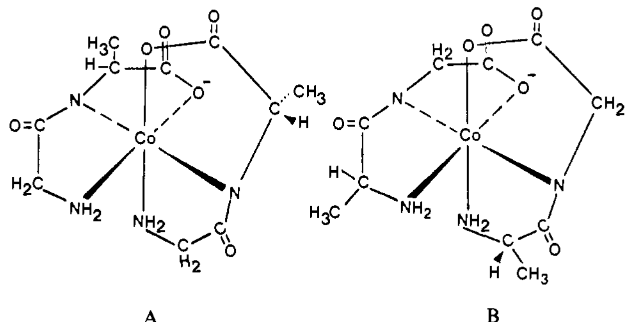
dipeptides racemized approximately 15-40 times faster than the corresponding complexed dipeptides. The L-alanine is known to racemize faster at the COOH terminal position than the NH_2 terminal position in the dipeptides.[17] But from Table II it is obvious that complexed L-alanine at the NH_2 terminal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



retard the formation of carbanion required for racemization. On the other hand, $[Co(Ala-Gly)_2]^-$ 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 $[Ni(L-Ala)_2]$ 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 $[Ni(L-Ala)_2]\cdot 2H_2O$ 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; $[Ni(L-ala)_2]$, 14040-30-3; $[Cu(L-ala)_2]$, 14263-10-6; $[Cr(L-ala)_3]$, 16483-21-9; *cis*-$[Co(L-ala)_3]$, 55448-50-5; *trans*-$[Co(L-ala)_3]$, 55328-27-3; $[Pd(L-ala)_2]$, 15276-20-7; $[Pt(L-ala)_2]$, 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.

 © 1983 American Chemical Society

Scheme I

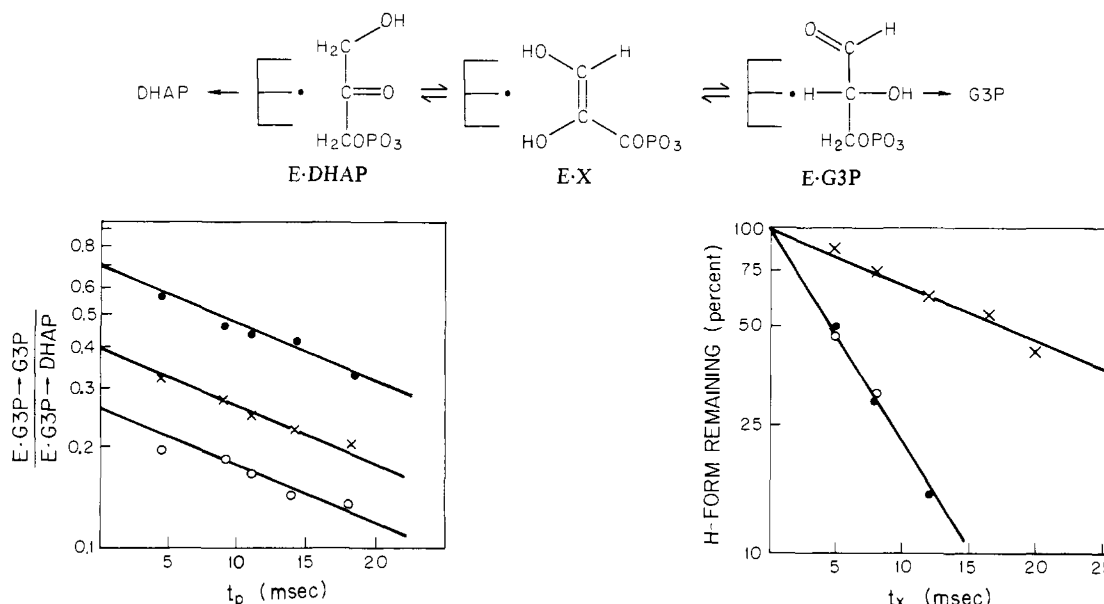


Figure 1. Partition of E·G3P exposed to D_2O for 10 ms or equilibrated with D_2O. [^{32}P]DHAP (4.8 nmol, 6974 cpm/nmol) and triose phosphate isomerase (82 nmol) in sodium cacodylate (0.02 M, pH 6.5, pD 6.9) in H_2O (○ and ×) or D_2O (●, 80%) in 50 μL was injected into a 4 grid mixer (Update Systems 1000) where it mixed with 200 μL of sodium cacodylate (0.02 M, pH 6.5, pD 6.9) in H_2O (○), 80% D_2O (●), or 100% D_2O (×) delivered by a second syringe. After 10 ms the effluent of the mixer was joined with 50 μL of the competitive inhibitor phosphoglycollate (0.5 M, pH 6.5) in a second mixer. After the aging time noted, t_p, the reaction was quenched with CCl_3COOH and analyzed for ^{32}P in DHAP and G3P. With time the earliest products ratio changes toward the eventual solution equilibrium, DHAP/G3P = 22, by action of the concentrated but highly inhibited enzyme. This technique and the methods used to analyze DHAP and G3P for isotope have been described.[2,3] When CCl_3CO_2H was used instead of phosphoglycollate, the G3P, all of it enzyme bound, contained 53% of the total ^{32}P. Detailed kinetic studies have shown[3] that the initial E·G3P is the only significant source of the G3P in the partition products. Therefore, if at 4.5 ms only 10% of the ^{32}P is present as G3P, the partition ratio for E·G3P would be 10/43 in favor of DHAP.

Figure 2. Rate of H/D exchange of TIM. A 50-μL pulse solution containing [^{32}P]DHAP (4.9 nmol, 4878 cpm/nmol) and triose phosphate isomerase (79 nmol) in sodium cacodylate (0.02 M, pH 6.5, ×) or triethanolamine (0.1 M (●) or 0.02 M (○) pH 8.5) was mixed with 4 volumes of sodium cacodylate (0.02 M, pD 6.9, 100% D_2O) or triethanolamine (0.1 M or 0.02 M, pD 8.9, 100% D_2O), respectively. Following the exposure to D_2O for 4.5–20 ms, t_x, the effluent from the mixer was joined with 4 volumes of phosphoglycollate (0.5 M in H_2O, pH 6.5 final). An interval of 8 ms was allowed for the partition to occur in all cases before quenching with CCl_3COOH (0.2 M final concentration). Partition ratios were also determined with enzyme equilibrated in H_2O or in 80% D_2O. The extent to which an observed partition has progressed between these limits is plotted as the percent of H form remaining vs. time allowed for exchange.

D_2O for several minutes. The partition of E·G3P itself was complete much before 4.5 ms. By this time the G3P had fallen from 100% as E·G3P to a value no greater than expected from the slow phase approach to solution equilibrium represented by the first-order disappearance of G3P at the rate of the inhibited enzyme. It has been concluded[3] that conversion of bound G3P to bound enediol–P is 3.2-fold faster than the dissociation of G3P, which as just noted is complete before the earliest sampling point. Therefore, catalysis would have equilibrated deuterium into the C-2 position of bound G3P within a millisecond so that its contribution to the D_2O effect could not be greater than that seen at 10 ms.

The rate at which the system acquires the 2.5-fold isotope effect was measured in more detail by varying the interval t_x, of exposure to 80% D_2O, from 5 to 20 ms. The interval spent in the inhibitor solution was held constant at 8 ms.[6] The conversion to the "deuterated state" follows first-order kinetics as seen in Figure 2 at pH 6.5 and 8.5. From these data it follows that (1) the exchange rate given by the slope is significantly increased by pH (pH 6.5, $k_x = 39.5$ s^{-1}; pH 8.5, $k_x = 153$ s^{-1}); (2) the rate at pH 8.5 is not buffer catalyzed; (3) the intercepts extrapolate to 100% H species of enzyme at $t_x = 0$, indicating that the apparent pK_a of the group(s) involved is >8.5. Otherwise, the unprotonated form(s) of the enzyme in H_2O at pH 8.5 would become deuterated immediately upon adjustment to pH 6.5 in D_2O and give a lower extrapolated value; (4) not being biphasic also implies that deuterium at C-2 of bound G3P has no effect on the partition ratio.

The observation in Figure 2, pH 6.5, that the D_2O effect develops with a half-time of 19 ms after solvent change and the difference at pH 8.5 seem to rule out effects due to changes in bulk solvent properties. The D_2O effect might reflect subtle effects on the pK's of functional groups contributing to a modified constellation of charges on the enzyme. This has already been ruled out by the finding that the same partition ratio is obtained for the mixture of substrate and enzyme in the pH range 5.5–8.5 as long as the partition step is done at a constant pH.[3]

The results therefore suggest that H/D exchange on the enzyme, occurring more slowly than catalysis, is required for the isotope effect to be seen. Because the isotope effect implies that a proton transfer within the enzyme occurs at some step of the reaction and because exchange is much slower than catalysis, the transfer must be reversed without exchange with the medium during product formation. It is proposed that a conformational change of E·G3P prior to the enolization is the step requiring such a proton transfer.[6]

The present work shows the value of measuring the rate of onset or loss of a D_2O effect to distinguish between enzyme-bound protons and protons of the medium as the cause of the effect. This alternative was examined in the important studies of Bender[7] on deacylation of chymotrypsin and papain in which the isotope effect in D_2O was lost within 10 s of dilution into H_2O and therefore was concluded to result from D_2O rather than from a deuterium-substituted enzyme. However, 10 s is a long interval for all but the hydrogen-bonded backbone hydrogens. With the usual

(6) Transfer of hydrogen in an internal ion pair has been proposed to explain a 4.5-fold D_2O effect on a conformation change seen with ribonuclease: French, T. C.; Hammes, G. G. *J. Am. Chem. Soc.* **1965**, *87*, 4667. Wang, M.-S.; Gandour, R. D.; Rodgers, J.; Haslamy, J. L.; Schowen, R. L. *Bioorg. Chem.* **1975**, *4*, 392–406.

(7) Bender, M. L.; Hamilton, G. A. *J. Am. Chem. Soc.* **1962**, *84*, 2570–2576. Brubacher, L. J.; Bender, M. L. *Ibid.* **1966**, *88*, 5871–5880.

rapid mixing device this interval can be shortened by at least 10^3, putting it in the range of many group ionizations.

Acknowledgment. This work was supported by United States Public Health Service Grants GM-20940 to I.A.R. and CA-06927 and RR-05539 to the Institute and also supported by an appropriation from the Commonwealth of Pennsylvania.

Registry No. Triose phosphate isomerase, 9023-78-3; D_2O, 7789-20-0.

Crystal Structure of a New Type of Two-Dimensional Organic Metal, $(C_{10}H_8S_8)_2ClO_4(C_2H_3Cl_3)_{0.5}$

Hayao Kobayashi*

Department of Chemistry, Faculty of Science
Toho University, Funabashi, Chiba 274, Japan

Akiko Kobayashi and Yukiyoshi Sasaki

Department of Chemistry, Faculty of Science
The University of Tokyo, Hongo, Tokyo 113, Japan

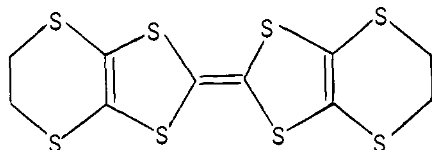
Gunzi Saito, Toshiaki Enoki, and Hiroo Inokuchi

Institute for Molecular Science, Okazaki 444, Japan
Received July 26, 1982

All organic metals known to date are composed of planar donor and/or acceptor molecules with π-conjugated systems. Most of them are regarded as quasi-one-dimensional compounds. The molecules are stacked face to face to form segregated columns. The one-dimensional properties are considered to arise from the intermolecular π–π interaction along the columns.[1-3] Since the CDW and SDW instabilities inherent in one-dimensional metals tend to break the metallic state,[4] interchain interactions must be introduced in order to stabilize this state. However, if the interchain interaction is sufficiently strong, the column structure will be unfavorable and the conduction pathway will become more complicated.

In this paper, we report on the structure of a new type of organic metal where the planar molecules are not stacked face to face but are arranged side by side to form a two-dimensional system. The two-dimensionality of the structure originates from short intermolecular contacts between the sulfur atoms of the donor molecules.

Saito et al. have grown the crystals of the perchlorate salt of bis(ethylenedithiolo)tetrathiafulvalene (abbreviated as BEDT-TTF)[5,6] by the electrochemical oxidation method. The single-



BEDT–TTF

crystal resistivity along the elongated direction ($\rho_\parallel$), which is close to the *a* axis in the (010) plane, decreases monotonically from the room-temperature value (3.8×10^{-2} Ω cm) to the minimum at 16 K (T_{min}, 1.0×10^{-3} Ω cm). Metallic conduction is retained even at the lowest measured temperature (1.4 K, 1.5×10^{-3} Ω cm). The anisotropy of the resistivity is very small in the *a*–*c* plane ($\rho_\parallel/\rho_\perp = 0.4$–$0.9$), but the room-temperature resistivity parallel to the b^* direction is 10^2–10^3 times bigger. The stoichiometry of BEDT-TTF to ClO_4 is 2:1, and the crystals contain solvent molecules (1,1,2-trichloroethane). The formal charge on the BEDT-TTF molecule is $^1/_2$. The crystal structure is shown in Figure 1.[7] The unit cell contains four BEDT-TTF, two ClO_4, and $C_2H_3Cl_3$. The two crystallographically independent BEDT-

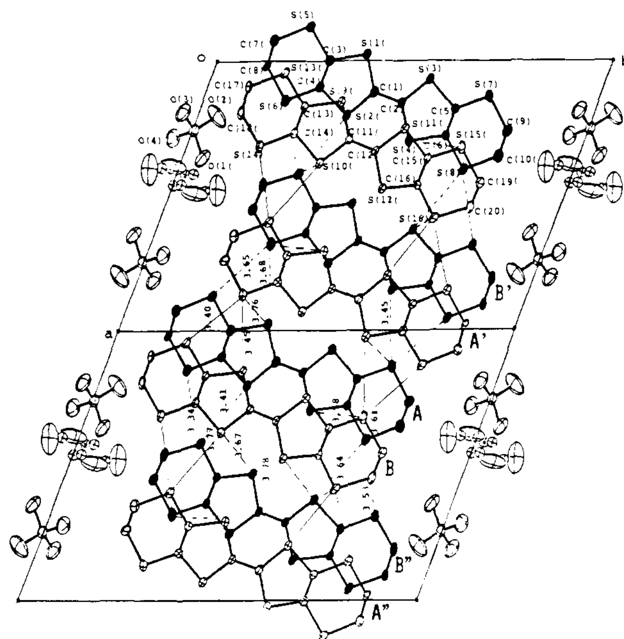


Figure 1. Crystal structure viewed along the c^* axis. Molecules A and B indicate two crystallographically independent BEDT-TTF molecules. The coordinates of the center of molecule A are (0.10, 0.48, -0.26) and those of molecule B are (0.34, 0.43, 0.21). Molecules A′ and A″ (B′ and B″) are the molecules related to A (B) by symmetry operations. The symmetry operations are A(x, y, z), B(x, y, z), A′($-x$, $1-y$, $-z$), B′($-x$, $1-y$, $-z$), A″($1-x$, $1-y$, $-z$), and B″($1-x$, $1-y$, $-z$). Molecules A, B′, and B″ are approximately on the same plane at $z = 0.25$, and molecules B, A′ and A″ are approximately on the plane at $z = 0.25$.

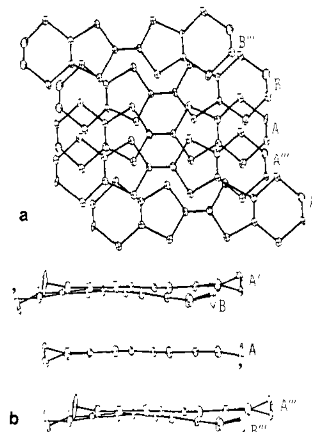


Figure 2. Arrangement of the neighboring BEDT-TTF molecules. The symmetry operations are A(x, y, z), A′($-x$, $1-y$, $-z$), A‴($-x$, $1-y$, $-1-z$), B(x, y, z), and B‴(x, y, $-1+z$) (see also Figure 1): (a) mode of the intermolecular overlapping; (b) side view of the moelcular planes.

(1) Andre, J. J.; Bieber, A.; Gautier, F. *Ann. Phys.* **1976**, 145–256.
(2) Kistenmacher, T. J. *Ann. N.Y. Acad. Sci.* **1978**, *313*, 333–342.
(3) Shibaeva, R. P. In "Extended Linear Chain Compounds"; Plenum Press: New York, 1982; pp 435–467.
(4) Gutfreund, H. In "Chemistry and Physics of One-Dimensional Metals"; Plenum Press: New York, 1977; pp 267–278.
(5) Saito, G.; Enoki, T.; Toriumi, K.; Inokuchi, H. *Solid State Commun.* **1982**, *42*, 557–560.
(6) The formal name of BEDT-TTF is 2-(5,6-dihydro-1,3-dithiolo[4,5-*b*][1,4]dithiin-2-ylidene)-5,6-dihydro-1,3-dithiolo[4,5-*b*][1,4]dithiin.

(7) The crystal data (room temperature) are as follows: $(C_{10}H_8S_8)_2ClO_4(C_2H_3Cl_3)_{0.5}$, triclinic; space group $P\bar{1}$; $a = 12.966$ (7), $b = 18.620$ (13), $c = 7.740$ (5) Å; $\alpha = 79.32$ (6), $\beta = 104.80$ (5), $\gamma = 110.85$ (5)°; $V = 1684$ (2) Å^3; $Z = 2$. A total of 8112 reflections were measured (Rigaku automated diffractometer) for $2\theta \leq 60°$ with Mo Kα radiation, of which 3797 reflections were considered to be observed ($|F_o| \geq 3\sigma|F_o|$). Block-diagonal least-squares refinement produced a final agreement factor $R = 0.087$. Fourier syntheses gave fairly large peaks corresponding to the position of chlorine atoms of the trichloroethane molecules, but they are highly disordered and could not be located definitely.

0002-7863/83/1505-0297$01.50/0 © 1983 American Chemical Society