

Suicide Inactivation of Dioldehydratase by Glycolaldehyde and Chloroacetaldehyde: an Examination of the Reaction Mechanism

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Dioldehydratase (DDH) is a coenzyme B₁₂-dependent enzyme that catalyzes the transformation of ethane-1,2-diol (**1**) and propane-1,2-diol (**2**) into the corresponding aldehydes.¹ In early studies of DDH, it was found that the substrate analogues glycolaldehyde (**3**)^{2a} and 2-chloroacetaldehyde (**4**)^{2b} rapidly caused its deactivation. 5'-Deoxyadenosine (Ado-H) was found to be one of the products in the deactivation process, but until recently, the fate of the substrate analogues was not known. In elegant EPR experiments, Frey and Reed and co-workers identified the product derived from both **3** and **4** as *cis*-ethanesemidione (**5**).^{3,4} They proposed mechanisms both for the formation of *cis*-ethanesemidione and for the inactivation process on the basis of these experimental results.^{3,4} The fact that glycolaldehyde (**3**) and 2-chloroacetaldehyde (**4**) inactivate DDH is very intriguing and makes a computational investigation of the detailed mechanism of the inactivation, and a determination of how and why it diverges from the functional catalytic mechanism, desirable. That is the purpose of the present study.

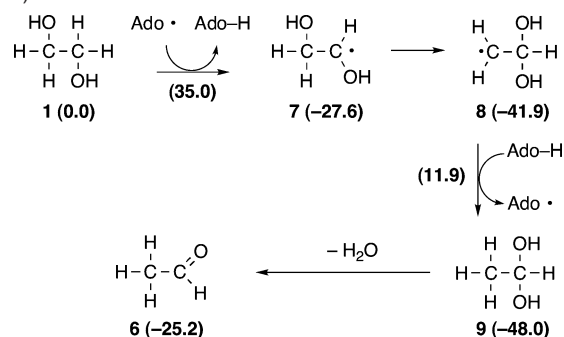
The accepted mechanism for the DDH-catalyzed transformation of ethane-1,2-diol (**1**) to acetaldehyde (**6**) is displayed in Scheme 1.^{1,5} After substrate-induced homolytic fission of the C–Co bond of adenosylcobalamin, the 5'-deoxyadenosyl radical (Ado•) is generated. This abstracts H from C1 of **1**, to give the 1,2-dihydroxyethyl radical (**7**). An OH 1,2-shift yields the 2,2-dihydroxyethyl radical (**8**). Reabstraction of H from Ado-H, leads to ethane-1,1-diol (**9**), from which enzyme-catalyzed elimination of water yields the product acetaldehyde (**6**).

The hydrates of glycolaldehyde and 2-chloroacetaldehyde, namely ethane-1,1,2-triol (**10**) and 1,1-dihydroxy-2-chloroethane (**11**), are structurally similar to the natural substrates for the DDH-catalyzed reactions. It is therefore not surprising that DDH is able to function (at least partially) on these substrate analogues. We have, therefore, examined computationally the process of suicide inactivation by these two substrates in the context of the mechanism depicted in Scheme 1.

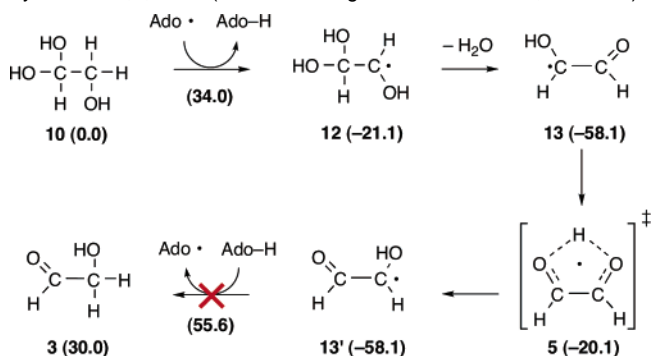
Geometries and scaled vibrational frequencies were obtained with the MPW1K/6-31+G(d,p) density functional theory procedure, with improved relative energies (enthalpies) at 0 K calculated using the high-level G3(MP2)-RAD methodology.^{6,7} We have chosen ethanol as a model for Ado-H as it has previously been shown to satisfactorily describe the H-abstraction steps for B₁₂-dependent processes.⁸

A mechanism for the DDH-catalyzed reaction of ethane-1,1,2-triol (**10**), closely related to that originally proposed,³ is depicted in Scheme 2.⁹ In a manner analogous to that pertaining to a catalytic substrate (Scheme 1), H abstraction by Ado• from C2 of **10** gives the 1,2,2-trihydroxyethyl radical (**12**), with a barrier of 34.0 kJ

Scheme 1. Proposed Mechanism for the DDH-Catalyzed Reaction of Ethane-1,2-diol (Relative Energies in Parentheses, kJ mol⁻¹)



Scheme 2. Proposed Mechanism of Suicide Inactivation of DDH by Ethane-1,1,2-triol (Relative Energies in Parentheses, kJ mol⁻¹)



mol⁻¹ and an exothermicity of 21.1 kJ mol⁻¹. These values are consistent with those calculated for ethane-1,2-diol (35.0 and 27.6 kJ mol⁻¹, Scheme 1). The presence of the additional hydroxyl group does not appear to have a significant effect on the initial H-abstraction step.

In the case of a natural substrate, such as ethane-1,2-diol (**1**), the next step involves hydroxyl group migration (Scheme 1).^{1,5} However, for **12** such a step would simply lead to an equivalent structure. Hence, it is proposed that dehydration of **12** occurs to give the glycolaldehyde radical (**13**), for which we calculate an exothermicity of 37.0 kJ mol⁻¹ (Scheme 2).

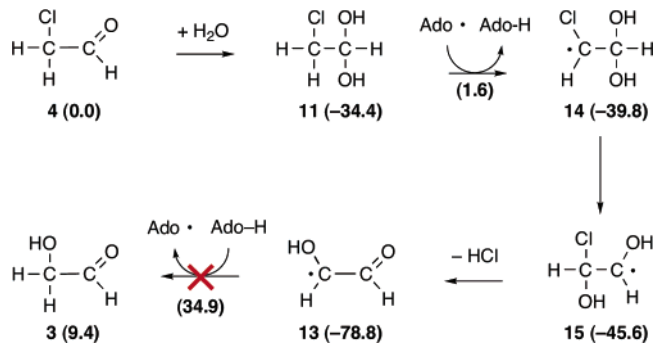
Interestingly, we find that the symmetric *cis*-ethanesemidione structure (**5**) deduced from EPR experiments³ is a transition structure (TS) lying 38.0 kJ mol⁻¹ above the glycolaldehyde radical (**13**) on the potential energy surface (PES). We will address this point further below. For the moment, we note that for a mechanism analogous to that of the catalytic pathway to continue, H abstraction by glycolaldehyde radical (**13'**) from Ado-H would be required. The calculated barrier for this process is an astounding 113.7 kJ mol⁻¹, with an endothermicity of 88.1 kJ mol⁻¹! By comparison, the barrier

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Scheme 3. Proposed Mechanism of Suicide Inactivation of DDH by 2-Chloroacetaldehyde (Relative Energies in Parentheses, kJ mol^{-1})



for reabstraction when ethane-1,2-diol (**1**) is the substrate is calculated to be 53.8 kJ mol^{-1} , with an associated exothermicity of 6.1 kJ mol^{-1} (Scheme 1).

Clearly, the glycolaldehyde radical (**13**) is a very stable species, a result that can be attributed to the captodative stabilization provided by the OH and CHO substituents at the radical center.^{9,10} The direct consequence is that **13** is unable to reabstract an H-atom from Ado-H. The indirect effect, and the essence of glycolaldehyde-induced suicide inactivation for DDH, is that Ado• is unable to be generated so as to recombine with the cob(II)alamin radical, and the latter remains tightly bound to DDH. The net effect is that DDH becomes an impotent enzyme.

Our calculations also indicate that glycolaldehyde (**3**) does not need to be hydrated for DDH inactivation. Thus, the barrier for H abstraction by Ado• from **3** itself, calculated as the reverse of the last step in Scheme 2, is 25.6 kJ mol^{-1} , which is even less than that for ethane-1,2-diol (**1**). Again, in a very exothermic step, **13'** plus Ado-H are generated, terminating the catalytic cycle.

The resemblance of the EPR spectra obtained when glycolaldehyde and 2-chloroacetaldehyde are the substrates implies a mechanism of the type shown in Scheme 3 for the latter.^{4,9} Hydration of 2-chloroacetaldehyde to give 1,1-dihydroxy-2-chloroethane (**11**) is exothermic by 34.4 kJ mol^{-1} . Subsequent H abstraction by Ado• has a barrier of 36.0 kJ mol^{-1} , and is exothermic by 5.4 kJ mol^{-1} . Migration of an OH group in the 2,2-dihydroxy-1-chloroethyl radical (**14**) is also calculated to be exothermic (by 5.8 kJ mol^{-1}). At this stage, loss of HCl from the 1,2-dihydroxy-2-chloroethyl radical (**15**) leads immediately to the glycolaldehyde radical (**13**) in a process that is exothermic by 33.2 kJ mol^{-1} . Again, as found in Scheme 2, any attempt to reabstract an H-atom from Ado-H is prevented by the biologically prohibitive barrier of $113.7 \text{ kJ mol}^{-1}$. Our calculations indicate a series of exothermic steps in the transformation of 2-chloroacetaldehyde (**4**) to the stable glycolaldehyde radical (**13**), and thus provide strong support for this mechanism for suicide inactivation of DDH (Scheme 3).⁴

Of additional interest in the suicide inactivation of DDH by glycolaldehyde (**3**) and 2-chloroacetaldehyde (**4**) is the observation that the presence of a monovalent cation such as K^+ is required.⁴ The presence of such an ion is also required in the normal DDH-catalyzed reactions, where it is believed to bind the substrate and facilitate OH migration.^{1b,5} For the suicide inactivators of DDH, the role of such a cation has yet to be determined experimentally.⁴ Accordingly, we have performed calculations to try to address this question.

Our calculations suggest that K^+ does not participate directly in suicide inactivation. Rather, its purpose is more likely to help bind the substrate in the active site, as in the case of the catalytic mechanism. During the course of catalysis leading to the glycol-

aldehyde radical (**13**), this type of binding of the K^+ ion effectively disappears.⁹ Our findings are consistent with EPR experiments that did not detect any magnetic interaction between a Ti^+ ion (used as a magnetically active model for K^+) and the inactivating radical species.⁴

As a final point, the nature of *cis*-ethanesemidione (**5**) warrants a brief examination. As noted above, we find that **5** is actually a TS on the PES, lying 38.0 kJ mol^{-1} above the glycolaldehyde radical (**13**). In contrast, the 77 K EPR studies have been interpreted in terms of a symmetric structure. At this temperature, interconversion of **13** and **13'** is likely to be slow on the EPR time scale. However, the initial incubation of the sample at 310 K would lead to a prior rapid equilibration, and the EPR spectrum would then reflect a (symmetric) mixture of **13** and **13'**.⁹

We have also examined the effect of partial deprotonation on **13** by a base such as Asp335 in DDH.¹¹ Using formate as a model, we find that significant deprotonation of **13** takes place, leading to a structure that could be described as the glyoxal radical anion interacting with formic acid. This structure is still asymmetrical, but the calculated barrier for interconversion of equivalent structures of this type is reduced to just 14.1 kJ mol^{-1} . This would facilitate the already postulated rapid equilibration of **13** and **13'**. The barrier for reabstraction of H from Ado-H with this model is again very high at $108.6 \text{ kJ mol}^{-1}$.

In summary, we have examined the ability of glycolaldehyde (**3**) and 2-chloroacetaldehyde (**4**) to trigger suicide inactivation of DDH. Both substrate analogues lead to the glycolaldehyde radical (**13**) that requires a barrier too high ($\sim 110 \text{ kJ mol}^{-1}$) for hydrogen reabstraction from Ado-H to be feasible. In addition, our results predict that the symmetric *cis*-ethanesemidione radical is not a stable species. The apparently symmetrical nature of the radical observed in EPR experiments is likely to be the result of a rapid prior equilibration of equivalent forms of the glycolaldehyde radical, partially deprotonated by a carboxylate moiety of the enzyme.

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Supporting Information Available: Details of the calculations, archive entries, and relevant total energies. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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