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EQUILIBRIUM CARBON ISOTOPE EFFECT ON A DECARBOXYLATION REACTION

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Carbon isotope exchange equilibrium between carbon dioxide and the β-carboxyl carbon of D-isocitric acid has been achieved by means of the enzyme isocitrate dehydrogenase. Measurements of the isotopic compositions at these two sites reveal that the carboxyl carbon is enriched in ^{13}C relative to carbon dioxide. The isotope exchange equilibrium constant is 1.0027 at 25°, pH 7.5.

INTRODUCTION

The variations in $^{13}C/^{12}C$ ratios in biological materials have been the subject of considerable study (1-3). Enzyme-catalyzed carboxylation and decarboxylation reactions are the principal means by which inorganic carbon (as CO_2) is exchanged with the carbon of living systems. Kinetic carbon isotope effects on enzyme-catalyzed decarboxylation reactions have provided information about the rate-determining steps and expected isotope fractionations in various decarboxylation reactions (4).

No previous measurements have been made of equilibrium carbon isotope effects on carboxylation/decarboxylation reactions, principally because of the paucity of such reactions having equilibrium constants of proper magnitude to allow measurements of isotope compositions of both reactant and product. We studied the oxidative decarboxylation of threo-D_S-isocitric acid by $NADP^+$-dependent isocitrate dehydrogenase (EC 1.1.1.42) of pig heart (eq. 1). The reaction is reversible, with an equilibrium constant

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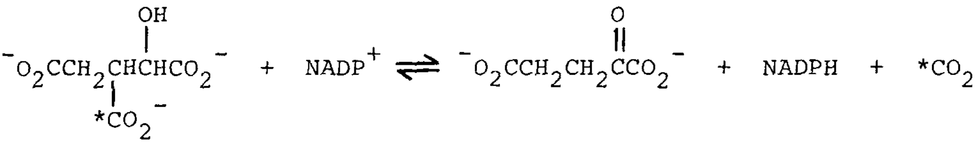

$$^{-}O_2CCH_2\underset{*CO_2^-}{\underset{|}{C}}H\overset{OH}{\overset{|}{C}}HCO_2^- + NADP^+ \rightleftharpoons {}^{-}O_2CCH_2CH_2\overset{O}{\overset{\|}{C}}CO_2^- + NADPH + *CO_2 \quad (1)$$

of 0.86 M at 25°, pH 7 (5). Kinetic isotope effects for this reaction have been measured (6,7).

MATERIALS AND METHODS

The equilibrium carbon isotope effect on the decarboxylation of isocitric acid was measured by allowing the β-carboxyl carbon of isocitric acid (10 mM) to equilibrate with CO_2 (P_{CO_2} 1 atm.; concentration of aqueous CO_2 34 mM; concentration of HCO_3^- 0.48 M) in the presence of isocitrate dehydrogenase, $NADP^+$ (0.025 mM), NADPH (2.5 mM), α-ketoglutarate (2.5 mM), and Mg^{2+} (5.0 mM) at pH 7.5 in 0.048 M tris-chloride buffer at 25°. After equilibrium had been attained (approximately 12 days) the CO_2 atmosphere was sampled, after which all CO_2 was removed by acidification and purging with N_2, and the isocitrate present was quantitatively decarboxylated by the coupled enzyme procedure used previously (6,7). Isotope ratio measurements on the two CO_2 samples following purification then provided directly the isotope effect on the isocitrate-$CO_{2(g)}$ equilibrium.

In order to ascertain that isotopic equilibrium was being achieved we conducted parallel experiments which approached equilibrium from both sides. CO_2 from a standard laboratory cylinder, which has an isotopic composition similar to that of the carboxyl carbon of the substrate being measured, and CO_2 from marine limestone, which is enriched in ^{13}C by about 35 per mil relative to the unexchanged carboxyl carbon, were used. The same isotope effect was obtained starting with either CO_2 source, indicating that isotopic equilibrium had been achieved.

All carbon isotope ratios are reported in the per mil notation (eq. 2) relative to the standard PDB (8).

$$\delta\ ^{13}C = \left[\frac{^{13}C/^{12}C\ (\text{sample})}{^{13}C/^{12}C\ (\text{standard})} - 1\right] \times 1000 \qquad (2)$$

RESULTS

Isotope ratios for tank CO_2, marine limestone CO_2, and for the β-carboxyl group of isocitric acid prior to exchange are given in the first part of Table I. In the second part of Table I are given isotopic compositions of these same materials following exchange, together with the calculated isotopic compositions of the dissolved CO_2 in isotope exchange equilibrium with CO_2(g) (9).

Table I. $\delta^{13}C$ values for CO_2 samples obtained in studies of CO_2-isocitrate exchange, relative to PDB. Isotopic compositions with no errors given are single determinations. When errors are given, the numbers represent the mean of at least 6 determinations.

Sample	$\delta^{13}C_{PDB}$ per mil	Equilibrium isotope effect
Prior to exchange		
tank CO_2	-40±0.1	
marine limestone CO_2	-0.8±0.1	
β-carboxyl carbon of isocitric acid	-35.1,-35.4	
After exchange		
tank CO_2(g)	-40.1±0.1	
tank CO_2(aq) (calculated)	-41.2	
β-carboxyl carbon of isocitric acid after exchange with tank CO_2	-37.9 -38.4	1.0033 1.0028
marine limestone CO_2(g)	-0.8±0.1	
marine limestone CO_2(aq)	-1.9	
β-carboxyl carbon of isocitric acid after exchange with marine limestone CO_2	+0.2 +1.3 +0.4	1.0021 1.0032 1.0023

Also given in Table I are values for the equilibrium isotope effect (eq. 3). At equilibrium at 25°C the β-carboxyl carbon of isocitric

$$\frac{K^{12}}{K^{13}} = \frac{(^{12}CO_2)_{(aq)}/(R^{12}CO_2^{-})}{(^{13}CO_2)_{(aq)}/(R^{13}CO_2^{-})} \qquad (3)$$

acid is enriched by 2.7 ± 0.5 per mil relative to the dissolved CO_2, so the equilibrium isotope effect (eq. 3) is 1.0027 ± 0.0005 for the ionized form of the carboxylic acid substrate.

DISCUSSION

The detailed theory of isotope effects is complex and has yet to find an important place in the understanding of isotope fractionations in living systems. The qualitative aspects of the theory, however, are of considerable use. Equilibrium isotope effects reflect the change in bonding to the atom in question on going from starting state to final state; the greater the change in bonding, the greater the isotope effect. Because the bonding around the carboxyl carbon is relatively constant for various carboxylic acids, we expect that the equilibrium carbon isotope effect in a decarboxylation reaction (eq. 4) will be nearly the same for all decarboxylations.

$$-\overset{|}{\underset{|}{C}}-CO_2^- + H^+ \rightleftharpoons -\overset{|}{\underset{|}{C}}-H + CO_2 \qquad (4)$$

The validity of the "cutoff procedure" in calculations of heavy-atom isotope effects provides some theoretical justification for this expectation (10).

If this assumption of constancy is correct, then we can use the recent measurements of the carbon isotope effect on the ionization of benzoic acid (11) to calculate the equilibrium isotope effect (eq. 3) on the decarboxylation of a protonated carboxylic acid. Thus, the equilibrium isotope effect for decarboxylation of protonated carboxylic acids is expected to be near 1.0041, and that for carboxylate ions is expected to be near 1.0027.

These equilibrium carbon isotope effects have at least two immediate uses in enzymology. In the first place, they provide expected values of carbon isotope effects in enzymatic decarboxylations in which the decarboxylation step itself is rapidly reversible and proceeds to an intermediate having enzyme-bound CO_2, as might occur, for example, in reversible decarboxylations. Kinetic isotope effects on the oxidative decarboxylation of isocitric acid (6,7) indicate that this reaction is not of this type. In the second place, because an equilibrium isotope effect must be equal to the ratio of the kinetic isotope effects for the forward and reverse reactions, these equilibrium isotope effects enable us to use kinetic isotope effects observed in decarboxylation reactions (4) to calculate kinetic isotope effects to be expected in carboxylation reactions.

There is an appreciable carbon isotope discrimination during the incorporation of CO_2 into plants (12). The simplest explanation of this discrimination is that photosynthetic carboxylation reactions show normal carbon isotope effects. However, Galimov (13) has recently proposed that carbon isotope ratios in biological systems are a consequence of equilibrium, rather than kinetic effects. The magnitude of the equilibrium carbon isotope effect in carboxylation indicates that if isotopic equilibrium were achieved during the carboxylation stage of photosynthesis, the plant should be enriched in ^{13}C relative to the CO_2 atmosphere, precisely contrary to what is observed. Thus, at least for the initial carboxylation phase of photosynthetic carbon metabolism, the theory of Galimov is clearly incorrect.

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