Decarboxylation of 2-Aminomalonic Acid Catalysed by Serine Hydroxymethyltransferase is, in fact, a Stereospecific Process

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Contrary to previous reports, 2-aminomalonic acid is decarboxylated stereospecifically by serine hydroxymethyltransferase; the newly introduced hydrogen occupies the 2-*pro*-*S* position of the glycine product.

Serine hydroxymethyltransferase (SHMT) is a ubiquitous pyridoxal 5'-phosphate (PLP) dependent enzyme, which catalyses the retro-aldol cleavage of L-serine to give glycine and formaldehyde.[1] The enzyme is unusual in that it shows a low regard for reaction-type specificity and, indeed, aldol/retro-aldol, transamination and decarboxylation reactions are catalysed with the appropriate substrates. Many of these reactions appear to occur non-stereospecifically.[2–4]

Of relevance to our interest in the mechanism of PLP-dependent decarboxylase reactions, it had been reported that the SHMT catalysed decarboxylation of aminomalonic acid, when conducted in tritiated water, gave equal quantities of (2*R*)- and (2*S*)-tritiated glycine.[4] Verification of the non-stereospecific nature of the reaction was obtained using (2*R*)[^{14}C]-aminomalonic acid as the substrate. In these experiments half of the radiolabel was lost as $^{14}CO_2$ and half was retained in the glycine product, Scheme 1.

The conclusion that SHMT catalysed a completely non-stereoselective decarboxylation reaction, therefore, seemed to be irrefutable and was of particular interest because it was the only reported example of a non-stereospecific decarboxylation catalysed by a PLP-dependent enzyme.[3] Furthermore, the result appeared to contradict the Dunathan's proposals regarding stereoelectronic control in PLP-dependent enzymes.[3]

In order to explain the apparent lack of stereospecificity Palekar *et al.*[4] suggested that the substrate, aminomalonic acid, might be able to bind in two equally populated conformations at the active site of the enzyme, such that each of the two carboxy groups was positioned correctly for decarboxylation at 90° to the plane of the coenzyme. If the decarboxylation and subsequent protonation steps occurred stereospecifically for each form, then the observed results would have been obtained, Scheme 2. Support for this proposal is provided by the fact that both D- and L-antipodes of several amino acid substrates bind to and are processed by the enzyme.[2]

On the other hand, if racemisation of the substrate occurred prior to decarboxylation, the same observations might have been expected. Prior racemisation it appeared, however, could be ruled-out in view of Palekar's earlier finding; that the PLP-dependent enzyme, aspartate β-decarboxylase, catalysed the stereospecific decarboxylation of aminomalonate.[5]

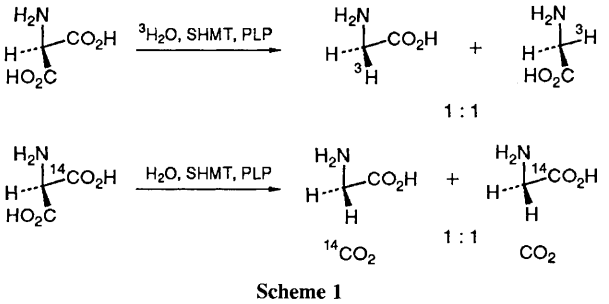
Our recent work with mammalian cytosolic and *E. coli* SHMT, using the chiral ^{13}C-isotopomers of the very slow decarboxylation substrate 2-amino-2-methylmalonic acid,[6,7] had shown that cleavage of the *pro*-*R* carboxy group of the substrate occurred with retention of configuration to give (2*R*)-alanine. Thus, in the context of the earlier work, either the bulkier substrate was only able to bind to the enzyme in one of the two conformations available to 2-aminomalonic acid (Scheme 2, conformation A) or, Palekar's explanation for non-stereospecificity with 2-aminomalonic acid as the substrate was incorrect.[6]

In order to test these possibilities, Palekar's experiments[4] with 2-aminomalonic acid were repeated except chemical assays for the chirality of glycine product were used.[8]

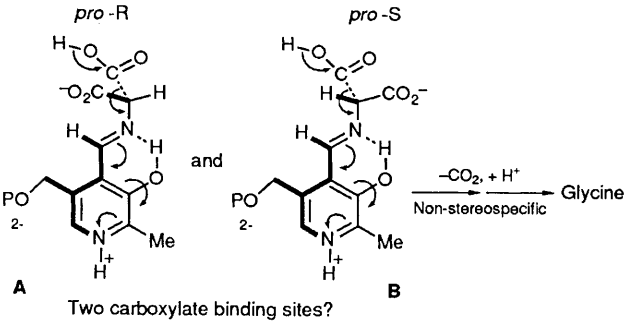
2-Aminomalonic acid (30 mmol dm^{-3} 3-times K_m) was incubated with SHMT and PLP in deuteriated buffer under the exact conditions described by Palekar *et al.* but, on a 20-fold larger scale, and the decarboxylation products were obtained. The glycine was treated with (1*S*,4*R*)-camphanoyl chloride to give the camphanamide derivative which was examined by NMR spectroscopy. The unlabelled derivative and the 2-dideuterio isotopomer were known to display well separated AB-type signals for the diastereotopic C-2 hydrogens of the glycine moiety in the ^{1}H and ^{2}H NMR spectra, H_R and H_S occur at δ 4.05 and 4.16, respectively in the 360 MHz ^{1}H NMR spectrum.[8] Hence, on the basis of the earlier work[4] the spectra of the derivative of the decarboxylation products were expected to show the incorporation of ~50 atom% solvent derived hydrogen in each of the C-2 positions. In actuality the incorporation of solvent hydrogen was close to 100 atom% for each of the C-2 positions as judged by the ^{1}H and ^{2}H NMR and mass spectrum of the camphanamide derivative. Thus, it was evident that either α-hydrogen exchange had occurred prior to decarboxylation or that the product glycine had racemised.

As Palekar *et al.*[4] had used tritium in their experiments and had analysed the chirality of the tritiated glycine product using D-amino acid oxidase, which is known to transfer the 2-*pro*-*S*-hydrogen of glycine to the solvent, their apparent results were identical with ours. Nevertheless, our result had shown that too much solvent-derived hydrogen had been incorporated into the glycine.

In order to determine whether non-enzymic α-hydrogen exchange between the substrate and the solvent could occur during the course of the enzymic decarboxylation reaction, the rates of α-deuterium atom incorporation into the unlabelled substrate were measured in a vast excess of deuterium oxide under pseudo-first-order conditions over the pD range 5.0–8.0



Scheme 1



Scheme 2

at 20 °C. The exchange reaction was followed by ^{1}H NMR spectroscopy using an internal dioxan concentration reference and nineteen spectra were accumulated at five minute intervals for a period of 100 min.

For the experiments conducted at pD 5.0, 5.5, 6.0 and 6.5, the half-lives for exchange, at 20 °C, were 15–20 min whilst those for the experiments conducted at pD 7.0, 7.5 and 8.0 were 35, 55 and 95 min, respectively. Thus, under Palekar's conditions, pH 6.0 at 37 °C, the half-life for exchange would have been ~5 min, a substantially shorter period than the duration of the experiments upon which Palekar performed the original stereochemical analyses (*e.g.* 30 min at 37 °C for the ^{3}H-experiment). Therefore, in these experiments, the concentration of the unexchanged substrate that was available to the enzyme would have been crucially dependent on the amount of enzyme used in the experiments and, of course, the initial concentration of the substrate and how long it was pre-exposed to the isotopically labelled buffer medium. It should be noted that arguments regarding hydrogen exchange are also relevant to pre-racemisation; an important consideration in the experiments in which chiral (carboxy group) labelled substrate was used.

Since it was now evident that a substantial amount of the 2-aminomalonic acid substrate had undergone α-hydrogen exchange or had been racemised prior to the actual enzyme-catalysed decarboxylation reaction in the original experiments, it was absolutely clear that SHMT, in fact, had not been shown to catalyse decarboxylation non-stereospecifically. From our kinetic analysis of the competition for the unlabelled substrate between the exchange/racemisation reaction, a pseudo-first-order process, and the enzyme-catalysed decarboxylation, an effectively zero-order process with respect to substrate at $[S] > K_m$ (the standard conditions at the start of the reactions) it seemed expedient to reassess the stereochemical course in incubations containing much larger amounts of enzyme.

Accordingly, 2-aminomalonic acid was decarboxylated under the conditions of Palekar *et al.* as before in deuteriated buffer but, in the presence of much more enzyme and for a shorter period of time, 10 min, when virtually all of the substrate had been consumed. The glycine products were isolated and were then converted to their camphanamide derivatives. Examination of the ^{1}H and ^{2}H NMR and mass spectra of the derivatives indicated that very little dideuterioglycine had been formed and that the monodeuteriated glycine was of the (2*S*)-configuration only. When a similar experiment was performed in protium oxide using [2-^{2}H]-2-aminomalonic acid as the substrate, the spectral analyses of the derivatised glycine indicated that only (2*R*)-glycine had been formed. Thus, SHMT, in fact, catalyses the stereospecific decarboxylation of 2-aminomalonic acid.

In the light of our earlier work with the methyl homologue of the substrate[6] and our findings with the enzyme L-methionine decarboxylase,[9] we suggest that cleavage of the pro-*R* carboxy group of the substate occurs with retention of configuration, such that protonation introduces the 2-*pro*-*S* hydrogen of glycine, and that this chemistry occurs on the 4′-*si*-face of the coenzyme, Scheme 2, conformation A.

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