The Stereospecificity of Oxaloacetate Decarboxylase: A Stereochemical Imperative?

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Received August 3, 1987

Bioorganic chemists commonly expect the same stereospecificity in enzymes catalyzing analogous reactions.[1] When stereochemical diversity is observed within a class of enzymes, it is often presumed to reflect an underlying mechanistic diversity.[1-3] For example, both β-decarboxylases that produce retention and β-decarboxylases that produce inversion are known. Further, stereospecificity appears to correlate with mechanism and substrate structure: β-ketoacid decarboxylases acting on substrates where the ketone is α to a carboxylate (Scheme I) require a metal ion and produce retention; decarboxylases not acting on α-keto acids do not require a metal ion and produce inversion (Table I).[4]

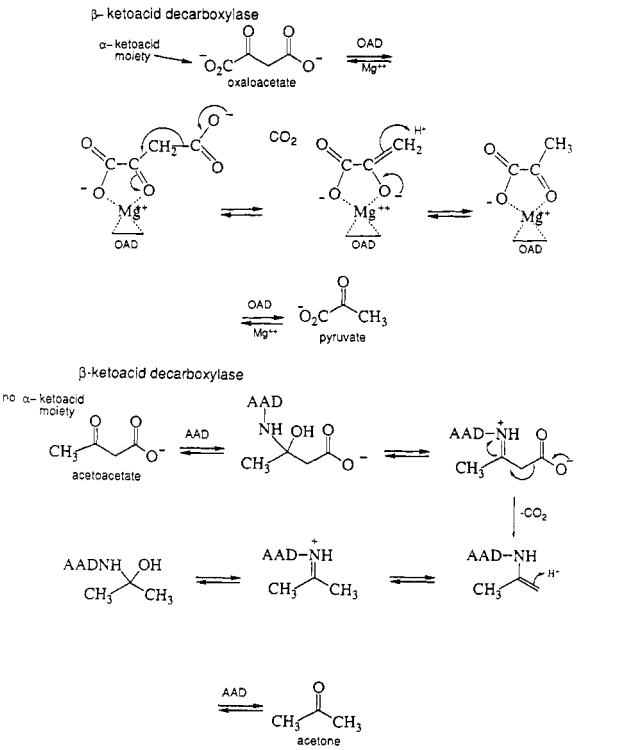
The correlation in Table I suggests that the stereochemical and mechanistic diversity observed in this class of enzyme is functional.[4] In the context of Darwinian evolution, this requires that natural selection favors one stereochemical course over the other for each structural class of substrates. We have often noted the importance of distinguishing between functional and nonfunctional behaviors of enzymes,[11] both to identify behaviors likely to reflect fundamental principles of protein structure and catalytic mechanism and to guide protein engineering.[12] Therefore, mechanistic explanations for the stereospecificity of decarboxylases need to be examined further.

The first independent test of the correlation produced a surprising result. Acetoacetate decarboxylase, predicted to produce inversion, produced essentially racemic product. This result was rationalized ad hoc by presuming that the reactivity of an intermediate enamine was sufficiently great to permit its protonation directly by solvent. While this ad hoc modification appeared to be consistent with stereochemical data for aspartate β-decarboxylase,[13] an independent test of the correlation remained elusive.

Table I. Substrates, Stereoselectivities, and Requirements for Metal Ions in Decarboxylations

enzyme	E.C. no.	keto acid substrate	stereoselectivity	metal ion	ref
isocitrate dehydrogenase ($NADP^+$)	1.1.1.42	yes	retention	yes	5
isocitrate dehydrogenase (NAD^+)	1.1.1.41	yes	retention	yes	6
malic enzyme	1.1.1.40	yes	retention	yes	7
phosphogluconate dehydrogenase	1.1.1.44	no	inversion	no	8
UDP-glucuronate decarboxylase	4.1.1.35	no	inversion	no	9
acetolactate decarboxylase	4.1.1.5	no	inversion	no	10
acetoacetate decarboxylase	4.1.1.4	no	racemization	no	4

Scheme I[a]



[a] OAD = oxaloacetate decarboxylase; AAD = acetoacetate decarboxylase.

Oxaloacetate decarboxylase (OAD) from *Pseudomonas putida* requires a divalent metal cation; the metal presumably chelates the α-keto acid moiety of the substrate providing an electron sink for decarboxylation (Scheme I).[14] The natural substrate is presumably oxaloacetate.[15] Consistent with the correlation, malic enzyme decarboxylates oxaloacetate with retention. Thus, based on both mechanistic considerations and by analogy, OAD from *Pseudomonas* is expected to catalyze the decarboxylation of oxaloacetate with retention.

We report here that this OAD produces *inversion*.

Because oxaloacetate enolizes rapidly,[16] a special reaction sequence was used to trap chirally labeled oxaloacetate generated in situ. (Scheme II). Incubation of (2*S*)-[2,3-^{3}H]aspartic acid with L-aspartase in D_2O yielded (2*S*,3*S*)-[^{2}H,^{3}H,^{2}H]aspartic

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(1) (a) Rose, I. A.; Hanson, K. R. *Crit. Rev. Biochem.* **1972**, *1*, 33–57. (b) Hanson, K. R.; Rose, I. A. *Acc. Chem. Res.* **1975**, *8*, 1–10.
(2) (a) Knowles, J. R.; Albery, W. J. *Acc. Chem. Res.* **1977**, *10*, 105–111. (b) Eigen, M.; Hammes, G. G. *Adv. Enzymol.* **1963**, *25*, 1–38. (c) Hammes, G. G. *Acc. Chem. Res.* **1968**, *1*, 321–329.
(3) (a) Benner, S. A. *Experientia* **1982**, *38*, 633–37. (b) Nambiar, K. P.; Stauffer, D. M.; Koloziej, P. A.; Benner, S. A. *J. Am. Chem. Soc.* **1983**, *105*, 5886–5890.
(4) (a) Rozzell, J. D.; Benner, S. A. *J. Am. Chem. Soc.* **1984**, *106*, 4937–4941. (b) Vennesland, B. *Top. Curr. Chem.* **1974**, *48*, 39–65.
(5) (a) Englard, S.; Listowsky, I. *Biochem. Biophys. Res. Commun.* **1963**, *12*, 356–360. (b) Lienhard, G. E.; Rose, I. A. *Biochemistry* **1964**, *3*, 185–190.
(6) Rose, Z. B. *J. Biol. Chem.* **1966**, *241*, 2311–2313.
(7) Rose, I. A. *J. Biol. Chem.* **1970**, *245*, 6052–6056.
(8) Lienhard, G. E.; Rose, I. A. *Biochemistry* **1964**, *3*, 190–195.
(9) Schutzbach, J. S.; Feingold, D. S. *J. Biol. Chem.* **1970**, *245*, 2476–2482.
(10) Hill, R. K.; Sawada, S.; Arfin, S. M. *Biorg. Chem.* **1979**, *8*, 175–189.
(11) Benner, S. A.; Nambiar, K. P.; Chambers, G. K. *J. Am. Chem. Soc.* **1985**, *107*, 5513–5517.
(12) Nambiar, K. P.; Stackhouse, J.; Presnell, S. R.; Benner, S. A. In *Enzymes as Catalysts in Organic Synthesis*; Schneider, M. P., Ed.; D. Reidel: 1986; pp 325–340.
(13) Chang, C.-C.; Laghai, A.; O'Leary, M. H.; Floss, H. G. *J. Biol. Chem.* **1982**, *257*, 3564–3569.
(14) (a) Steinberger, R.; Westheimer, F. H. *J. Am. Chem. Soc.* **1949**, *71*, 4158–4159. (b) Steinberger, R.; Westheimer, F. H. *Ibid.* **1951**, *73*, 429–435.
(15) The specific activity of OAD was greater than 250 international units/mg, and the enzyme displayed no activity as a pyruvate kinase[21] or as a malic enzyme.
(16) Pogson, C. I.; Wolfe, R. G. *Biochem. Biophys. Res. Commun.* **1972**, *46*, 1048–1054.

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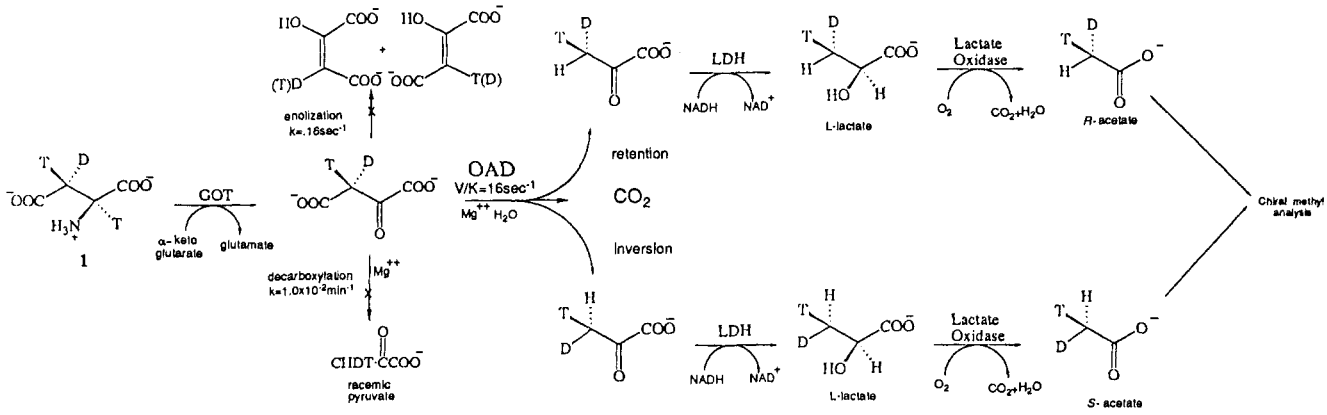
Scheme II



acid (**1**), stereospecifically labeled at C-3 with deuterium and tritium (Scheme II).[17] Incubation of **1** with glutamate–oxaloacetate transaminase (GOT) and α-ketoglutarate resulted in generation of (3*S*)-[^{2}H,^{3}H]oxaloacetate, which was decarboxylated in situ by OAD to yield [^{2}H,^{3}H]pyruvate. Pyruvate was reduced in situ to lactate with NADH and lactate dehydrogenase to prevent loss of chirality via enolization. Chiral lactate was isolated by anion exchange chromatography (Dowex-1 formate),[18] oxidized to acetate with lactate oxidase, and analyzed for chirality.[19] (*S*)-[^{1}H,^{2}H,^{3}H]acetate was formed from (3*S*)-oxaloacetate, showing that decarboxylation was with inversion. This conclusion was confirmed by the production of (*R*)-acetate starting with (3*S*)-[3-^{1}H,^{3}H]aspartate.[20]

An OAD that produces inversion violates the correlation in Table I. Indeed, the result is inconsistent with any functional explanation for stereospecificity in decarboxylases based on a property intrinsic in the substrate, as OAD from *Klebsiella aerogenes* (biotin dependent),[21] pyruvate carboxylase (biotin dependent),[7] and malic enzyme all produce retention.[7] In the last case, the enzyme-producing retention appears to operate via the same mechanism as the one reported here producing inversion.[22] Therefore, mechanistic diversity does not appear to accompany stereochemical diversity. However, the result is also inconsistent with "historical" explanations that presume common ancestry for enzymes catalyzing analogous reactions.[11] Such explanations are prominent in the analysis of stereospecificity in many enzymatic reactions and are weakened by the results presented here.

The enzyme class "β-ketoacid decarboxylases" thus displays a full range of stereochemical diversity: retention, inversion, and racemization. This result is consistent with the emerging notion that enzymatic distinctions between diastereomeric transition states often reflect functional adaptation, while distinctions between enantiomeric transition states do not.[23]

However, for β-decarboxylases, three explanations must be considered for these results: (a) Several independent pedigrees of decarboxylases descendent from several ancestral decarboxylases, where stereospecificity is nonfunctional but highly conserved; (b) stereospecificity as a nonfunctional trait capable of facile neutral "drift" as homologous enzymes diverge; or (c) stereospecificity as a functional trait, where a mechanistic imperative is hidden in the details of the individual reactions or environments of the organism.

Acknowledgment. We thank David Berkowitz for helpful discussion. This research was supported by the Swiss National Science Foundation, Sandoz, Ciba-Geigy, Hoffmann-La Roche, the Searle Foundation, and the National Institutes of Health.

(17) The presumed stereospecificity of the aspartase reaction was confirmed by converting aspartate to malate with use of glutamate–oxaloacetate transaminase, malate dehydrogenase, and NADH. Incubation of the radiolabeled malate with fumarase showed 97% of the label to be in the pro-*S* position of C-3.

(18) Englard, S.; Hanson, K. R. *Methods Enzymol.* **1969**, *13*, 567–601.

(19) (a) Cornforth, J. W.; Redmond, J. W.; Eggerer, H.; Buckel, W.; Gutshow, C. *Eur. J. Biochem.* **1970**, *14*, 1–13. (b) Luethy, J.; Retey, J.; Arigoni, D. *Nature (London)* **1969**, *221*, 1213–1215.

(20) "*F* values"[19] were 0.26 and 0.61.

(21) Dimroth, P. *Eur. J. Biochem.* **1981**, *115*, 353–358.

(22) Creighton, D. J.; Rose, I. A. *J. Biol. Chem.* **1976**, *251*, 61–68.

(23) Benner, S. A.; Ellington, A.; Piccirilli, J. A. *Crit. Rev. Biochem.* **1987**, submitted for publication.

Charge-Transfer Photooxygenation of Sulfides in a Cryogenic Oxygen Matrix: IR Spectroscopic Observation of Persulfoxides

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Received June 1, 1987

The oxygenation of organic sulfur compounds with molecular oxygen continues to yield fascinating results.[1-8] Much attention

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(1) For reviews, see: (a) Ando, W. *Sulfur Report* **1981**, *1*, 143. (b) Ando, W.; Takata, T. In *Singlet O_2*; Frimer, A. A., Ed.; CRC Press: Florida, 1985; Vol. 3, Chapter 1, p 1.

(2) Krauch, C. H.; Hess, D.; Schenck, G. O, unpublished results quoted by Gollnick (Gollnick, K. *Adv. Photochem.* **1968**, *6*, 1).

(3) (a) Foote, C. S.; Denny, R. W.; Weaver, L.; Chang, Y.; Peters, J. W. *Ann. N. Y. Acad. Sci.* **1970**, *171*, 139. (b) Foote, C. S.; Peters, J. W. *J. Am. Chem. Soc.* **1971**, *93*, 3795. (c) Foote, C. S.; Peters, J. W. *IUPAC Congr., 23rd, Spec. Lect.* **1971**, *4*, 129. (d) Kacher, M. L.; Foote, C. S. *Photochem. Photobiol.* **1979**, *26*, 765. (e) Gu, C.-L.; Foote, C. S.; Kacher, M. L. *J. Am. Chem. Soc.* **1981**, *103*, 5949. (f) Gu, C.-L.; Foote, C. S. *J. Am. Chem. Soc.* **1982**, *104*, 6060. (g) Liang, J.-J.; Gu, C.-L.; Kacher, M. L.; Foote, C. S. *J. Am. Chem. Soc.* **1983**, *105*, 4717. (h) Jensen, F.; Foote, C. S. *J. Am. Chem. Soc.* **1987**, *109*, 1478.

(4) (a) Ando, W.; Nagashima, T.; Saito, K.; Kohmoto, S. *J. Chem. Soc., Chem. Commun.* **1979**, 154. (b) Ando, W.; Kabe, Y.; Miyazaki, H. *Photochem. Photobiol.* **1980**, *31*, 191. (c) Ando, W.; Kabe, Y.; Kobayashi, S.; Takyu, C.; Yamagishi, A.; Inaba, H. *J. Am. Chem. Soc.* **1980**, *1025*, 4526. (d) Ando, W.; Miyazaki, H.; Akasaka, T. *Tetrahedron Lett.* **1982**, *23*, 2655. (e) Akasaka, T.; Ando, W. *J. Chem. Soc., Chem. Commun.* **1983**, 1203. (f) Takata, T.; Tamura, Y.; Ando, W. *Tetrahedron* **1984**, *41*, 2133. (g) Akasaka, T.; Ando, W. *Tetrahedron Lett.* **1985**, *25*, 5049. (h) Ando, W.; Sonobe, H.; Akasaka, T. *Tetrahedron Lett.* **1986**, *27*, 4473.

(5) (a) Skold, C. N.; Schlessinger, R. H. *Tetrahedron Lett.* **1970**, 791. (b) Wasserman, H. H.; Strehlow, W. *Tetrahedron Lett.* **1970**, 795. (c) Murray, R. W.; Jindal, S. L. *Photochem. Photobiol.* **1972**, *16*, 147. (d) Murray, R. W.; Jindal, S. L. *J. Org. Chem.* **1972**, *37*, 3516. (e) Casagrande, M.; Gennari, G.; Cauzzo, G. *Gazz. Chim. Ital.* **1974**, *104*, 1251. (f) Stary, F. E.; Jindal, S. L.; Murray, R. W. *J. Org. Chem.* **1975**, *40*, 58. (g) Corey, E. J.; Ouannes, C. *Tetrahedron Lett.* **1976**, 4263. (h) Martin, C. D.; Martin, J. C. *J. Am. Chem. Soc.* **1977**, *99*, 3511. (i) Cauzzo, G.; Gennari, G.; Da Re, F.; Curci, R. *Gazz. Chim. Ital.* **1979**, *109*, 541. (j) Monroe, B. M. *Photochem. Photobiol.* **1979**, *29*, 761.

0002-7863/87/1509-8085$01.50/0