A Role for Zinc in OMP Decarboxylase, an Unusually Proficient Enzyme

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In the final step in pyrimidine biosynthesis, orotidine 5′-monophosphate is decarboxylated in a reaction (Figure 1) whose spontaneous rate appears to be slower than that of any other biological reaction that has been described ($t_{1/2} = 78 \times 10^6$ years). The enzyme orotidine 5′-monophosphate decarboxylase (ODCase, EC 4.1.1.23) enhances the rate of this reaction by a factor of more than 10^{17}-fold.[1] A single lysine residue (Lys-93) has been identified as essential to enzyme activity.[2] However, added amines fail to catalyze the nonenzymatic reaction,[1] which has also been found to be remarkably insensitive to solvent environment, proceeding at similar rates in water, concentrated sulfuric acid, and several nonaqueous solvents.[3] In view of this lack of susceptibility of the spontaneous reaction to catalysis by simple acids or bases, or by desolvation, and the reported absence of metals or other cofactors from ODCase,[4] the sources of this enzyme's remarkable proficiency as a catalyst remain mysterious. Because carbonic anhydrase and oxaloacetate decarboxylase use divalent cations to promote elimination of CO_2 from their respective substrates, we considered it worthwhile to reexamine the possibility that metal atoms might be present in ODCase.

Pure yeast ODCase[5] was dialyzed extensively against HEPES buffer (pH 7.0, 2×10^{-3} M), which had been rendered metal-free by treatment with Chelex 100. Plastic vessels were soaked overnight in 20% HNO_3 to remove contaminating zinc, and dialysis tubing (Spectrapor, molecular weight cutoff 12000−14000 Da) was treated with EDTA (10^{-4} M) followed by multiple washings in metal free water at 70 °C. After dialysis, enzyme activity[6] was assayed by monitoring the decrease in absorbance at 280 nm as OMP was converted to UMP (where $\Delta\epsilon_M = -1650$ cm^{-1}) at 25 °C in MOPS buffer, pH 7.2 (1.5×10^{-3} M), containing EDTA (10^{-4} M) and 1,4-dithiothreitol (10^{-3} M). Analysis of the dialyzed enzyme, using a flame atomic absorption/emission spectrophotometer (Instrumentation Laboratories S-12), revealed the presence of approximately two atoms of zinc in each monomer of active ODCase.[7] Significantly lower amounts of zinc were detected in a mutant enzyme, containing cysteine in place of Lys-93[2] (Table 1).

To determine whether zinc might be required for activity, the enzyme was dialyzed overnight in the presence of EDTA (0.01 M), followed by extensive washing in metal-free HEPES buffer (pH 7.0, 2×10^{-3} M). After this treatment, ODCase was devoid of spectroscopically detectable zinc (less than 0.1 mol of Zn per mole of enzyme) and displayed less than 3% of wild-type activity.[6] After removal of zinc by treatment with EDTA, efforts to restore activity by addition of zinc were unsuccessful. When the zinc-depleted enzyme was denatured with urea (6 M) and then dialyzed against a buffer (2×10^{-3} M HEPES) containing 10^{-4} M $ZnSO_4$, in an effort to remove the denaturant and restore enzyme-bound zinc, no significant recovery of activity was observed. Other zinc-chelating agents were also found to inactivate ODCase, as shown in Table 2. Loss of ODCase activity was found to be particularly rapid in the presence of 2,3-dimercapto-1-propanol (5×10^{-3} M) with a half-time of 20 s in MOPS buffer (pH 7.2, 1.5×10^{-3} M).

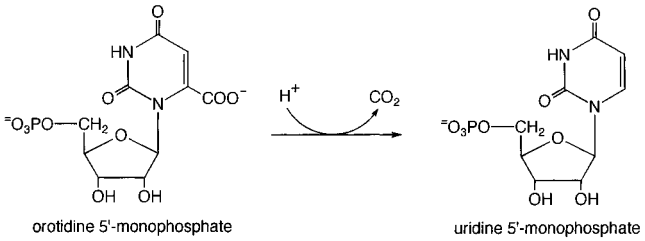

Figure 1. The decarboxylation of orotidine 5′-monophosphate (OMP) catalyzed by ODCase.

Table 1. Zinc Content of ODCase

enzyme	mol of Zn/mol of protein
native ODCase	1.87 ± 0.24
ODCase treated with EDTA	<0.1
ODCase + BMP treated with EDTA	0.7 ± 0.2
K93C mutant ODCase	0.41 ± 0.18

Table 2. Inactivation of ODCase

zinc chelator	concentration	$t_{1/2}$ of inactivation
EDTA	5.0×10^{-3}	25.5 min
dipicolinic acid	2.5×10^{-4}	1.8 min
2,3-dimercapto-1-propanol	5.0×10^{-4}	21.6 s

Is zinc present at the active site of ODCase? In other enzymes, "structural" zinc atoms tend to be coordinated by cysteine or histidine residues, whereas zinc atoms with a direct role in catalysis are coordinated by a variety of amino acids.[8] Examination of the amino acid sequence of yeast ODCase[9] does not suggest obvious homologies with other zinc-containing enzymes, but the sequence contains several highly conserved aspartate and two lysine residues, any of which might in principle furnish a ligand to zinc. Indeed lysine and aspartate residues have been implicated in zinc coordination in polynuclear zinc enzymes such as leucine aminopeptidase,[10] and carbamylated lysines have been discovered in the structurally related binuclear metal centers of urease and phosphotriesterase.[11] Accordingly, it is of special interest that a mutant ODCase, in which cysteine replaces the conserved Lys-93, shows a lower zinc content than the native enzyme (Table 1). The reduced zinc content of this mutant enzyme, and the attendant lack of catalytic activity, would be understandable if mutation of lysine 93 (possibly modified by carbamylation) to cysteine disturbed the coordination of a zinc atom at the active site.

In another set of experiments, 1-β-D-ribofuranosylbarbituric acid 5′-phosphate (BMP) was found to afford the enzyme some protection against the loss of zinc. With a K_i value of approximately 10^{-11} M, BMP is bound by ODCase nearly 4 orders of magnitude more tightly than the substrate OMP, in accord with its postulated resemblance to the transition state in OMP decar-

(1) Radzicka, A.; Wolfenden, R. *Science* **1995**, *267*, 90−93.
(2) Smiley, J. A.; Jones, M. E. *Biochemistry* **1992**, *31*, 12162−12168.
(3) Beak, P.; Siegel, B. *J. Am. Chem. Soc.* **1976**, *98*, 3601−3606.
(4) Shostak, K.; Jones, M. E. *Biochemistry* **1992**, *31*, 12155−12161.
(5) Bell, J. B.; Jones, M. E. *J. Biol. Chem.* **1991**, *266*, 12662−12667.
(6) ODCase dialyzed in the absence of EDTA retained 100.9% activity.
(7) We do not understand the failure of earlier efforts (ref 4) to detect Zn in yeast ODCase by atomic absorption spectroscopy. The negative results obtained using diphenylthiocarbazone to detect Zn in ODCase, which we confirm, can probably be attributed to steric hindrance, combined with the enzyme's high affinity for Zn.
(8) For a recent review: see Lipscomb, W. N.; Sträter, N. *Chem. Rev.* **1996**, *96*, 2375−2433.
(9) Rose, M.; Grisafi, P.; Botstein, D. *Gene* **1984**, *29*, 113−124.
(10) Sträter, N.; Lipscomb, W. N. *Biochemistry* **1995**, *34*, 9200−9206.
(11) Holm, L.; Sander, C. *Proteins: Struct., Funct., Genet.* **1997**, *28*, 72−82.

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boxylation.[12] When this transition-state analogue inhibitor was present at a concentration of 10^{-4} M during dialysis against EDTA (5×10^{-3} M), the enzyme retained 0.7 ± 0.2 mol of zinc per mole of protein, whereas, in the absence of BMP, zinc levels were less than the detectable limit of 0.2 mol per subunit.

As OMP contains no π orbitals into which electrons can be delocalized as CO_2 is eliminated, it would be of interest to know how this proficient catalyst stabilizes anionic intermediates to such an usual degree. Beak and Siegel[3] suggested that ODCase might act by stabilizing zwitterionic forms of substrate OMP and product UMP, as shown in Figure 2; and the ^{13}C NMR spectrum of the enzyme's complex with BMP[13] suggests that this transition-state analogue inhibitor is tightly bound in a form that resembles these zwitterions. One possibility is that this stabilization might be accompanied by proton transfer from the enzyme to the substrate's 2-oxygen atom from an acidic group at the active site.[2] Alternatively, it has been proposed that proton transfer from the enzyme's critical lysine residue to the substrate's 4-oxygen atom offers a pathway that may be more favorable energetically.[14]

The present results suggest that zinc, rather than a proton, may be largely responsible for neutralizing charge development in the transition state for OMP decarboxylation. In a possibly related reaction, Cu^+ ion in quinoline solution has been found to promote the decarboxylation of aromatic carboxylic acids in the presence of organic bases.[15] Detailed structural information about ODCase, when that becomes available, should help to establish the extent to which stabilization involves either direct zinc interaction with C-6 at which the carbanion is generated (Figure 2), or interaction with O-2, O-4, or the π electrons of the aromatic system. The remarkable factor (10^{17}-fold) by which binding affinity increases as the ODCase reaction proceeds from the ground state to the transition state[1] could well be associated with a major increase in the strength and intimacy of contact between OMP and zinc. In this connection, it is of special interest that the mutant enzyme K93C, lacking zinc, binds substrate normally but does not catalyze its decarboxylation.[2] A direct, and structurally discriminating, interaction between site-bound zinc and substrate OMP would also be consistent with Kalman's observation[16] that ODCase binds the competitive inhibitor 6-thiocarboxamido-UMP almost 5 orders of magnitude more tightly than 6-carboxamido-UMP.

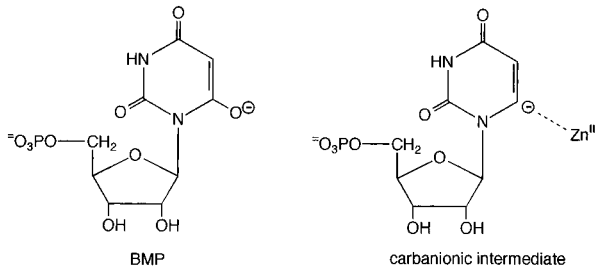

Figure 2. Structures of the postulated transition-state analogue 1-β-D-ribofuranosylbarbituric acid 5′-phosphate (BMP) and of a carbanionic intermediate stabilized by enzyme-bound Zn^{II}.

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(12) Levine, H. L.; Brody, R. S.; Westheimer, F. H. *Biochemistry* **1980**, *19*, 4990−4999.
(13) Acheson, S. A.; Bell, J. B.; Jones, M. E.; Wolfenden, R. *Biochemistry* **1990**, *29*, 3198−3202.
(14) Lee, J. K.; Houk, K. N. *Science* **1997**, *276*, 942−945.
(15) Cairncross, A.; Roland, J. R.; Henderson, R. M.; Sheppard, W. A. *J. Am. Chem. Soc.* **1970**, *92*, 3187−3189.
(16) It is of special interest that the design of 6-thiocarboxamido-UMP was predicated on the possibility that zinc, with its high affinity for sulfur ligands, might be present at the active site (Kalman, T. In *Drug Action and Design: Mechanism-Based Enzyme Inhibitors*; (Kalman, T., Ed.; Elsevier North Holland, Inc.: New York, 1979; pp 285−287).