar & Banerjee, 1995). In a subsequent step the deoxyadenosyl radical abstracts a hydrogen atom from the methyl group of methylmalonyl-CoA (**I**; see Figure 1) to generate a primary radical (**II**). The latter then rearranges by an as yet unknown mechanism to the secondary radical of succinyl-CoA (**III**). Reabstraction of a hydrogen from the cofactor to give the product succinyl-CoA (**IV**) and

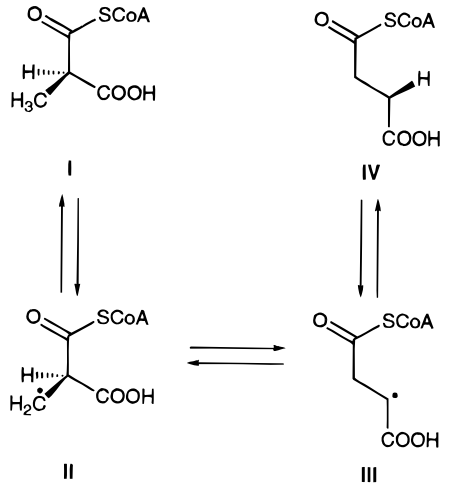

FIGURE 1: Mechanistic scheme for the reaction catalyzed by methylmalonyl-CoA mutase. AdoCbl is omitted from this figure.

AdoCbl completes the reaction cycle. The involvement of AdoCbl as hydrogen acceptor and hydrogen donor had been demonstrated by several groups using tritium-labeled substrate and tritium-labeled cofactor (Erfle et al., 1964; Rétey & Arigoni, 1966; Cardinale & Abeles, 1967).

Kinetic tritium isotope effects have been measured for hydrogen transfer from coenzyme to product on several AdoCbl-dependent enzymes catalyzing 1,2 rearrangements. The values obtained for diol dehydrase ($k_H/k_T = 125$; Essenberg et al., 1971) and for ethanolamine ammonia lyase ($k_H/k_T = 160$; Weisblat & Babior, 1971) are about 10 times higher than expected for a radical hydrogen abstraction. In the case of ethanolamine ammonia lyase this high isotope

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[1] Abbreviations: AdoCbl, adenosylcobalamin or coenzyme B_{12}; TFA, trifluoroacetic acid.

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effect has been rationalized by invoking the existence of a second hydrogen pool of protein-bound hydrogens which can equilibrate with the cofactor (Cleland, 1982; O'Brien et al., 1985). In contrast, the tritium isotope effect of glutamate mutase was recently found to lie in the normal range for a primary kinetic tritium isotope effect, $k_H/k_T = 13.5-18$ (Marsh, 1995).

For methylmalonyl-CoA mutase, only overall kinetic deuterium isotope effects have previously been measured, using fully deuterated substrates. Their values were determined as effects on V_{max} and are $k_H/k_D = 6.2$ for the substrate [$^2H_3$]methylmalonyl-CoA (Michenfelder et al., 1987) and $k_H/k_D = 3.5$ for the substrate [$^2H_4$]succinyl-CoA (Wölfle et al., 1986). In this paper the fate of tritium released from mutase holoenzyme and the tritium isotope effect for hydrogen abstraction from AdoCbl are reported. The results are discussed in the context of previously observed isotope effects for AdoCbl-dependent enzymes.

MATERIALS AND METHODS

Materials. The purification of methylmalonyl-CoA mutase from recombinant *E. coli* strains has been described previously (McKie et al., 1990). Radiolabeled 5′-[^{3}H]-AdoCbl with a specific activity of 6700 dpm/nmol was added to the apoenzyme before the final purification step, and methylmalonyl-CoA mutase was eluted as holoenzyme. Enzymic synthesis and purification of 5′-[^{3}H]-AdoCbl were done as described by Marsh (1995). [^{14}C]-DL-methylmalonyl-CoA was purchased from NEN DuPont and purified prior to use by HPLC (Beckman Ultrasphere RP C18 column, 1 × 25 cm, linear gradient of methanol in 100 mM phosphate buffer, pH 5.0, flow rate 1 mL/min, detection at 256 nm). All other chemicals were purchased from commercial suppliers and used without further purification.

AdoCbl Exchange Rate. To a 5 mL solution of 1 μM methylmalonyl-CoA mutase in 50 mM Tris-HCl, pH 7.5 at 4 °C, was added 10 μM 5′-[^{3}H]-AdoCbl with a specific activity of 6700 dpm/nmol. One milliliter samples were taken after intervals of 0.5, 1, 2, 4, and 24 h, respectively, and injected onto a 1 mL HiTrap Q column (Pharmacia FPLC system). AdoCbl and mutase holoenzyme were separated with a potassium chloride gradient in 50 mM Tris-HCl, pH 7.5. Free AdoCbl did not bind to the column and eluted after one column volume. Mutase holoenzyme eluted at about 0.3 M KCl. Fractions were taken and counted for tritium content.

Standard Assay Conditions for Methylmalonyl-CoA Mutase. To a 5 μM solution of methylmalonyl-CoA mutase in 50 mM Tris-HCl, pH 7.5 at 30 °C, succinyl-CoA was added to a final concentration of 100 μM to start the reaction. Samples (100 μL) were withdrawn after various periods of time and quenched on ice using 100 μL of 0.5% TFA.

Tritium Transfer from AdoCbl to the Protein. An experiment was set up under standard conditions. Samples were taken after 1, 2, 5, and 10 min, respectively, and quenched. The samples were injected onto a reverse-phase column (Pharmacia μRPC C2/C18, 3.2 × 30 mm) attached to a SMART system (Pharmacia), which had been preequilibrated with 0.1% TFA in water. Protein was separated from CoA esters and AdoCbl by a linear gradient of 0.1% TFA in acetonitrile at a flow rate of 200 μL/min, and fractions containing the protein were pooled and counted for tritium.

Tritium Transfer from AdoCbl to Water. An experiment was set up under standard conditions. Samples were taken after 5 and 10 min, respectively, and were lyophilized. The volatile compounds were collected in a cold trap cooled with liquid nitrogen and counted for tritium.

Quench Flow Assays. The measurements were made on a Bio Logic quench flow apparatus at either 25 or 30 °C. Three stock solutions were made up in 50 mM Tris-HCl buffer, pH 7.5: stock solution A was 9 μM methylmalonyl-CoA mutase, stock solution B was 2.25 mM CoA ester substrate, and the quench stock solution was 0.5% TFA. Reactions were started by mixing 160 μL of substrate with 80 μL of enzyme during 60 ms, and the reaction mixture was allowed to age for a variable period of time (0−1600 ms). Then 200 μL of quench solution was added to 200 μL of the reaction mixture over a time period of 50 ms. Quenched samples were transferred into Eppendorf tubes and immediately frozen on dry ice and stored at −20 °C until HPLC analysis.

HPLC Analysis of Radiolabeled Substrates and Products. Methylmalonyl-CoA, succinyl-CoA, and AdoCbl were separated at 10 °C by HPLC on a reverse-phase column (Pharmacia μRPC C2/C18, 2.1 × 100 mm) attached to a SMART system (Pharmacia). Samples of 100 μL were injected onto the column, which had been preequilibrated with 100 mM phosphate buffer, pH 5.0, and the compounds were eluted with a linear gradient of methanol. Methylmalonyl-CoA eluted at 34%, succinyl-CoA at 36%, and AdoCbl at 50% methanol. The flow rate was 100 μL/min, and compounds were detected by simultaneously monitoring the absorbance at 256 and 520 nm. The recovery of radiolabeled substrates was determined with standard samples and found to be >95%.

Scintillation Counting. Scintillation counting was done on a Beckman LS3801 instrument using OptiPhase HiSafe scintillation liquid. Pooled fractions of methylmalonyl-CoA, succinyl-CoA, and AdoCbl were counted for tritium and/or ^{14}C using either single or dual channel counting. A quench curve was established, and commercially available standards were used to correct for quenching of the samples.

Data Calculation. To correct for pipetting errors, total radioactivity of methylmalonyl-CoA, succinyl-CoA, and AdoCbl was normalized. The data were plotted and curves fitted using the KaleidaGraph program (Abelbeck Software).

RESULTS

Exchange of Free with Enzyme-Bound AdoCbl. The rate of exchange of free AdoCbl and of enzyme-bound cofactor was measured by adding 5′-[^{3}H]-AdoCbl to a solution of the holoenzyme bearing unlabeled cofactor. After varying periods of time a sample was injected onto an anion-exchange column, and free AdoCbl was separated from the holoenzyme by elution using a gradient of increasing ionic strength. Fractions containing free AdoCbl and holoenzyme, respectively, were pooled and counted for tritium in a scintillation counter. From the data it was established that even after 24 h less than 5% of the enzyme-bound AdoCbl had exchanged (data not shown).

Tritium Transfer from AdoCbl to the Protein. An experiment was set up under standard conditions, and samples were taken after 1, 2, 5, and 10 min, respectively. The analysis of the protein fractions showed that less than 1% of the total

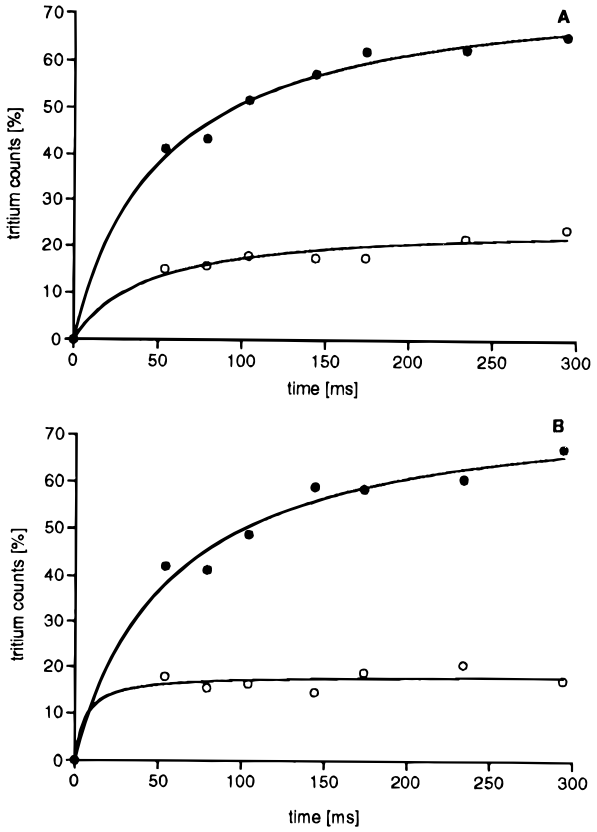

FIGURE 2: Tritium appearance in methylmalonyl-CoA (○) and in succinyl-CoA (●), respectively. Tritium loss from AdoCbl is not shown but is essentially the same as shown in Figure 3B. (A) Methylmalonyl-CoA was added as substrate; (B) succinyl-CoA was added as substrate.

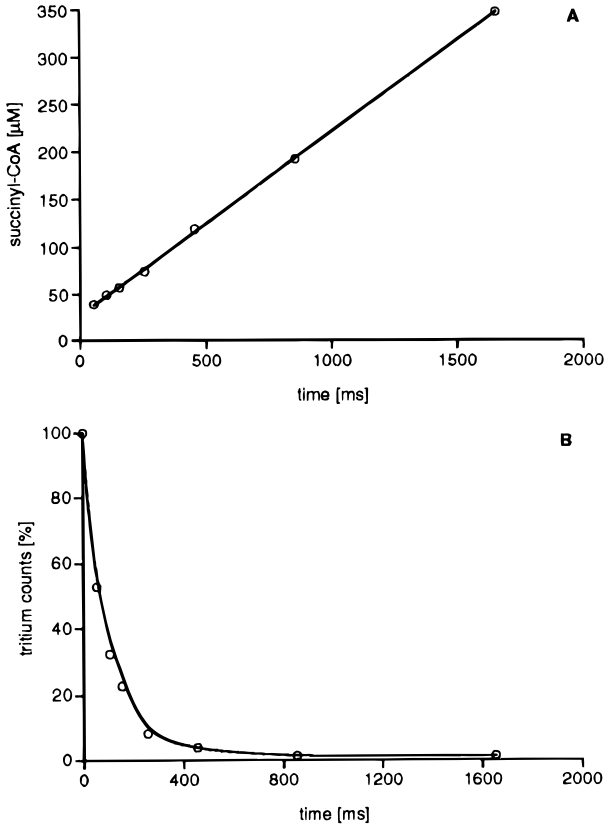

FIGURE 3: (A) Formation of succinyl-CoA from methylmalonyl-CoA. The reaction remained linear for the first 1600 ms. (B) Percentage of total tritium remaining in AdoCbl. Most of the tritium is lost from the cofactor after about 500 ms.

counts were associated with the protein and the number of counts was not time dependent (data not shown). This suggests that tritium does not become stably bound to the protein during transfer from the cofactor to the CoA ester.

Tritium Transfer from AdoCbl to Water. An experiment was set up under standard conditions, and samples were taken after 5 and 10 min, respectively. Analysis of the volatile compounds showed that less than 1% of the total counts was in the volatile fraction and the number of counts was not time dependent (data not shown). This result confirms previous observations that tritium from the cofactor does not exchange with solvent during the reaction (Overath et al., 1962; Erfle et al., 1964).

Partitioning of Tritium. Preliminary experiments showed that tritium was completely washed out from the cofactor after less than 1 s. The subsequent kinetic measurements were therefore done using a stopped-quench flow apparatus. No additional 5′-[^{3}H]-AdoCbl was added to the reaction mixture, because methylmalonyl-CoA mutase binds AdoCbl very tightly (see above).

The results of the experiment with methylmalonyl-CoA as substrate are shown in Figure 2A. Tritium is released from AdoCbl very rapidly, and about 90% of the tritium is washed out after 300 ms (data not shown but essentially as in Figure 3B). Tritium was found in the fractions containing both CoA esters, the substrate methylmalonyl-CoA, and the product succinyl-CoA. The ratio of tritium distribution was 1 to 3 in favor of succinyl-CoA, and this ratio was identical for each sample taken.

The experiment with succinyl-CoA as substrate was done under the same conditions as described above, and the results are shown in Figure 2B. Tritium from AdoCbl is almost completely released after 300 ms (data not shown but essentially as in Figure 3B), and it appears in both CoA esters, methylmalonyl-CoA, and succinyl-CoA. The distribution of tritium, 1 to 3 in favor of succinyl-CoA, is identical to the one found in the experiment with the substrate methylmalonyl-CoA.

Tritium Isotope Effect. The experimental arrangement to measure the tritium isotope effect was the same as for the partitioning experiments. [^{14}C]Methylmalonyl-CoA was used as substrate to allow accurate measurement of the reaction velocity. After separation of the two CoA esters and AdoCbl by HPLC, pooled fractions of the respective CoA esters were counted for ^{14}C and tritium. The pooled fractions for AdoCbl were counted for tritium alone. To correct for pipetting errors, the total amount of ^{14}C counts was normalized to 100%. Turnover of methylmalonyl-CoA to succinyl-CoA was linear during the first 1600 ms, and the calculated turnover number in this forward reaction was $k_{Hf} = 66\ s^{-1}$ (see Figure 3A). Because of the relatively low tritium counts compared to the ^{14}C counts of CoA esters, tritium content in the CoA esters could not be determined accurately. Therefore, it had to be determined indirectly by measuring the tritium release from AdoCbl. The loss of tritium from AdoCbl was fitted to a first-order rate constant of $k_T = 9.1\ s^{-1}$ (see Figure 3B). From the data obtained in the experiments without a ^{14}C label (Figure 2A), it was clear that only 75% of the tritium released from the cofactor goes into the product succinyl-CoA. The release of tritium in the forward direction was therefore calculated as $k_{Tf} = 6.8\ s^{-1}$. Assuming a rapid rotation of the methyl group of enzyme-

bound deoxyadenosine (Gaudemer et al., 1981), a statistical factor of 2 has to be taken into consideration. The calculated tritium isotope effect is therefore $k_H/k_T = 66\ s^{-1}/2 \times 6.8\ s^{-1} = 4.9$.

DISCUSSION

Free AdoCbl does not exchange with cofactor bound to the purified methylmalonyl-CoA mutase holoenzyme on the time scale of these experiments. This is in contradiction to an observation made by Rétey and co-workers, who found that free AdoCbl easily exchanged with cofactor bound to purified mutase (Gaudemer et al., 1981). The fact that our purified recombinant enzyme bound AdoCbl very tightly left two ways of purifying mutase holoenzyme binding 5′-[^{3}H]-AdoCbl: the first one would have involved a time-consuming procedure to obtain homogeneous apoenzyme, to which [^{3}H]-AdoCbl could be added to give the holoenzyme (Francalanci et al., 1986). More conveniently, advantage could be taken of the fact that mutase apoenzyme and mutase holoenzyme elute at different ionic strengths from an anion-exchange column (McKie et al., 1990). Tritium-labeled AdoCbl was therefore added to the pooled fractions of the apoenzyme, and pure holoenzyme was eluted from the anion-exchange column. The fact that free AdoCbl and enzyme-bound cofactor do not easily exchange had the advantage that—unlike the glutamate mutase system (Marsh, 1995)—this equilibrium did not interfere with the determination of the rate of tritium release from adenosylcobalamin.

Although kinetic deuterium isotope effects have been measured for methylmalonyl-CoA mutase and have been shown to be in the normal range (Wölfle et al., 1986; Michenfelder et al., 1987), these results did not exclude involvement of a second hydrogen pool as shown in the case of ethanolamine ammonia lyase. The deuterium isotope effect of $k_H/k_D = 7.4$ of this enzyme was in the expected range, but the tritium isotope effect of $k_H/k_T = 160$ was much higher than would be expected for a direct tritium transfer (Weisblat & Babior, 1971). The apparent contradiction in the two observed isotope effects was resolved by obtaining evidence for the existence of a second, protein-bound, hydrogen pool (Cleland, 1982; O'Brien et al., 1985).

Our results show that, for methylmalonyl-CoA mutase, a protein-based hydrogen pool can be excluded. After the addition of either methylmalonyl-CoA or succinyl-CoA to a mutase solution with tritium-labeled cofactor, all the tritium could be found in the two CoA esters and in AdoCbl. No radioactivity was washed out into the solvent, which is in good agreement with previous observations (Overath et al., 1962; Erfle et al., 1964), and no radioactivity could be found associated with the protein. This makes a protein-based hydrogen pool very unlikely to be involved in the reaction.

The partitioning of hydrogen isotope from an enzyme-bound intermediate has often proved mechanistically informative. For example, such measurements of hydrogen isotope exchange were exploited by Knowles and co-workers in their work on triose-phosphate isomerase. Experiments in tritiated water were used to measure the partitioning of an enediol intermediate between dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. The partitioning ratio is determined by the rate of product release and the kinetic tritium isotope effect in each direction (Maister et al., 1976; Fletcher et al., 1976). When combined with other data, these results allowed a complete free energy profile to be deduced for this enzyme-catalyzed reaction (Albery & Knowles, 1976a,b).

To our knowledge, measurements of partitioning during hydrogen isotope transfers in AdoCbl-dependent enzymes have not been reported. We assumed that tritium released from AdoCbl during the reaction catalyzed by methylmalonyl-CoA mutase, similar to the one found for triose-phosphate isomerase, could partition between substrate and product, under conditions where the reverse reaction was negligible. The results of such partitioning would depend on the shape of the free energy profile. Three alternative free energy profiles and their consequences for the partitioning are shown in Figure 4 to illustrate the point. Figure 4A,D shows the case where tritium released from the cofactor partitions very unequally in favor of the product P when starting with S as substrate. Therefore, when starting with P as substrate, tritium is expected to be found largely in substrate P rather than in product S. Figure 4B,E shows the case where the rate-limiting step of the reaction is the rearrangement, and therefore most of the tritium transferred from AdoCbl to CoA esters will be found in the substrate rather than in the product, regardless of whether S or P is the substrate. Finally, Figure 4C,F shows the case where the substrate radical and product radical interconvert rapidly, and a partitioning is expected in both directions. In this way, the partitioning results provide a qualitative free energy profile of the catalyzed reaction.

After the addition of methylmalonyl-CoA to an enzyme solution, tritium appears in the substrate methylmalonyl-CoA and the product succinyl-CoA from the very start of the reaction (Figure 2A). The ratio of the tritium content in the two CoA esters does not change during the first 300 ms and is always 1 to 3 in favor of succinyl-CoA. This means that 25% of the methylmalonyl-CoA radicals **II** (Figure 1) abstract a hydrogen from the cofactor, leading to the starting material methylmalonyl-CoA, and 75% of radicals **II** rearrange to the succinyl-CoA radical **III**, which, upon abstraction of a hydrogen from the cofactor, gives succinyl-CoA. The ratio of 1 to 3 can be seen as the ratio of the rate constant of the backward reaction and of the rate constant of the forward reaction. It should be noted that the equilibrium constant favors the product succinyl-CoA by 20:1 (Kellermeyer et al., 1964) and that all the kinetic experiments were performed on a very short time scale so that only the forward reaction is studied. Figure 3B shows that turnover of methylmalonyl-CoA is linear for the first 1600 ms, representing about 40% conversion of (2*R*)-methylmalonyl-CoA into product, and that the reverse reaction can be neglected.

When succinyl-CoA is added as substrate, tritium appears in both CoA esters in a constant ratio of 1 to 3 in favor of succinyl-CoA (Figure 2B). Surprisingly, this ratio is identical to the one measured for the substrate methylmalonyl-CoA; that is, 75% of the radicals **III** reabstract a hydrogen from the cofactor to give the starting material succinyl-CoA and 25% of the radicals **III** rearrange to the radical **II**. This same ratio of 1 to 3 clearly demonstrates that the interconversion of substrate radical and product radical does not contribute to limiting the overall rate. Such partitioning of tritium was not explicitly measured for glutamate mutase (Marsh, 1995) and might explain the small discrepancy between the rate constants for tritium release from cofactor and for tritium appearance in the product.

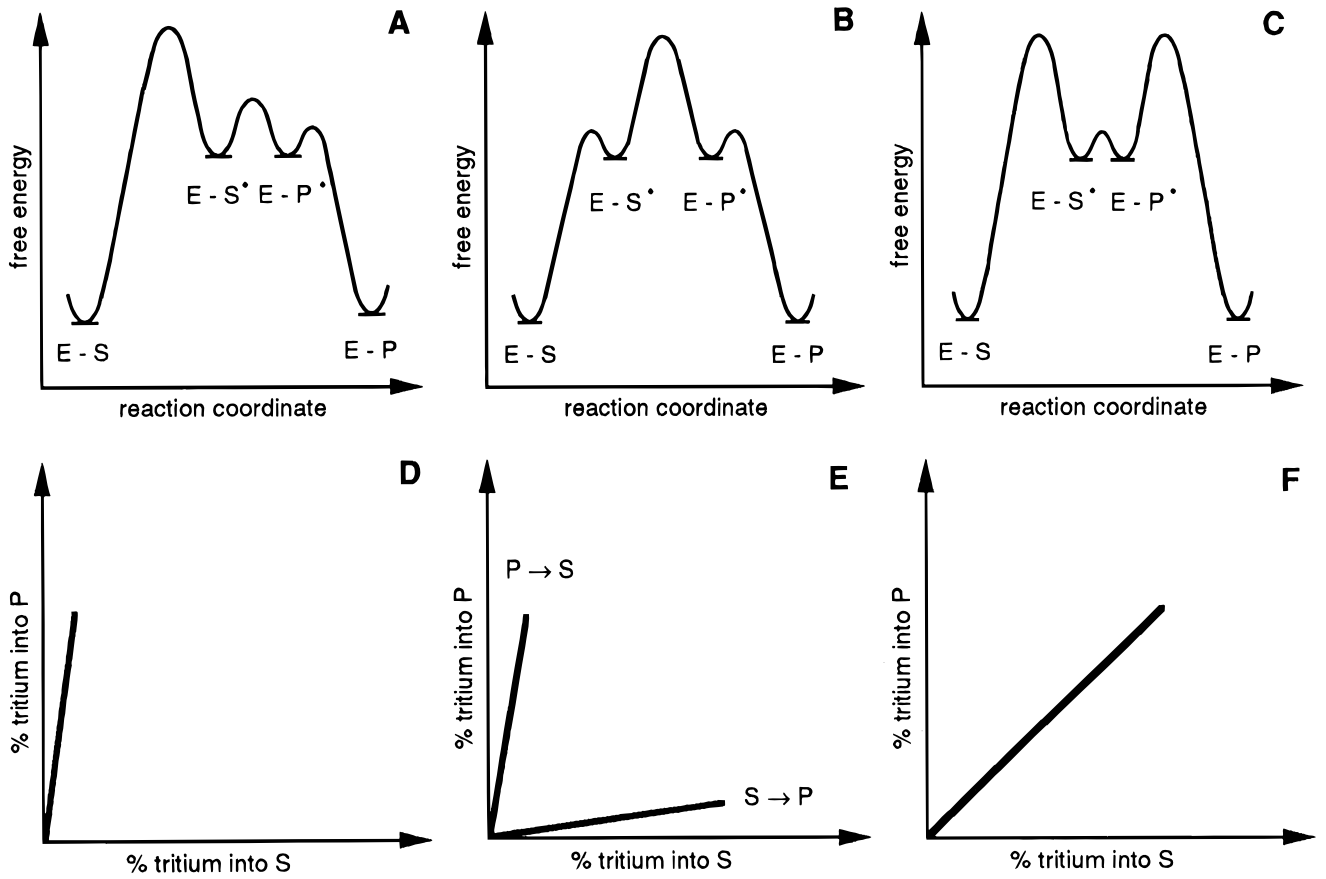

FIGURE 4: (A−C) Three alternative free energy profiles of the mutase-catalyzed reaction, where the steps for substrate binding and product release are omitted from the figure. (D−F) Tritium appearance in substrate and product as predicted from the energy profile and under the assumption that product release is fast. (A, D) Same, unequal partitioning ratio for either direction with tritium always found in P. (B, E) Different partitioning ratio for each direction with tritium always found in the starting material. (C, F) Same, balanced partitioning ratio found, regardless of whether S or P serves as substrate.

Measuring turnover of the enzyme with ^{14}C-labeled methylmalonyl-CoA allows the overall rate of rearrangement in the forward direction to be determined, whereas the loss of tritium from AdoCbl gives a number for the total turnovers involving the coenzyme. This last number must be corrected for partitioning of the released tritium between substrate and product, in order to calculate a tritium isotope effect. In our experiments a very low tritium isotope effect (k_H/k_T = 4.9) was observed. This low value strongly indicates that the abstraction of a hydrogen from deoxyadenosine to regenerate AdoCbl and the product is not fully rate limiting and there have to be other rate-limiting steps in the overall reaction. Inspection of the high-resolution X-ray crystal structure of the enzyme shows that the substrate is very tightly bound to the $(\beta/\alpha)_8$ TIM barrel of the larger subunit of the protein (Mancia et al., 1996). Further work is required to confirm it, but we propose that slow release of product contributes substantially to limiting the overall rate in the forward direction.

So far, the tritium isotope effects on tritium release from cofactor to product of four different AdoCbl-dependent mutases have been measured. There seem to be two classes of mutase, one consisting of diol dehydrase (k_H/k_T = 125; Essenberg et al., 1971) and ethanolamine ammonia lyase (k_H/k_T = 160; Weisblat & Babior, 1971), which both show a very high value for the kinetic tritium isotope effect. Evidence for an involvement of a second, protein-bound, hydrogen pool was obtained in the case of ethanolamine ammonia lyase (O'Brien et al., 1985), and a similar hydrogen pool has been proposed for diol dehydrase (Cleland, 1982). On the other hand, the two members of the second class, glutamate mutase (k_H/k_T = 13.5−18; Marsh, 1995) and methylmalonyl-CoA mutase (k_H/k_T = 4.9, this paper), show tritium isotope effects that are either normal or partly suppressed. These differences, like the previously observed inconsistency in the stereochemical course of 1,2 rearrangements, are not necessarily incompatible with any unified mechanistic pathway for AdoCbl-dependent mutases (Beatrix et al., 1995), but the molecular basis for them is not yet evident.

A qualitative free energy profile representing all the kinetic data obtained in this study is shown in Figure 5. The data from the partitioning experiments show that, regardless of the substrate fed (e.g., **I** or **IV**), 25% of the tritium released from the cofactor is found in methylmalonyl-CoA and 75% is found in succinyl-CoA. The energy barrier of the rearrangement step (step 3), compared to the hydrogen abstraction barriers (steps 2 and 4), must therefore be very low. The constant ratio of tritium distribution of 1 to 3 is reflected by the ratio of the peak heights of step 2 and of step 4. The low tritium isotope effect shows that substrate binding and product release also contribute to the overall reaction rate. This is represented by the two peaks on either side of the energy profile. The energy minima of the enzyme-bound substrates and of the intermediates **II** and **III** are arbitrarily set at the same level. Under the assumption that the intrinsic kinetic tritium isotope effect of tritium transfer is about k_H/k_T = 20, the rate constant of product

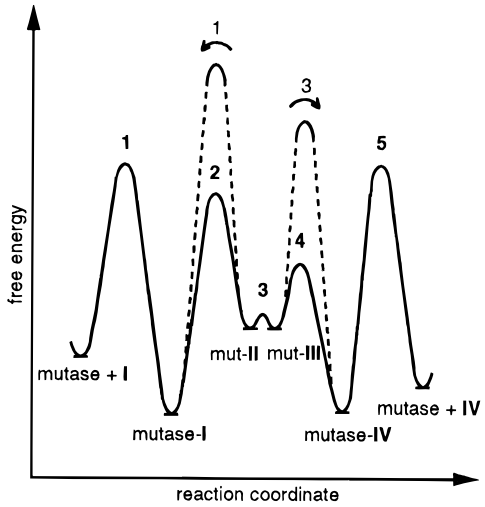

FIGURE 5: Free energy profile of the reaction catalyzed by methylmalonyl-CoA mutase. In the absence of precise data, the values of the energy minima are chosen arbitrarily. The solid line represents the energy profile for hydrogen, whereas the dashed line is a superimposed energy profile for tritium. The partitioning ratio of 1:3 in favor of succinyl-CoA is indicated.

release can be calculated to lie between the rate constant for hydrogen release and tritium release from cofactor. Although further kinetic information will be required to define the free energy profile more fully, it is already evident that, in this enzyme, the energetics are relatively evenly balanced. The availability of a high-resolution crystal structure for *P. shermanii* methylmalonyl-CoA mutase, together with a convenient system for mutagenesis, means that such kinetic studies can now be coupled to protein engineering in order to define more completely the energetics of catalysis and to illuminate the roles of individual active site residues in the rearrangement.

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