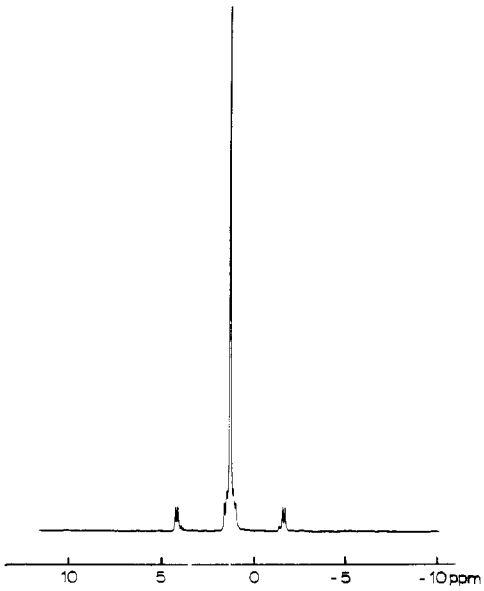


Figure 2. ^{31}P NMR spectrum (40.5 MHz, 25 °C, toluene-d_8 solvent) of $W_2(OCH_2$-t-$Bu)_6(PMe_3)_2$. Chemical shift δ 1.5 relative to external H_3PO_4 (85% aq); $^1J_{^{183}W-^{31}P}$ = 240 Hz, $^2J_{^{183}W-^{31}P}$ = 20 Hz, $^3J_{^{31}P-^{31}P}$ = 5.4 Hz.

chelated amine adduct $W_2(OEt)_6(Me(H)NCH_2CH_2N(H)Me)$ is isolated as a red-orange crystalline compound.[5] The molecular structure deduced from an X-ray study[10] shows that the chelated amine straddles the W≡W bond so that the molecule has virtual C_2 symmetry and the two ends of the molecule are almost perfectly staggered (see Figure 1). In solution, the diamine is tightly bound and not easily replaced by other donor ligands. The ^1H NMR spectrum in toluene-d_8 shows three types of OEt ligands: each ethyl group appears as an ABX_3 spectrum. The ^1H NMR signals associated with the chelated amine also indicate a rigid structure. Evidently, enantiomerization is not rapid on the NMR time scale, and the diamine, which is firmly coordinated, denies EtOH access to the $(W{\equiv}W)^{6+}$ center, which is necessary for oxidative addition.

(3) When hydrocarbon solutions of $W_2(NMe_2)_6$ are allowed to react with alcohols in the presence of PMe_3 (≥2 equiv) at room temperature, the phosphine adducts $W_2(OR)_6(PMe_3)_2$ are formed (R = i-Pr, CH_2-t-Bu, and Et).[5] The molecular structure of the neopentoxide reveals two roughly square-planar WO_3P units joined by a W≡W bond of distance 2.362 (2) Å. The conformation is staggered with the P–W–W–P torsion angle of 114°. In solution, rotation about the W≡W bond is rapid on the ^1H NMR time scale and the PMe_3 ligands are tightly bound as evidenced by $J_{^{183}W-^{31}P}$ and $J_{^{31}P-^{31}P}$ couplings (see Figure 2).

(4) Addition of pivalic acid, t-BuCOOH, which is a much stronger acid than either i-PrOH or EtOH and is known to oxidize tungsten(0) to the 4+ oxidation state in reactions involving $W(CO)_6$,[12] reacts smoothly with $W_2(O$-t-$Bu)_6$ in hydrocarbon solvents to give $W_2(O_2C$-t-$Bu)_6$, which may be isolated as a yellow crystalline solid.[5] This compound has not been structurally characterized by an X-ray study because its spectroscopic properties indicate a structure analogous to that found for $W_2(O_2CNMe_2)_6$.[13] In the mass spectrometer, the ion of highest mass corresponds to $W_2(O_2C$-t-$Bu)_6^+$ and many daughter W_2-containing ions are observed. The ^1H NMR spectrum shows three types of methyl signals in the integral ratio 1:1:1.

In the reaction between $W_2(O$-t-$Bu)_6$ and t-BuCOOH, each replacement of an alkoxy ligand by a carboxylate group allows an increase in coordination number of one if the carboxylate group acts as a bidentate ligand. The intermediate $W_2(O$-t-$Bu)_4(O_2C$-t-$Bu)_2$ in the formation of $W_2(O_2C$-t-$Bu)_6$ probably has a structure akin to that found for $M_2(O$-t-$Bu)_4(O_2CO$-t-$Bu)_2$[14] compounds and $Mo_2(O$-t-$Bu)_4(O_2CPh)_2$.[15] In the reactions described in 1–3 above, it is evident that monodentate donors, L, or bidentate donors, L–L, can coordinate to the dinuclear center to give $W_2(OR)_6L_2$ and $W_2(OR)_6(L$–$L)$ compounds, and depending upon the relative binding abilities of the ligand, this may either serve to suppress (L = $HNMe_2$, R = i-Pr) or completely block (L = PMe_3 or L–L = $Me(H)NCH_2CH_2N(H)Me$) the oxidative addition reaction that takes $(W{\equiv}W)^{6+}$ to $(W{=}W)^{8+}$. The pathway leading to oxidative addition finds a parallel with that noted recently by Nubel and Brown wherein 1,2-$Re_2(CO)_8L_2$ compounds (L = H_2O or pyridine) give $(\mu$-$H)Re_2(CO)_8(\mu$-X) compounds (X = OH or C_5H_4N).[16]

Further studies are in progress.[17]

Registry No. $W_2(NMe_2)_6$, 54935-70-5; i-PrOH, 67-63-0; EtOH, 64-17-5; $NHMe_2$, 124-40-3; $W_2(OPr$-$i)_6(HNMe_2)_2$, 84028-40-0; PMe_3, 594-09-2; $W_2(OPr$-$i)_6(PMe_3)_2$, 84028-42-2; $W_2(OEt)_6(PMe_3)_2$, 57125-20-9; t-BuCOOH, 75-98-9; $W_2(O_2CBu$-$t)_6$, 84028-44-4; W, 7440-33-7.

Supplementary Material Available: Table of atomic coordinates and thermal parameters for $W_2(OEt)_6(MeN(H)CH_2CH_2N$-(H)Me) (3 pages.) Ordering information is given on any current masthead page. Complete Molecular Structure Center reports are available, in microfiche form only, from the Indiana University Library.

(10) Crystal data for $W_2(OEt)_6(MeN(H)CH_2CH_2N(H)Me)$ at −61 °C: space group $P2_1/n$, a = 19.050 (14) Å, b = 15.627 (9) Å, c = 8.793 (4) Å, β = 104.27 (4)°, Z = 4, d_{calcd} = 1.901 g cm^{-3}. Of the 2667 reflections collected with use of Mo Kα radiation, $6° \le 2\theta \le 40°$, the 1739 reflections having $F > 2.33\ F$ were used in the full-matrix refinement. Due to the excessive thermal parameters, the hydrogen atoms were included as fixed idealized atoms, and no attempt was made to refine them. The final residuals are R_F 0.063 and R_{wF} = 0.056.

(11) Indiana University, MSC Report No. 82009.

(12) Bino, A.; Cotton, F. A.; Dori, Z.; Koch, S.; Kuppers, H.; Millar, M. *Inorg. Chem.* **1978**, *17*, 3245.

(13) Chisholm, M. H.; Cotton, F. A.; Extine, M. W.; Stults, B. R. *Inorg. Chem.* **1977**, *16*, 603.

(14) Chisholm, M. H.; Cotton, F. A.; Extine, M. W.; Reichert, W. W. *J. Am. Chem. Soc.* **1978**, *100*, 1727.

(15) Chisholm, M. H.; Huffman, J. C.; Kirkpatrick, C. C., submitted for publication in *Inorg. Chem.*

(16) Nubel, P. O.; Brown, T. L. *J. Am. Chem. Soc.* **1982**, *104*, 4955.

(17) We thank the Department of Energy, Chemical Science Division, the Wrubel Computing Center, and the taxpayers of the state of Indiana for support.

Effect of Nickel(II) and Cobalt(III) and Other Metal Ions on the Racemization of Free and Bound L-Alanine[1]

Grant Gill Smith,* Awni Khatib, and G. Sudhakar Reddy

Department of Chemistry and Biochemistry
Utah State University, Logan, Utah 84322

Received April 12, 1982
Revised Manuscript Received November 24, 1982

The catalytic effects of transition-metal ions on the reactivity of amino acids and their derivatives are well-known.[2] Increased reactivity occurs when metal ions are present with amino acids in base-catalyzed aldol-type condensations,[3] isotope exchange,[4]

(1) Presented in part at the Fifth IUPAC Conference on Physical Organic Chemistry in Santa Cruz, CA, Aug 17–22, 1980, and in part at the 183rd National Meeting of the American Chemical Society Las Vegas, NV, March 28, 1982. Abstracted from an M.S. Thesis submitted by Awni Khatib to the Graduate School at Utah State University, 1982.

(2) For reviews see: (a) R. D. Gillard, *Inorg. Chim. Acta Rev.*, **1**, 69, (1967); (b) A. E. Martell, In "Metal Ions in Biological Systems", H. Sigel, Ed., Marcel Dekker, New York, 1973, Vol. 2, pp 207–293; (c) A. Pasini and L. Casella, *J. Inorg. Nucl. Chem.*, **36**, 2133, (1974); (d) D. A. Phipps, *J. Mol. Catal.*, **5**, 81 (1979); (e) R. W. Hay and D. R. Williams, in "Amino acids, Peptides and Proteins", Chemical Society, London, 1981, Vol. 11, pp 475–503.

(3) (a) S. Akabori, T. T. Otani, R. Marshall, M. Winitz, and J. P. Greenstein, *Arch. Biochem. Biophys.*, **83**, 1 (1959); (b) R. D. Gillard and P. M. Harrison, *J. Chem. Soc. A*, 1657 (1967); (c) J. P. Aune, P. Maldonado, G. Larcheres, and M. Pierrot, *J. Chem. Soc., Chem. Commun.*, 1351 (1970); (d) J. R. Brush, R. J. Magee, M. J. O'Connor, S. B. Teo, R. J. Geue, and M. R. Snow, *J. Am. Chem. Soc.*, **95**, 2034 (1973); (e) D. A. Phipps, *Inorg. Chim. Acta*, **27**, L103 (1978); (f) L. Casella, A. Pasini, R. Ugo, and M. Visca, *J. Chem. Soc., Dalton Trans.*, 1655 (1980); (g) M. G.-Weller and W. Beck, *Inorg. Chim. Acta*, **57**, 107 (1982).

0002-7863/83/1505-0293$01.50/0

Table I. Reaction Rate Constants for the Racemization of Free and Complexed L-Alanine[a]

metal ion	structure of complex	L-Ala, 10^7k, s^{-1} free	L-Ala, 10^7k, s^{-1} complex	racemization conditions pH	racemization conditions temp, °C
Ni(II)	$[Ni(L\text{-}ala)_2]\cdot 2H_2O$	1.78	0.96, 1.06[b]	8.2	119.6 ± 0.2
Cu(II)	$[Cu(L\text{-}ala)_2]$	1.64	4.21[c]	8.0	120.0 ± 0.2
Cr(III)	$[Cr(L\text{-}ala)_3]$	1.74	4.63	8.0	120.0 ± 0.2
Co(III)[d]	$[Co(L\text{-}ala)_3]$	0.15	7.65	7.0	100.0 ± 0.2
Co(III)[e]	$[Co(L\text{-}ala)_3]$	0.44	55.5	7.6	106.0 ± 0.2
Pd(II)	$[Pd(L\text{-}ala)_2]$	1.71	59.6	8.0	120.0 ± 0.2
Pt(II)	$[Pt(L\text{-}ala)_2]$	1.82	162	8.0	120.0 ± 0.2

[a] For preparation, see ref 14. All the complexes gave a satisfactory analysis data. The kinetics were obtained in triplicate, and the correlation coefficient in each pseudo-first-order plot was 0.993. [b] In excess, L-ala. [c] The racemization of the L-alanine in the Ni(II) complex in excess alanine did not follow linear first-order kinetics for this reversible reaction. A logical explanation is given in ref 11. [d] Mixture of cis and trans isomers.[14] [e] Trans isomer.[14]

racemization,[5] the formation and hydrolysis of esters and peptides,[6] and Schiff base formation.[7] It was proposed that metal–ligand bonding[8] and the charge on the complex[9] are responsible for the increase.

Stadtherr and Angelici[10] studied the effect of various metals on the base-catalyzed racemization and deuterium exchange at the methine hydrogen of *N,N*-bis(carboxymethyl)-D-phenylglycine and reported the effect to be 5000 times faster than the uncomplexed amino acid derivative. A similar effect was reported by Gillard and O'Brien[11] for L-alanine in the presence of Cu(II). Recently, Norman and Phipps[8] carried out a systematic investigation of the effect of various metal ions on ethylenediaminetetraacetic acid (EDTA) by ^1H NMR. Among the 30 metals studied, only 7, all transition metals, enhanced the activation in the methylene groups of the ligand. Ni(II) ion exhibited a true catalytic activity, inducing complete exchange even with a large excess of the ligand. An enhanced activity of amino acids in monodipeptide complexes, $[CoL_3(AA_1AA_2)]^+$ (where L = NH_3, AA_1AA_2 = dipeptide), is also known.[12]

Contrary to the above published results, we report that Ni(II) when complexed to L-alanine and Co(III) when complexed to Gly-Ala or Ala-Gly retard the rate of racemization of the L-alanine. The earlier methods used to measure the racemization rates of metal complexes of amino acids largely involved the techniques of optical rotation, optical rotatory dispersion (ORD), and/or circular dichroism (CD). For stable complexes, these methods do not specify whether changes occur at the central metal ion or at the chiral center of the ligand. Further, their accuracy largely depends upon concentration, which often varies due to precipitation, ligand exchange, or other chemical processes.

We employed a more accurate method involving the resolution of enantiomers by capillary GC. This reliable technique[13] has provided an opportunity to investigate the kinetics of racemization of the free amino acids, of amino acids bound in peptides, and of amino acids complexed to Ni(II) and Co(III) and also prompted reinvestigations of the kinetics of alanine complexed to other metals, which have been previously reported by using other analytical techniques.

Table II. Reaction Rate Constants for the Racemization of Free and Complexed Dipeptides

dipeptide	free 10^7k, s^{-1}	complex[a] (Co(III) ion)	racemization conditions pH	racemization conditions temp, °C
Gly-L-Ala	71.4	1.71	8.5	119.8 ± 0.2
L-Ala-Gly	52.6	3.33	8.5	119.8 ± 0.2

[a] Mixture of *R* and *S* isomers.[14]

The racemization experiments were carried out on 0.05 M solutions in sealed Pyrex tubes at carefully controlled pH and temperature. The metal complexes[14] appeared stable throughout the racemization experiment as measured by absorption spectroscopy.[15] After racemization, the amino acids or dipeptides were freed from the complexes either by precipitating the metal ions as their sulfides or by adding a few drops of 0.1 M EDTA solution. The dipeptides were hydrolyzed to the corresponding free amino acids. The D- and L-alanine were quantified by GC with a chiral phase. First-order rate constants for the racemization reaction were calculated by plotting $0.5 \ln [(1 + D/L)/(1 - D/L)]$ vs. time, where *D* and *L* are the concentrations of D- and L-alanine, respectively.

The rate constants for the racemization are shown in Table I. The free alanine racemized approximately 1.5–2 times faster than the alanine complexed to nickel, while the other metal ions enhanced the rate of racemization appreciably. These results point to the unique character of Ni(II) on amino acid racemization. We have observed the retarding affect by Ni(II) ion also on Val, Ile, and Leu. Further, a detailed study on the pH profile on the free and Ni(II)-complexed L-alanine[16] clearly indicated that above pH 4 the free amino acid racemized faster than the complex. At lower pH values (e.g., 3) the amino acid in the complex gets protonated and becomes monodentate. The retarding effect of Ni(II) ion is very significant and has importance in the field of geochronology and biogeochemistry. Pt(II) and Co(III) ions, of all the metals studied, showed the greatest enhancing effect on racemization. This pronounced effect was reversed when a dipeptide containing the L-alanine moiety complexed to Co(III) was racemized. Reaction rate constants for the racemization of free and Co(III)-complexed dipeptides are given in Table II. The

(4) (a) D. H. Williams and D. H. Busch, *J. Am. Chem. Soc.*, **87**, 4644 (1965); (b) D. A. Buckingham, L. G. Marzilli, and A. M. Sargeson, *ibid.*, **89**, 5133 (1967); (c) J. D. Sudmeier and G. Occupati, *Inorg. Chem.*, **7**, 2524 (1968); (d) R. D. Gillard and D. A. Phipps, *J. Chem. Soc., Chem. Commun.*, 800 (1970); (e) R. D. Gillard, S. H. Laurie, D. C. Price, D. A. Phipps, and C. F. Weick, *J. Chem. Soc., Dalton Trans.*, 1385 (1974); (f) P. R. Norman and D. A. Phipps, *Inorg. Chim. Acta*, **24**, L35 (1977).

(5) (a) B. Halpern, A. M. Sargeson, and R. K. Turnbull, *J. Am. Chem. Soc.*, **88**, 4630 (1966); (b) D. A. Buckingham, L. G. Marzilli, and A. M. Sargeson, *ibid.*, **89**, 3428 (1967); (c) M. M. Ghandehari, T. N. Anderson, R. D. Boone, and H. Eyring, *ibid.*, **92**, 6466 (1970); (d) I. J. Legg, D. R. Willett, E. R. Caputo, and E. W. Keyes, *Ibid.*, **98**, 6939 (1976); (e) A. Dempsey and D. A. Phipps, *Inorg. Chim. Acta*, **36**, L425 (1979); (f) G. G. Smith and R. C. Evans, in "Biogeochemistry of Amino Acids", P. E. Hare, T. C. Hoering, and K. King, Eds., Wiley Interscience, New York, 1980, pp 257–282; (g) R. Liardon and R. Jost, *Int. J. Pept. Protein Res.*, **18**, 500 (1981); (h) L. Casella, *Inorg. Chim. Acta*, **55**, L9 (1981); (i) A. Miyanaga, U. Sakaguchi, Y. Morimoto, Y. Kushi, and H. Yoneda, *Inorg. Chem.*, **21**, 1387 (1982).

(6) (a) H. Kroll, *J. Am. Chem. Soc.*, **74**, 2036 (1952); (b) W. A. Connor, M. M. Jones, and D. L. Tuleen, *Inorg. Chem.*, **4**, 1129 (1965); (c) J. E. Hix, and M. M. Jones, *ibid.*, **5**, 1863 (1966); (d) R. W. Hay and L. J. Porter, *J. Chem. Soc. A*, 127 (1969); (e) R. W. Hay and C. R. Clark, *J. Chem. Soc., Dalton Trans.*, 1993 (1977).

(7) (a) J. Olivard, D. E. Metzler, and E. E. Snell, *J. Biol. Chem.*, **199**, 669 (1952); (b) K. Toi, Y. Izumi, and S. Akabori, *Bull. Chem. Soc. Jpn.*, **36**, 829 (1963); (c) K. Hirota, K. Miyamota, and Y. Izumi, *ibid.*, **40**, 178 (1967); (d) M. Ando and S. Emoto, *ibid.*, **48**, 1655 (1975); (e) R. D. Gillard, P. O'Brien, P. R. Norman, and D. A. Phipps, *J. Chem. Soc., Dalton Trans.*, 1988 (1977).

(8) P. R. Norman and D. A. Phipps, *Inorg. Chim. Acta*, **17**, L19 (1976).

(9) J. C. Dabrowiak and D. W. Cooke, *Inorg. Chem.*, **14**, 1305 (1975).

(10) L. G. Stadtherr and R. J. Angelici, *Inorg. Chem.*, **14**, 925 (1975).

(11) R. D. Gillard and P. O'Brien, *J. Chem. Soc., Dalton Trans.*, 1444 (1978).

(12) I. G. Browning, R. D. Gillard, J. R. Lyons, P. R. Mitchell, and D. A. Phipps, *J. Chem. Soc., Dalton Trans.*, 1815 (1972).

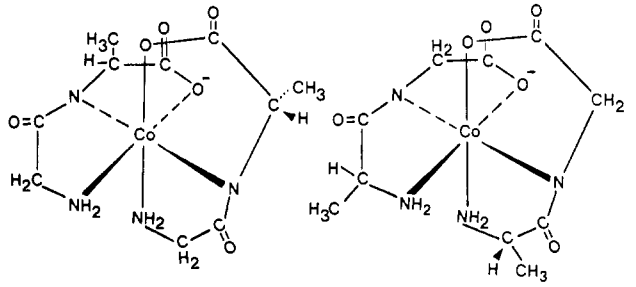
(13) G. G. Smith and D. M. Wonnacott, *Anal. Biochem.*, **109**, 414 (1980).

(14) For the preparation of complexes known procedures were adopted with some modifications. $Ni(L\text{-}Ala)_2\,2H_2O$; K. Nakamoto, Y. Morimoto, and A. E. Martell, *J. Am. Chem. Soc.*, **83**, 4528 (1961). $Cu(L\text{-}Ala)_2$: ref 7e. $Co(L\text{-}Ala)_3$: R. G. Denning and T. S. Piper, *Inorg. Chem.*, **5**, 1056 (1966). $Cr(L\text{-}Ala)_3$: S. S. Minor, G. Witte, and G. W. Everett, Jr., *ibid.*, **15**, 2052 (1976). $Pd(L\text{-}Ala)_2$ and $Pt(L\text{-}Ala)_2$: J. F. Jackovitz, J. A. Durkin, and J. L. Walter, *Spectrochim. Acta*, **23A**, 67 (1967). $KCo(Gly\text{-}L\text{-}Ala)_2$ and $KCo(L\text{-}Ala\text{-}Gly)_2$: T. Yasui, H. Kawaguchi, and T. Ama, *Bull. Chem. Soc. Jpn.*, **54**, 2918 (1981).

(15) A. Khatib, M.S. Thesis, Utah State University, Logan, UT, 1981.

(16) The reaction rate constants ($k \times 10^8$ s^{-1}) for racemization of free and Ni(II)-complexed L-alanine at 121 °C and various pH values. pH 2: free 2.88, complex 4.45. pH 3: free 8.82, complex 11.3. pH 7: free 17.4, complex 10.6. pH 10: free 37.2, complex 18.8.

dipeptides racemized approximately 15–40 times faster than the corresponding complexed dipeptides. The L-alanine is known to racemize faster at the COOH terminal position than the NH_2 terminal position in the dipeptides.[17] But from Table II it is obvious that complexed L-alanine at the NH_2 terminal position racemized 2 times faster than the corresponding COOH terminal analog. This is explained due to the fact that the methine carbon of L-alanine in $[Co(Gly\text{-}Ala)_2]^-$ is sandwiched between two negative charges (deprotonated amide nitrogen and carboxyl anion of dipeptide), as shown in A. This positioning would appreciably



A B

retard the formation of carbanion required for racemization. On the other hand, $[Co(Ala\text{-}Gly)_2]^-$ has only one negative amide nitrogen (B), resulting in a relatively high racemization rate.

A rationale for the surprising results observed in the case of $[Ni(L\text{-}Ala)_2]$ and $K[Co(dipeptide)_2]$ complexes may be drawn from the following arguments. The mechanism of racemization in free and in metal-complexed amino acids involves the abstraction of the α-proton by base, forming a carbanion.[18] Norman and Phipps[8] in explaining metal ion catalysis in the metal–EDTA complexes assumed that the electron-withdrawing power of the central metal ion played an important role in defining the overall reactivity of the complex. The effect of the metal ion has been correlated with the degree of covalency in the metal–ligand bond. The metal complexes studied by these workers exhibited significant covalent character in their metal to ligand bonds, as substantiated by IR.[19] In the present study, the IR spectra of $[Ni(L\text{-}Ala)_2]\cdot 2H_2O$ and Co(III)–dipeptide complexes were measured to determine the nature of the bonding between the carboxyl group of the ligand and the metal ion. The symmetric COO^- stretch at 1415 cm^{-1} and the asymmetric stretch at 1590 cm^{-1} strongly indicated that in these complexes the M–COO^- bond is primarily ionic, not covalent. The complexation of amino acids to metal ions generally reduces the negative charge on the carboxyl group of the ligand, which results in the increased rates of racemization. In the case of nickel complex and cobalt–dipeptide complexes, however, the bonding may be more ionic than covalent. It may be assumed that the methine carbon is oriented in a way such that it cannot attain coplanarity, a situation required for racemization to occur.[18] Additional investigations are required to establish the generality and an understanding of the retarding effect observed in case of nickel and cobalt complexes.

Acknowledgment. We express our sincere thanks to the National Aeronautics and Space Administration, Planetary Biology Program NSG-7038, and Utah State University for their generous support.

Registry No. L-Alanine, 56-41-7; Ni(II), 14701-22-5; Co(III), 22541-63-5; Cu(II), 15158-11-9; Cr(III), 16065-83-1; Pd(II), 16065-88-6; Pt(II), 22542-10-5; $[Ni(L\text{-}ala)_2]$, 14040-30-3; $[Cu(L\text{-}ala)_2]$, 14263-10-6; $[Cr(L\text{-}ala)_3]$, 16483-21-9; *cis*-$[Co(L\text{-}ala)_3]$, 55448-50-5; *trans*-$[Co(L\text{-}ala)_3]$, 55328-27-3; $[Pd(L\text{-}ala)_2]$, 15276-20-7; $[Pt(L\text{-}ala)_2]$, 74868-20-5; gly-L-ala, 3695-73-6; L-ala-gly, 687-69-4.

(17) G. G. Smith and B. S. de Sol, *Science (Washington, D.C.)* **207**, 765 (1980).

(18) (a) R. D. Gillard and M. S. Laurie, in "Amino Acids, Peptides and Proteins", T. G. Young, Ed., Chemical Society, London, 1969, Vol. 1, pp 262–293; (b) P. R. Norman and D. A. Phipps, *Inorg. Chim. Acta*, **28**, L161 (1978); (c) J. L. Bada and E. H. Man, *Earth Sci. Rev.*, **16**, 21 (1980).

(19) D. T. Sawyer and J. M. McKinnie, *J. Am. Chem. Soc.*, **82**, 4191 (1960).

The D_2O Effect on Catalysis by Triose Phosphate Isomerase Requires Isotope Exchange on the Enzyme

Irwin A. Rose* and Radha Iyengar[1]

Institute for Cancer Research
Fox Chase Cancer Center
Philadelphia, Pennsylvania 19111

Received August 19, 1982

When at equilibrium with its substrates, triose phosphate isomerase occurs in the three liganded forms with dihydroxyacetone phosphate (DHAP), D-glyceraldehyde 3-phosphate (G3P) and the enediol phosphate in the ratio 0.8, 1.0, and 0.1, respectively.[2] When an equilbrium solution having a great excess of enzyme, so that free triose phosphates are negligible, is diluted with a compound that forms a strong complex with free enzyme, the subsequent initial appearance of DHAP and G3P will depend on the relative magnitude of the first-order rate constants of steps shown in Scheme I.

Studies of this kind in which the initial equilibration, the pulse, was made in tritiated water and the large dilution in the presence of competitive inhibitor, the chase, was done in normal water allowed us to estimate the sources of the free DHAP and G3P because tritium incorporated in each bound substrate is almost completely exchanged with H_2O at the E·X stage in the chase. Thus only direct dissociation of free ligand would lead to tritiated free DHAP and G3P. The partition of each of the liganded forms to free species was in the direction required by Scheme I, i.e., the ratio of DHAP to G3P formed was greatest for E·DHAP followed by E·enediol–P (E–X) and least for E·G3P: >10, 3, and 1.4, respectively.[3] The partition of E·G3P was of special interest for two reasons: (1) The free G3P derived from E·[2-^{3}H]G3P after mixing with the competitive inhibitor phosphoglycollate in normal water retained most of its tritium. This contrasts with the observation that in the formation of G3P from DHAP in TOH, tritium is incorporated with almost no discrimination,[4] implying that release of G3P from E·G3P in the steady state is slower than its enolization. The contradiction arises because one expects a significant discrimination against tritium in the enolization step and may be resolved if one assumes that E·G3P must undergo a rate-limiting conformational change to a thermodynamically less stable species prior to its conversion to the enediol–P intermediate.[3] (2) When, prior to the experiment, the enzyme–substrate equilibrium mixture was established in D_2O instead of H_2O, the partition of bound G3P to free G3P vs. free DHAP increased 2.5-fold.[3] Because of the absence of discrimination against [2-^{3}H]G3P in the same experiment, the D_2O effect could not be due to the presence of ^{2}H at C-2 of G3P and seemed best explained if the proposed conformational change step was appreciably slower in D_2O. This could be a "medium effect", in which case it would be observed immediately after shifting E·G3P from an H_2O to a D_2O medium. On the other hand, were the onset of the isotope effect measureably slow it could be attributed to an H/D exchange of a functional group on the enzyme.

Preliminary experiments showed that the full D_2O effect on the partition of E·G3P was seen with enzyme after as little as 10 s in D_2O. Most of the backbone amide hydrogens of the protein would not have exchanged within this time.[5] With the technique of partition analyses[3] detailed in Figure 1 in which exposure of the E·ligand complexes to D_2O was shortened to 10 ms at 4 °C, the partition of E·G3P, given by the zero time extrapolation, was less than midway between the value obtained with a fully H_2O medium and the value found with enzyme that had been kept in

(1) Present address: Institute for Enzyme Research, University of Wisconson, Madison, WI 53706.

(2) Iyengar, R.; Rose, I. A. *Biochemistry* **1981**, *20*, 1223–1229.

(3) Rose, I. A.; Iyengar, R. *Biochemistry* **1982**, *21*, 1591–1597.

(4) Maister, S. G.; Pett, C. P.; Albery, W. J.; Knowles, J. R. *Biochemistry* **1976**, *15*, 5607–5612.

(5) Browne, C. A.; Waley, S. G. *Biochem. J.* **1965**, *141*, 4667. Wang, M.-S.; Gandour, R. D.; Rodgers, J.; Haslamy, J. L.; Schowen, R. L. *Bioorg. Chem.* **1975**, *4*, 392–406.

0002-7863/83/1505-0295$01.50/0