Substrate Binding Mode and Molecular Basis of a Specificity Switch in Oxalate Decarboxylase

Wen Zhu, Lindsey M. Easthon, Laurie A. Reinhardt, Chingkuang Tu, Steven E. Cohen, David N. Silverman, Karen N. Allen, and Nigel G. J. Richards

Department of Chemistry & Chemical Biology, Indiana University Purdue University Indianapolis, Indianapolis, IN 46202, Department of Chemistry, Boston University, Boston, MA 02215, Department of Biochemistry, University of Wisconsin-Madison, Madison, WI 53726, and the Department of Pharmacology & Therapeutics, University of Florida, Gainesville, FL 32610.

Quantitative Analysis of the Observed ^{13}V/K IE Measurements for the DASN OxDC Variant.

Observed ^{13}V/K IEs were determined using the following equation (Eqn. 1):

$$IE_{app} = \frac{\ln(1-f)}{\ln[(1-f)\frac{R_S}{R_0}]} \qquad \text{(Eqn. 1)}$$

where f is the fraction of reaction, and R_s and R_0 are the ^{13}C/^{12}C isotopic ratios in the CO_2 produced in the DASN-catalyzed reaction and initial oxalate, respectively. Based on the proposal of Bornemann et al.[1] (Scheme 2 in the main text) the following minimal mechanism can be written for the DASN-catalyzed reaction up to, and including, the first irreversible step, which we assume to be decarboxylation.[2]
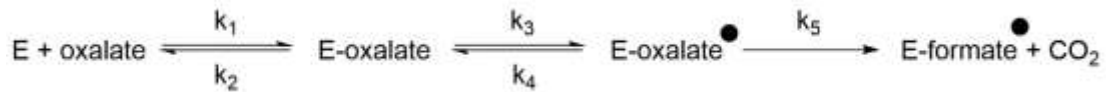
In addition, for reasons that we have discussed elsewhere,[3] this minimal mechanism yields the following equation (Eqn. 2) assuming that isotopic substitution has an effect only on the rate constants k_3, k_4 and k_5:

$$^{13}\left(\frac{V}{K}\right) = \frac{^{13}K_{eq3}\,^{13}k_5 + ^{13}k_3\left(\frac{k_5}{k_4}\right) + \frac{k_5 k_3}{k_4 k_2}}{1+\left(\frac{k_5}{k_4}\right)\left(1+\frac{k_3}{k_2}\right)} \qquad \text{(Eqn. 2)}$$

As in our previous studies,[3,4] we made the following three assumptions in order to analyze the ^{13}V/K isotope effects for the CO_2 produced from the oxidase activity of the DASN variant at pH 5.7. First, $^{13}K_{eq3}$ and $^{13}k_3$ were both set to unity given that there will be a negligible effect from ^{13}C for removal of the proton on the carboxyl acid group of the mono-protonated substrate.[5] Second, we set the value of $^{13}k_5$

to be 1.04, which is an average value for decarboxylation reactions.[6] Finally, we assumed k_3/k_2 to be

small given the slow reaction rate at this pH (see Figure 5 in the main text). As a result, eqn. 2 becomes:

$$1.006 = \frac{1.04 + \dfrac{k_5}{k_4}}{1 + \dfrac{k_5}{k_4}}$$

Solving this equation gives k_5/k_4 = 5.7, which is very similar to the value of 4.0 reported for WT OxDC

under these conditions.[3]

At pH 4.2, setting k_5/k_4 = 5.7 (i.e. assuming that the decarboxylation step is unaffected by solution pH),

$^{13}K_{eq3}$ and $^{13}k_3$ to unity, and $^{13}k_5$ to 1.04 gives the following expression:

$$1.0036 = \frac{1.04 + (5.7)(1 + \dfrac{k_3}{k_2})}{1 + (5.7)(1 + \dfrac{k_3}{k_2})}$$

Solving this expression gives a value of 0.78 for k_3/k_2, which is comparable to that reported previously

for WT OxDC under these conditions.[3]

References

1. Burrell, M. R., Just, V. J., Bowater, L., Fairhurst, S. A., Requena, L., Lawson, D. M., and Bornemann, S. (2007) Oxalate decarboxylase and oxalate oxidase activities can be interchanged with a specificity switch of up to 282,000 by mutating an active site lid, *Biochemistry 46*, 12327-12336.

2. Muthusamy, M., Burrell, M. R., Thorneley, R. N., and Bornemann, S. (2006) Real-time monitoring of the oxalate decarboxylase reaction and probing hydron exchange in the product, formate, using Fourier transform infra-red spectroscopy, *Biochemistry 45*, 10667-10673.

3. Reinhardt, L. A., Svedruzic, D., Chang, C. H., Cleland, W. W., and Richards, N. G. J. (2003) Heavy atom isotope effects on the reaction catalyzed by the oxalate decarboxylase from *Bacillus subtilis*. *J. Am. Chem. Soc. 125*, 1244-1252.

4. Svedruzic, D., Liu, Y., Reinhardt, L. A., Wroclawska, E., Cleland, W. W., and Richards, N. G. J. (2007) Investigating the roles of putative active site residues in the oxalate decarboxylase from *Bacillus subtilis*, *Arch. Biochem. Biophys. 464*, 36-47.

5. Bayles, J. W., Bron, J., and Paul, S. O. (1976) Secondary carbon-13 isotope effect on the ionization of benzoic acid, *J. Chem. Soc., Faraday Trans. 1* 1546-1552.

6. In this analysis, we note that ^{13}C IEs on decarboxylation are usually 4-5% when the heavy atom is located in the carboxylate group that is undergoing reaction. This relatively large effect arises due to the requirement that the carbon must move to become collinear with the two oxygen atoms as the reaction proceeds to yield CO_2. Assuming a slightly different value will modify the derived ratios to a small extent but not the overall conclusions of these experiments. Support for our choice is provided by experiments on the oxidative decarboxylation of malate by malic enzyme: Edens, W. A., Urbauer, J. L., and Cleland, W. W. (1997) Determination of the chemical mechanism of malic enzyme by isotope effects, *Biochemistry 36*, 1141-1147.