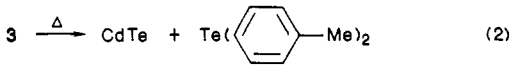
The yellow solid shows solubility similar to, and a proton NMR essentially identical with, the corresponding mercury complex. Pyrolysis of **3** in the solid state (200 °C, 16 h) gives CdTe and bis(4-methylphenyl)tellurium in 98% and 82% yields, respectively (after purification) (eq 2).



(2)

Owing to the drastic differences in reaction conditions, the relevance of the present work to thin-film growth by OMVPE with use of ditelluride source compounds is not clear, although one point is noteworthy. In the OMVPE reactor we found[1] that the ditelluride itself is stable, in the absence of the cadmium source, under conditions which, when cadmium is present, CdTe is produced. This implies that the cadmium source (or its decomposition products) reacts with the ditelluride. One mechanism which would explain this is that the cadmium source decomposes to give cadmium atoms which subsequently react with the ditelluride to give a complex such as **3** which decomposes to give CdTe.

(7) A slight excess of (4-methylphenyl)(trimethylsilyl)tellurium was added to $CdCl_2$, suspended in THF, and the mixture was stirred at room temperature 14 h. The yellow solid was isolated, washed with pentane, and extracted with toluene/PMe_3. Evaporation gave **3** as a bright yellow solid: yield 32%; ^1H NMR (C_6D_6 with PEt_3) δ 2.02 (s, $-CH_3$), 6.75 (d, J = 6.6 Hz), 8.14 (d, J = 6.6 Hz), AB quartet due to aromatic protons. Anal. Calcd for $C_{14}H_{14}Te_2Cd$: C, 30.58; H, 2.57. Found: C, 30.24, H, 3.29.

The Catalytic Base of Enolase Is a Sulfhydryl Group

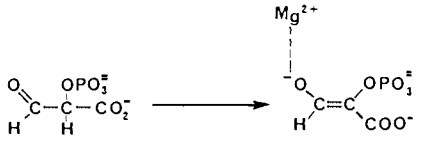
Paul M. Weiss, Renee J. Boerner, and W. W. Cleland*

Department of Biochemistry, University of Wisconsin Madison, Wisconsin 53706

Received July 13, 1987

We have made use of the partial chemistry undergone by the competitive inhibitor, D-tartronate semialdehyde phosphate (D-TSP), in the presence of enolase, to investigate the acid–base catalyst of enolase responsible for proton removal from D-TSP and presumably from the substrate, D-2-phosphoglyceric acid (D-2PGA). From the differential inhibition of D-2-proteo-TSP (D-TSPH) and D-2-deuterio-TSP (D-TSPD) we conclude that the base responsible for this process is a sulfhydryl group of a cysteine residue.

It has been known for some time that enolase catalyzes the enolization of D-TSP.[1,2] The C-2 proton is removed by an enzymic base, facilitating formation of the enzyme:Mg:enolate complex.



The nature of this base, however, has remained unknown despite extensive studies on enolases from various sources. We have been able to compare the inhibition of yeast and muscle enolase by D-TSPH and D-TSPD under conditions where isotopic discrimination can be detected in the form of a deuterium isotope effect on the inhibition constant, ^DK_i.

TSP was prepared by reacting β-hydroxypyruvate and MgATP with pyruvate kinase at pH 9.[3] The stereochemistry of this TSP has been found to be racemic;[4] that is, the phosphorylated enol is the actual product of the pyruvate kinase reaction. This tautomerizes in solution to the aldehyde form, which, in turn, hydrates to >95%.[5] TSPD was synthesized by carrying out the pyruvate kinase reaction in D_2O rather than H_2O.

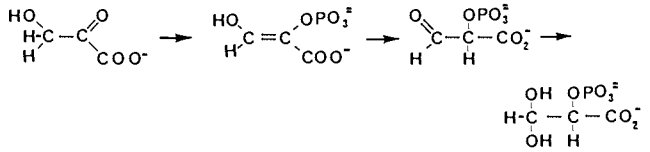


Table I. Michaelis and Inhibition Constants for Yeast and Muscle Enolase[a]

enolase source	D-TSPH $K_{D\text{-}2PGA}$, μM	D-TSPH K_i, μM	D-TSPD $K_{D\text{-}2PGA}$, μM	D-TSPD K_i, μM	^DK_i = K_i(D-TSPH)/K_i(D-TSPD)
yeast	13.5 ±0.9	0.46 ±0.04	12.1 ±0.7	1.02 ±0.08	0.45 ±0.05
yeast[b]	3.9 ±0.4	0.23 ±0.02	4.9 ±0.5	0.50 ±0.07	0.46 ±0.08
yeast	12.8 ±0.06	0.48 ±0.02	12.2 ±0.07	1.36 ±0.09	0.35 ±0.03
muscle	4.3 ±0.7	0.10 ±0.01	4.4 ±0.6	0.25 ±0.03	0.40 ±0.06
muscle	5.8 ±0.5	0.13 ±0.01	5.3 ±0.7	0.24 ±0.03	0.54 ±0.08
muscle	4.7 ±0.4	0.14 ±0.01	4.6 ±0.4	0.31 ±0.03	0.45 ±0.05

[a] Values for these constants were obtained by fitting the initial velocities to the equation for competitive inhibition ($v = VA/[K_{D\text{-}2PGA}(1 + I/K_i) + A]$ where v, V, A and I are the initial velocity, maximum velocity, D-2PGA concentration, and D-TSPH or D-TSPD concentration, respectively) by using a Fortran program[11] and microcomputer. [b] Despite the fact that these values are significantly lower than the other yeast enolase values, ^DK_i is in excellent agreement with the other ^DK_i values. The ratio of K_i/K_m is the value most accurately determined in these experiments, and these values are in better agreement with the other K_i/K_m ratios for yeast enolase.

The inhibition constants for D-TSPH and D-TSPD were determined at 25 °C and pH 7.4. Initial velocities were obtained by varying the concentration of D-2PGA (added last to initiate the reaction) at various fixed concentrations of each inhibitor. The concentration of free Mg^{2+} was maintained at 8.5 mM. A pyruvate kinase and lactate dehydrogenase coupled assay was used to monitor the enolase reaction. The phosphoenolpyruvate (PEP) is converted into pyruvate, which is subsequently reduced to lactate with the concomitant oxidation of NADH ($\Delta\epsilon = -6220\ M^{-1}$). The stock concentrations of D-TSPH and D-TSPD were accurately determined by reduction of the aldehyde to the corresponding alcohol (D-2PGA) with $NaBH_4$.[6] End-point assays for D-2PGA, and thus D-TSP, were carried out with use of the same coupled assay described above.

From six independent experiments, in which the D-TSPH and D-TSPD were each resynthesized and recalibrated, the K_i values shown in Table I were obtained. The ratio of the K_i values, ^DK_i, is also shown for each experiment. The weighted average of these ratios is 0.41 ± 0.02. Since these K_i values are dissociation constants, the value of 0.41 represents the equilibrium isotope effect for the two-step process of binding and enolization of D-TSP. Further, this value suggests that the fractionation factor[7] for the proton transferred from C-2 of D-TSP to the enzymic base has decreased drastically, from 1.19 when on D-TSP (the value for the C-2 proton of D-2PGA[8] and assumed to be the same for the C-2 proton of D-TSP) to ~0.4 when on the enzymic base. The only reasonable functional group with a fractionation factor so

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(6) The pyruvate kinase reaction mixture was passed through an ultrafiltration apparatus to remove enzyme. The TSP was used without further purification, as the coupled assays also contained K^+, Mg^{2+}, and ADP at levels similar to those in the TSP solution. There was no remaining β-hydroxypyruvate by lactate dehydrogenase assay.
(7) The fractionation factor of a hydrogen is the equilibrium constant for exchange with deuterium in one-half of a solvent water molecule. Thus a fractionation factor less than unity implies depletion of deuterium in the group relative to water.
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far below unity is the sulfhydryl group of a cysteine residue (0.43[8]). Presumably in the enolase-catalyzed dehydration of D-2PGA to PEP, the base is in the E—S^- protonation state, whereas for the reverse hydration reaction, it is in the E—SH form.

We have been able to inactivate both yeast and muscle enolase with *p*-mercuribenzoate. Substrate protection against inactivation was observed, with Mg^{2+} not as effective as D-2PGA, which was not as effective as Mg^{2+} and D-2PGA. The inactivation was in all cases reversed by treatment with dithiothreitol.

Yeast enolase contains a single cysteine residue per monomer,[9] and it will be of interest to see if confirmation of this cysteine residue as the acid–base catalyst will come from the crystal structure of yeast enolase. Preliminary crystallographic data have been reported;[10] however, a high resolution structure has not yet been obtained.

Acknowledgment. This work was supported by a grant from the National Institutes of Health (GM18938).

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Mn^{2+}/Mn^{3+} and Mn^{3+}/Mn^{4+} Mixed Valence Binuclear Manganese Complexes of Biological Interest

G. C. Dismukes,* J. E. Sheats,† and J. A. Smegal‡

Department of Chemistry, Princeton University
Princeton, New Jersey 08544
Received June 29, 1987

Binuclear and multinuclear manganese containing enzymes which occur in biology are involved in functions such as hydrogen peroxide decomposition in bacteria[1] and the oxidation of water to oxygen during photosynthesis[2], respectively. In the case of the water-oxidizing complex, the so-called S_2 oxidation state has been characterized by EPR spectroscopy to be a mixed valence manganese cluster.[3] This can be produced in two forms. The native form, which is found in active O_2-evolving samples, is considered to be either tetranuclear[4,5] or trinuclear[6] in Mn, based upon interpretation of its broad 1500 G-wide 19-line "multiline" EPR signal and unusual temperature dependence. A partially "decoupled" form is found in inactivated samples having a narrower width (1345 G), with only 16 lines and a more Curie-like temperature dependence, all features which are found in typical Mn^{3+}/Mn^{4+} binuclear centers (16-line EPR, spin $S = {}^1/_2$ ground state).[6] For the native form both different oxidation states and different numbers for the manganese ions in the cluster have been proposed in order to account for the larger spectral width due to the hyperfine interaction with ^{55}Mn. Both binuclear[7] Mn^{2+}/Mn^{3+} and tetranuclear[4] $3Mn^{3+}/Mn^{4+}$ states have been considered. Notably lacking in this analysis has been EPR data for binuclear complexes in which both types of mixed valence oxidation states, Mn^{3+}/Mn^{4+} and Mn^{2+}/Mn^{3+}, are compared within the same ligand system. The present report gives the first experimental evidence showing how ^{55}Mn hyperfine data can easily distinguish between binuclear manganese complexes having these mixed valence oxidation states.

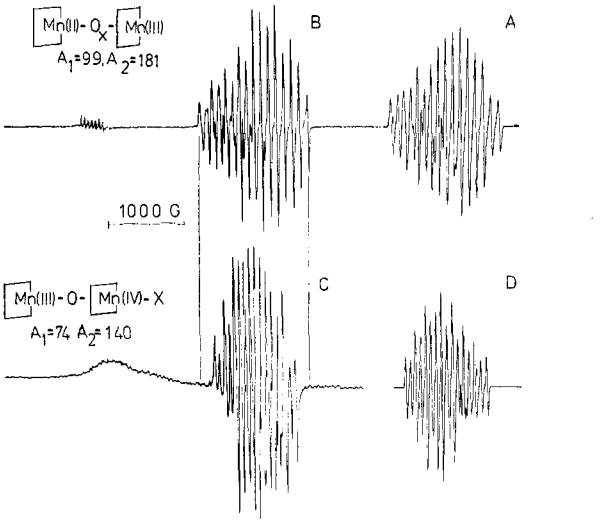

Figure 1. X-band EPR spectra at 10 K for (B) **2a** or **2b**, $[(TPP)Mn^{II}OOMn^{III}(TPP)]^-$, or $[(TPP)Mn^{II}OMn^{III}(TPP)]^+$; (C) **1**, $(TPP)Mn^{III}OMn^{IV}(TPP)X$, X = OI(Br)Ph or Br; (A) simulation of B; and (D) simulation of C using the following parameters:[12] A_1 = 99 G, A_2 = 181 G, Δ_0 = 25 G, a_1 = 3.0, a_2 = 3.0 and A_1 = 74 G, A_2 = 140 G, Δ_0 = 25 G, a_1 = −3.0, a_2 = 3.0 G with an M_I dependent Gaussian line width: $\Delta = \Delta_0 + a_1M_1 + a_2M_2$.

The tetraphenylporphyrin ligand (TPP) forms stable complexes with Mn^{2+}, Mn^{3+}, and Mn^{4+} and was employed in this study. Preparation of the Mn^{2+}/Mn^{3+} binuclear species was performed by air oxidation of $(TPP)Mn^{II}$ in chlorobenzene. $(TPP)Mn^{II}$ was prepared from (TPP)MnCl by anaerobic reduction with $NaBH_4$ in methanol and isolated by crystallization prior to use.[8] The reaction with O_2 was followed by EPR at 10 K. Prior to reaction with O_2 the only visible EPR signal was the resonance for the high spin $(TPP)Mn^{II}$ at $g = 6$.[9] Oxidation with O_2 caused this signal to decrease with concomitant appearance of a narrow free radical signal at $g = 2.0$. Subsequently, a broad underlying multiline signal appears at $g = 2$ as the free radical decays. When the reaction is complete, only the multiline signal remains and exhibits the EPR spectrum shown in Figure 1B. Here it is compared to the EPR spectrum for the mixed valence complex obtained from the room temperature decomposition of $[Mn^{IV}(TPP)X]_2O$,[10] X = OI(Br)Ph^-, given in Figure 1C. An identical spectrum has been assigned as a mixed valence Mn^{3+}/Mn^{4+} complex for the case of X = OCN^- and N_3^-.[11] The spectrum shown in Figure 1C can therefore be assigned to the analogous mixed valence complex $(TPP)Mn^{III}OMn^{IV}(TPP)X$, **1**, where X = OI(Br)Ph^- or Br^-. Both spectra arise from the ground state, as seen by a limited temperature study between 5 and 100 K (not shown).

As can be seen, both species yield 16-line "multiline spectra". Temperature dependence and hyperfine structure of this type indicate antiferromagnetic electron exchange coupling between inequivalent (valence trapped) Mn oxidation states in which the ground state has spin $S = {}^1/_2$.[12] However, the spectra differ greatly in the magnitude of their ^{55}Mn hyperfine fields; the Mn^{2+}/Mn^{3+} complex has a total width of 1410 G, while the Mn^{3+}/Mn^{4+} complex is considerably narrower at 1070 G and also exhibits somewhat larger line shape anisotropy (axial). Computer simulations of these spectra using a second-order perturbation solution to an isotropic spin hamiltonian[12] are given in Figure 1A and D. The agreement with the positions of all transitions is

†Present address: Rider College, Lawrenceville, NJ 08648.
‡Present address: Shell Development, Houston, TX 77082.

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