1 I. Bertini, G. Canti, C. Luchinat and F. Mani, *J. Am. Chem. Soc., 103,* 7784 (1981).
2 M. E. Fabry, S. H. Koenig and W. E. Schillinger, *J. Biol. Chem., 245,* 4256 (1970); I. Bertini, R. D. Brown, III, S. H. Koenig and C. Luchinat, *Biophys. J.,* in press.
3 I. Bertini, G. Canti and C. Luchinat, *J. Am. Chem. Soc., 104,* 4943 (1982).

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Catalysis by Zn^{2+}-Metalloenzymes Proceeds via a Penta-Coordinate Metal–OH_2 Complex

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The active site of a variety of metalloenzymes contains a catalytically essential tetra-coordinate Zn^{2+} ion to which a water molecule is a ligand. It is generally assumed that the metal-bound water molecule has no role in catalysis. However, for both the native Zn^{2+}- and Co^{2+}-reconstituted forms of carboxypeptidase A (CPA) and liver alcohol dehydrogenase (LADH), we have identified metal ion dependent ionizations that govern steady state kinetic parameters and can be ascribed only to a metal-bound water molecule in catalytically competent reaction intermediates [1, 2]. To assign the coordination environment of the Co^{2+} ion in these reaction intermediates, we have identified spectroscopic parameters of the high-spin Co^{2+} ion that serve as a direct index of coordination number.

Our studies [3] show on the basis of a group theoretical treatment of spin–orbit coupling, together with relationships derived through second-order perturbation theory, that the magnitude of the zero-field splitting (ZFS) of the high-spin Co^{2+} ion is a direct monitor of crystal field symmetry and that it follows the order $ZFS_4 < ZFS_5 < ZFS_6$ where the subscript indicates the coordination number. Measurement of the ZFS in structurally defined coordination complexes confirms this relationship and indicates that the magnitude of $2|D|$ is 0–13 cm^{-1} for tetra-coordinate complexes, 20–60 cm^{-1} for penta-coordinate complexes, and 90–130 cm^{-1} for hexa-coordinate complexes. Application of this theory to measurements of the ZFS of CoCPA and CoLADH shows that the active site metal ion is tetra-coordinate in the free enzyme and is altered to a penta-coordinate complex in true reaction intermediates. In these intermediates the metal ion coordinates both the substrate and a water molecule simultaneously. Together with kinetic studies identifying ionizations of a metal-bound water molecule and showing that the reaction pathway is identical for Zn^{2+}- and Co^{2+}-enzymes, the results require that, upon formation of a penta-coordinate Zn^{2+}–OH_2 complex, breakdown of the acyl-enzyme (mixed anhydride) reaction intermediate of CPA occurs via metal–hydroxide nucleophilic attack on the carbonyl carbon of the scissile bond while in the physiologically active, ternary enzyme–NAD^+–alcohol complex of LADH a neutral metal-bound water molecule serves as the base catalyst for proton abstraction. The results of these studies show that the metal-bound water has an integral catalytic role in CPA and LADH. It is probable that the metal–OH_2 complex has similarly a functional role in other Zn^{2+}-metalloenzymes. (Supported by NIH grant GM 21900).

1 M. W. Makinen, L. C. Kuo, J. J. Dymowski and S. Jaffer, *J. Biol. Chem., 254,* 356 (1979).
2 (a) M. W. Makinen, W. Maret and M. B. Yim, *Proc. Natl. Acad. Sci. U.S.A., 80,* in press (1983); (b) W. Maret and M. W. Makinen, *J. Biol. Chem.,* submitted for publication.
3 (a) M. W. Makinen and M. B. Yim, *Proc. Natl. Acad. Sci. U.S.A., 78,* 6221 (1981); (b) L. C. Kuo and M. W. Makinen, *J. Am. Chem. Soc.,* to be submitted.