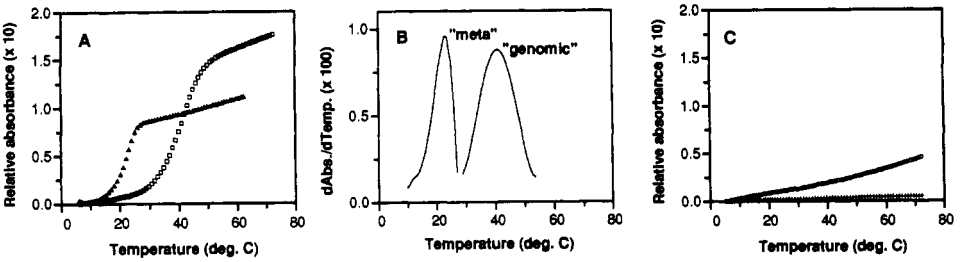

Figure 3. (A) UV absorbance profiles versus temperature for meta-dA_{12}/meta-dU_{12} (△) and genomic dA_{12}/dT_{12} (□). Measurements were determined by monitoring absorbance at 260 nm. The sample buffer in all cases contained 1 M NaCl, 10 mM sodium phosphate, and 0.1 mM EDTA in H_2O at pH 7. Oligonucleotide concentration was 2.5 μM in each strand. All results are derived from duplicate experiments. Profiles have been normalized to zero absorbance at 5 °C. (B) First derivatives of the UV absorbance profiles versus temperature for meta-dA_{12}/meta-dU_{12} and genomic dA_{12}/dT_{12}. (C) UV absorbance profiles versus temperature for meta-dA_{12} (○) and meta-dU_{12} (+). See part A above for experimental conditions.

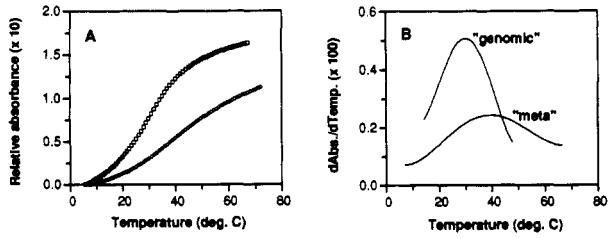

Figure 4. (A) UV absorbance profiles versus temperature for meta-d-$(AU)_6$ (○) and genomic $d(AT)_6$ (□). The oligonucleotide concentration was 5 μM; see the caption of Figure 3A for additional experimental details. Profiles have been normalized to zero absorbance at 5 °C. (B) First derivatives of the UV absorbance profiles versus temperature for meta-$d(AU)_6$ and genomic $d(AT)_6$.

determination of the melting temperatures (T_m's) of the meta and genomic mutually complementary dodecamers as 22.8 and 40.8 °C, respectively. It is also apparent from the derivative curves that the denaturation of the meta dodecamers is more cooperative than that of the genomic ones. Somewhat different behavior was observed for the self-complementary meta-$d(AU)_6$ (T_m = 39.3 °C) and genomic $d(AT)_6$ (T_m = 29.9 °C) (Figure 4). Here, in contrast to the previous case, the denaturation of the self-complementary meta dodecamer is *less* cooperative and occurs with a *higher* T_m value than that of the genomic one.

To probe the nature of the structure in the first example involving meta-dA_{12} and meta-dU_{12}, controls were performed where absorbance profiles versus temperature were determined for pure meta-dA_{12} and pure meta-dU_{12}, separately (Figure 3C). These profiles rule out the possibilities of purinic self-association[13] or pyrimidinic self-association,[14] leading to the profile that resulted from the admixture of meta-dA_{12} and meta-dU_{12} (Figure 3A), and permit the observation that meta-dA_{12} and meta-dU_{12} complex with one another in a manner consistent with classical, Watson-Crick base pairing.[15]

Comparison of the T_m values for the mutually complementary dodecamers in the complexes meta-dA_{12}/meta-dU_{12} and genomic dA_{12}/dT_{12} indicates that the genomic complex is more stable.[16] However, the opposite behavior is observed for the self-complementary dodecamers meta-$d(AU)_6$ and genomic $d(AT)_6$. Here, a comparison of the T_m values indicates that the meta dodecamer structure is the more stable.

If one accepts the premise that an RNA or RNA-like polymer served a central role in the origins of life, then there is a need to reconcile the biotic predominance of the 3′,5′-internucleotide linkage with the likely prebiotic bias toward a 2′,5′-linkage. One way to do this would be to postulate an ancestral nucleic acid that contained a sugar different from ribose.[17] An alternative scenario would consider 2′,5′-linked nucleotides to be ancestral to genetic material composed of 3′,5′-linked nucleotides. On the basis of the findings reported in this work, there is no apparent reason why 2′,5′-linked nucleotides could not both replicate accurately and assume a tertiary structure conducive to catalysis.[4] Evidence for these possibilities awaits further experiments.

In summary, the self-associative property of 3′,5′-linked nucleotides is shared by 2′,5′-linked ones. As a consequence, any scenario in which the latter precede the former during prebiotic or biotic evolution would appear reasonable, but will require an explanation for natural selection[18] of the present-day genomic linkage.

Acknowledgment. Acknowledgement is made to the National Institutes of Health (GM-47375), the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the University of California for support of this research.

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(14) Young, P. R.; Kallenbach, N. R. *J. Mol. Biol.* **1978**, *126*, 467.
(15) A similar control experiment is not possible for the self-complementary meta-DNA dodecamer. When the dodecamer concentration is varied by 1 order of magnitude, the T_m does not change, consistent with a monomolecular hairpin structure.
(16) The stability of the meta complex relative to the genomic one is actually greater than simple comparison would suggest, as the stability of the genomic complex is enhanced by the hydrophobic methyl groups in the dT residues, which the dU residues of the meta complex lack. See: Zmudzka, B.; Bollum, F. J.; Shugar, D. *J. Mol. Biol.* **1969**, *46*, 169.
(17) Joyce, G. F.; Schwartz, A. W.; Miller, S. L.; Orgel, L. E. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 4398.
(18) One probable selective advantage of the RNA 3′,5′-internucleotide linkage would be a greater stability than the 2′,5′-linkage toward hydrolysis when in a right-handed helical conformation, see: Usher, D. A. *Nature (London), New Biol.* **1972**, *235*, 207. Usher, D. A.; McHale, A. H. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 1149; this work does not address the stability of a 2′,5′-linkage in a purely, or a predominantly, 2′,5′-linked RNA strand, however.

Stereochemistry of Enzyme-Catalyzed Decarboxylation of α-Methyl-α-phenylmalonic Acid

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We have recently demonstrated a highly enantioselective and effective decarboxylation of α-methyl-α-phenylmalonic acid (**1**) by *Alcaligenes bronchisepticus* KU 1201 into (*R*)-α-phenylpropionic acid (**2**).[1] Because this was the first example of enantioselective enzymatic decarboxylation of a synthetic substrate,[2] the stereochemical course of the reaction is of great interest. Two substrate enantiomers **1** containing ^{13}C at either one of the two carboxyl groups were synthesized. The starting material, [1-^{13}C]phenylacetic acid (**3**) (99% ^{13}C), was commercially available.

As shown in Scheme I, methylation of **4** followed by benzyloxymethylation and deprotection afforded [1-^{13}C]-α-methyltropic

(1) (a) Miyamoto, K.; Ohta, H. *J. Am. Chem. Soc.* **1990**, *112*, 4077. (b) Miyamoto, K.; Ohta, H. *Biocatalysis* **1991**, *5*, 49.
(2) Although some efforts have been made by chemical methods, enantiomeric excesses of the products were very low: (a) Toussaint, O.; Capdevielle, P.; Maumy, M. *Tetrahedron Lett.* **1987**, *28*, 539. (b) Verbit, L.; Halbert, T. R.; Patterdon, R. B. *J. Org. Chem.* **1975**, *40*, 1649.

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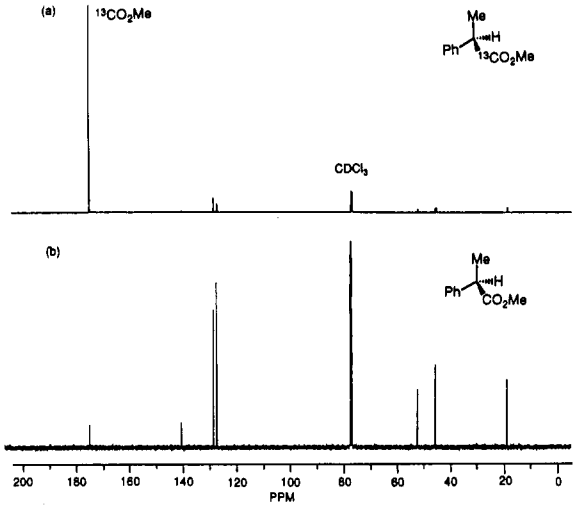
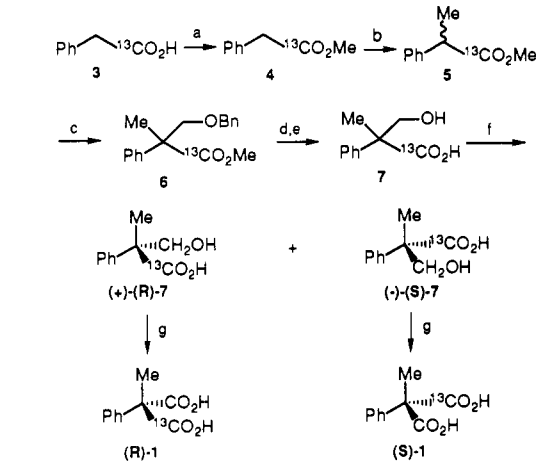


Figure 1. ^{13}C NMR spectra (100 MHz, $CDCl_3$) of the methyl ester of α-phenylpropionic acid (**2**) with ^{13}C (a) and without ^{13}C (b).

Scheme I

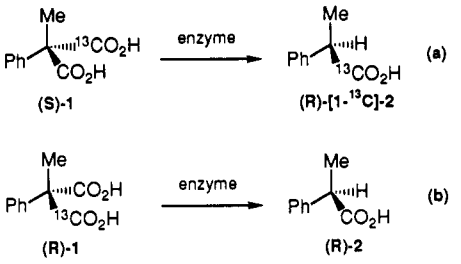


(a) MeOH, TsOH; (b) LDA, MeI; (c) LDA, $ClCH_2OBn$; (d) H_2/Pd-C; (e) KOH/EtOH; (f) quinine resolution; (g) Jones' oxidation;

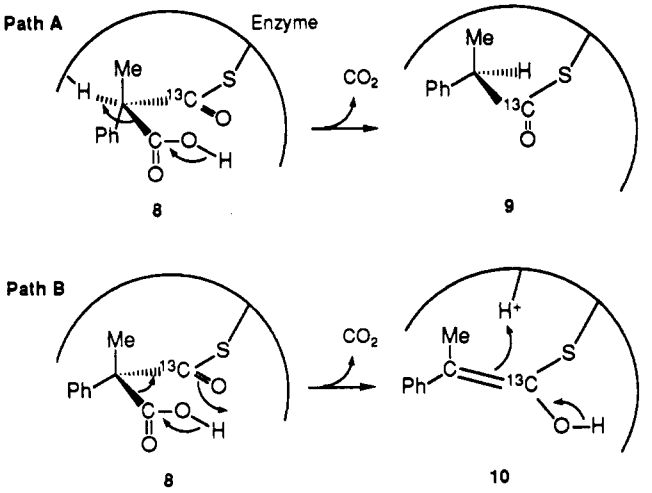
acid (**7**), which was resolved with the aid of quinine:[3] (−)-(*S*)-[1-^{13}C]-**7**, $[\alpha]^{22}_D$ − 26.6° (*c* 2.0, EtOH), 98% ee; (+)-(*R*)-[1-^{13}C]-**7**, $[\alpha]^{21}_D$ +26.0° (*c* 2.0, EtOH), 98% ee. The absolute configuration of both enantiomers is unambiguous from the signs of optical rotation.[4] Moreover, Curtius rearrangement of (−)-**7** to the known compound (*S*)-2-amino-2-phenylpropanol ($[\alpha]^{18}_D$ +13.7° (*c* 1.08, EtOH)) gave further confirmation of the configuration.[5] The % ee was determined by ^1H NMR analysis of the corresponding MTPA esters.[6] Jones' oxidation of (*R*)- and (*S*)-**7** gave (*R*)- and (*S*)-[1-^{13}C]-**1** of defined configuration, respectively. Decarboxylation was carried out using either intact cells or the enzyme isolated from the cell,[7] both giving the same results.

The enzymatic reaction was performed at 30 °C for 2 h in a volume of 1 mL of 250 mM phosphate buffer (pH 6.5) containing 50 mM of KOH, 32 U/mL of the enzyme, and [1-^{13}C]-**1** (10 mg). The product was isolated as the methyl ester. When (*S*)-**1** was employed as the substrate, ^{13}C remained completely in propionate **2**, as confirmed by ^{13}C NMR (Figure 1a) and HRMS (165.0853, calcd for $^{12}C_9{}^{13}CH_{12}O_2$ 165.0869). In addition, spin–spin coupling between ^1H and ^{13}C was observed in the product, and the frequency of the C–O stretching vibration shifted lower to 1690 cm^{-1} (cf. 1740 cm^{-1} for ^{12}C–O). On the contrary, reaction of (*R*)-**1** resulted in the formation of (*R*)-**2** containing ^{13}C only within natural abundance (Figure 1b, HRMS 164.0864, calcd for $^{12}C_{10}H_{12}O_2$ 164.0936). These results clearly indicate that the *pro-R* carboxyl group of **1** is eliminated to form (*R*)-**2** with *inversion of configuration* (Scheme II). This is in sharp contrast to the known decarboxylation reaction by malonyl CoA decarboxylase[8] and serine hydroxymethyltransferase,[9] which proceeds with retention of configuration.

Scheme II



Scheme III



The study on additive effects and cofactor requirement has revealed that this enzyme contains an SH group at the active site and attains its full activity without the aid of coenzyme A.[7] Thus, the first step of the reaction is believed to be thiol ester formation between a cysteine residue of the enzyme and the *pro-S* carboxyl group of **1** (Scheme III). Thereafter there may be two possible pathways leading to the observed results. One is an S_E2 type reaction with complete inversion. The second possibility is the formation of enolate **10** followed by the enantioselective attack on proton from the *si*-face of the double bond. Although there is no evidence available to prove which pathway is correct at present, it must be taken into consideration that the substrate must be bound tightly to the enzyme until the completion of decarboxylation. Thus, the intermediary formation of enolate **10** seems more likely as it requires less change in enzyme conformation than the concerted inversion.

Supplementary Material Available: Experimental details for the preparation of (*R*)- and (*S*)-[^{13}C]-α-methyl-α-phenylmalonic acid and copies of ^{13}C NMR spectra of methyl α-phenylpropionate with and without ^{13}C (8 pages). Ordering information is given on any current masthead page.

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(4) Knabe, J.; Junginger, H.; Geismar, W. *Liebigs Ann. Chem.* **1970**, *15*, 739.
(5) Reference $[\alpha]^{23}_D$ +14.3° (*c* 0.98, EtOH). Yamada, S.; Terashima, S.; Achiwa, K. *Chem. Pharm. Bull.* **1966**, *14*, 800.
(6) The chemical shifts due to OCH_3 of the racemic MTPA esters were δ 3.67 (d, J = 3.9 Hz, ^{13}CH) and δ 3.63 (d, J = 3.9 Hz, ^{13}CH).
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