in Figure 3B is due to the fact that in this experiment the faradaic current from the redox chemistry of the poly-**1** film is superimposed on the ohmic current between the Au and GC electrodes. This can be demonstrated by plotting $i_{GC} + i_{Au}$ vs. $E_{Au}$ (for small $\Delta E$) which nearly quantitatively reproduces the CV of the polymer (i.e., Figure 3A). The potential offsets between the positive and negative scans, respectively, match up well with the peak currents for the CV and thus are attributable to the slow response of the thick, highly cross-linked polymer film used to obtain Figure 3. Once the steady state is reached, however, $-i_{Au} = i_{GC}$ within experimental error.[6]

While the specific resistance of reduced poly-**1** is significantly larger than those of many other organic electronic conductors,[7-13] it does approach that of many semiconductors. The fact that the electronic conductivity is as large as it is is unusual, considering that (1) poly-**1** is, by X-ray diffraction, amorphous, (2) it lacks any long-range conjugation, and (3) the redox sites are all, within experimental error, in a single oxidation state. Detailed studies of this behavior are presently under further investigation.

(6) It should be noted here that, because of the use of the polymer electrolyte PDDP, at potentials negative of ~−1.00 V (cf. Figure 1) only a single oxidation state of poly-**1** (i.e., zero) is accessible by either electrode. Thus the mode of conduction between the electrodes cannot be due to redox conduction (an oxidation state concentration gradient).

(7) Shacklette, L. W.; Chance, R. R.; Ivory, D. M.; Miller, G. G.; Baughman, R. H. *Synth. Met.* **1979**, *1*, 307.

(8) Shiradawa, H.; Louis, E. J.; MacDiarmid, A. G.; Chiang, C. K.; Heeger, A. J. *J. Chem. Soc., Chem. Commun.* **1977**, 578.

(9) Rabolt, J. R.; Clarke, T. C.; Kanazawa, K. K.; Reynolds, J. R.; Street, G. B. *J. Chem. Soc., Chem. Cummun.* **1980**, 347.

(10) Chance, R. R.; Shacklette, L. W.; Miller, G. G; Ivory, D. M.; Sowa, J. M.; Eisenbaumer, R. L.; Baughman, R. H. *J. Chem. Soc., Chem. Commun.* **1980**, 348.

(11) Perlstein, J. *Angew. Chem., Int. Ed. Engl.* **1977**, *16*, 519.

(12) Schramm, C. J.; Scaringe, R. P.; Stojakonic, D. R.; Hoffman, B. M.; Ibers, J. A.; Marks, T. J. *J. Am. Chem. Soc.* **1980**, *102*, 6702.

(13) Kanazawa, K. K.; Diaz, A. F.; Greiss, R. H.; Gill, W. D.; Kwak, J. F.; Logan, J. A.; Rabolt, J. F.; Street, G. B. *J. Chem. Soc., Chem. Commun.* **1979**, 854.

## *N*-Carboxybiotin Formation by Pyruvate Carboxylase: The Stereochemical Consequence at Phosphorus

David E. Hansen and Jeremy R. Knowles*

*Department of Chemistry, Harvard University Cambridge, Massachusetts 02138*

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The mechanism of the ATP-dependent enzyme-catalyzed reaction of bicarbonate with biotin to give $N_1$-carboxybiotin, ADP, and $P_i$ has been debated since the critical observation of Boyer and co-workers in 1962 that when $HC^{18}O_3^-$ is used as the substrate for propionyl-CoA carboxylase in unlabeled water, $^{18}O$ is incorporated into the products methylmalonyl-CoA and $P_i$ in the ratio of 2:1.[1] This result, along with the large amount of mechanistic data that has subsequently been published on the six enzymes[2] that catalyze the ATP-dependent carboxylation reaction,[3] has led to the three favored pathways shown in Figure 1. In mechanism **1** ("stepwise"), ATP reacts with bicarbonate to form the reactive mixed anhydride carboxy phosphate, which is then attacked by the apparently nonnucleophilic[4] ureido $N_1$ nitrogen atom of biotin

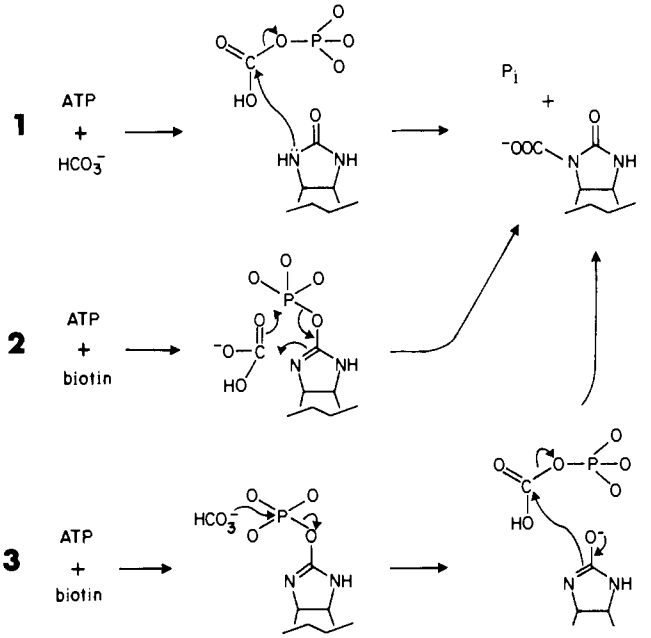


**Figure 1.** Three postulated mechanisms for the formation of $N_1$-carboxybiotin.

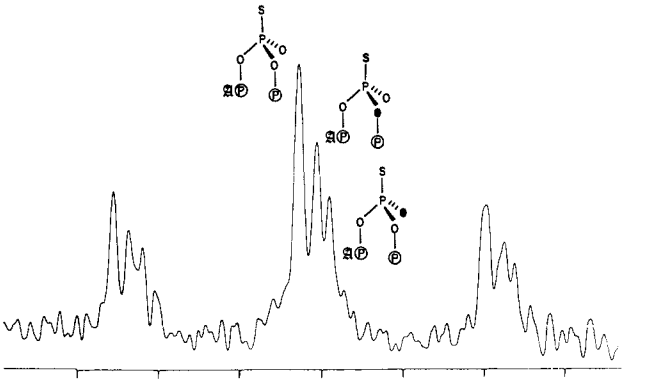

**Figure 2.** $^{31}P$ NMR spectrum of the $\beta$-phosphorus of ATP$\beta$S derived from the stereochemical analysis of the inorganic [$^{16}O$,$^{17}O$,$^{18}O$]thiophosphate obtained in the pyruvate carboxylase reaction. The spectrum was obtained on a Bruker WM-300 instrument at 121.5 MHz with a deuterium field lock and broad-band decoupling: spectral width 600.24 Hz; acquisition time 13.65 s; pulse width 24.5 s; number of transients 3000; Gaussian multiplication with Gaussian broadening, 0.016 Hz, and line broadening, −0.300 Hz. The chemical shifts for the nine major reasonances:[24] 29.9544, 29.9353, 29.9179, 29.7259, 29.7039, 29.6887, 29.4983, 29.4744, 29.4617 ppm; downfield from external 85% phosphoric acid. Scale: 0.10 ppm per division. The $\alpha$- and $\gamma$-phospho groups of ATP$\beta$S are abbreviated by ℗, adenosine by a, and $^{18}O$ by ●.

to form the products. In mechanism 2 ("concerted"), ATP is first attacked by the ureido oxygen atom of biotin[5] to give *O*-phosphobiotin, which then undergoes a chemically unprecedented six-electron electrocyclic process with bicarbonate. In mechanism 3 ("composite"), *O*-phosphobiotin is formed as above but is attacked by bicarbonate to form carboxy phosphate and the enolic form of biotin (in which the $N_1$ nitrogen is now nucleophilic[6]), and these two intermediates then react to give the products. This third mechanism is supported by chemical precedent and is analogous to the preferred pathway for the enzyme phosphoenolpyruvate carboxylase.[7]

To narrow the mechanistic possibilities, we have determined the stereochemical fate of the terminal phospho group of adenosine

(1) Kaziro, Y.; Hass, L. F.; Boyer, P. D.; Ochoa, S. *J. Biol. Chem.* **1962**, *237*, 1460.

(2) Wood, H. G.; Barden, R. E. *Annu. Rev. Biochem.* **1977**, *46*, 385. Biotin is normally covalently attached to an enzyme by an amide linkage to a lysyl residue.

(3) Attwood, P. V.; Keech, D. B. *Curr. Top. Cell. Regul.* **1984**, *23*, 1; Kluger, R.; Davis, P. P.; Adawadkar, P. D. *J. Am. Chem. Soc.* **1979**, *101*, 5995.

(4) Caplow, M. *J. Am. Chem. Soc.* **1965**, *87*, 5774.

(5) Bruice, T. C.; Hegarty, A. F. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *65*, 805.

(6) Hegarty, A. F.; Bruice, T. C.; Benkovic, S. J. *J. Chem. Soc. Chem. Commun.* **1969**, 1173.

(7) Hansen, D. E.; Knowles, J. R. *J. Biol. Chem.* **1982**, *257*, 14795.

0002-7863/85/1507-8304$01.50/0 

5′-$O(\gamma S_p)$-[$\beta\gamma$-$^{17}O$,$\gamma$-$^{17}O$,$^{18}O$](3-thiotriphosphate)(chiral ATP$\gamma$S)[8] with chicken liver pyruvate carboxylase (EC 6.4.1.1).[9] This enzyme catalyzes the overall reaction

$$\underset{\text{pyruvate}}{CH_3COCO_2^-} + HCO_3^- + ATP \rightleftharpoons \underset{\text{oxaloacetate}}{{}^-O_2CCH_2COCO_2^-} + ADP + P_i$$

via the intermediacy of $N_1$-carboxybiotin. The chiral ($S_p$)-ATP$\gamma$S was incubated[10] with pyruvate, bicarbonate, Co(II), and enzyme,[11] and the absolute configuration of the product inorganic [$^{16}O$,$^{17}O$,$^{18}O$]thiophosphate[12] was determined to be $R_p$ by the $^{31}P$ NMR method independently developed by Webb and Trentham[13] and by Tsai[14] (Figure 2). That is, of the stereochemically informative resonances (the second and third peak of each set) of the adenosine 5′-$O$-(2-thiotriphosphate) (ATP$\beta$S) that derives from the inorganic thiophosphate product, the second peak is larger than the third. Pyruvate carboxylase from chicken liver therefore catalyzes the conversion of ATP to ADP and $P_i$ with overall *stereochemical inversion of the configuration at phosphorus.* Control experiments demonstrated that no ATP$\gamma$S was broken down when incubated under identical conditions with enzyme that had been inactivated with avidin,[15] a potent biotin-binding protein.

Of the three mechanisms outlined above, both the stepwise (**1**) and the concerted (**2**) are predicted to go with overall inversion at phosphorus, while the composite (**3**) is predicted to go with retention. The stepwise mechanism involves the direct transfer of the phospho group of ATP to bicarbonate (with inversion[16]) followed by the expulsion of inorganic phosphate. The concerted mechanism also begins with a direct transfer of the phospho group, this time to the ureido oxygen of biotin (with inversion), followed by a six-electron $\pi_2S + \pi_2S + \sigma_2S$ electrocyclic rearrangement (with retention[7]). The composite mechanism, although chemically and enzymatically precedented, must be incorrect because it involves two direct transfers (each with inversion) and would thus result in overall retention at phosphorus.

In an effort to distinguish between mechanisms **1** and **2**, we may look to three critical results in the literature. First, when carbamoyl phosphate, a putative carboxyphosphate analogue, is allowed to react with ADP and either sheep liver pyruvate carboxylase[17] or *E. coli* acetyl-CoA carboxylase,[18] ATP is produced (however, see Kluger et al.[3]). Second, phosphonacetic acid, a stable carboxy phosphate analogue, is a noncompetitive inhibitor of ATP with the sheep liver enzyme.[17] Third, in the absence of bicarbonate, there is no positional isotope exchange of ATP[19] with chicken liver pyruvate carboxylase[20] and apparently no ADP–ATP isotope exchange with the sheep liver enzyme.[17] These results are most simply accommodated by the stepwise mechanism **1** (but do not unequivocally rule out mechanism **2**). Furthermore, it is becoming apparent that the few enzymes that might in principle catalyze concerted electrocyclic mechanisms, e.g., anthranilate synthase[21] or phosphoenolpyruvate carboxylase,[7] evidently follow stepwise pathways.

Finally, in mechanism **1**, how does the enzyme "activate" the nonnucleophilic ureido $N_1$ nitrogen atom of biotin so that it may attack carboxy phosphate? If by using acid–base chemistry the enzyme could tautomerize the ureido group into the enolic form (a mechanism for which has been proposed[22]), the $N_1$ nitrogen would become approximately $10^{10}$ times more nucleophilic.[6] Interestingly, Mildvan, Lane, and co-workers have recently shown that the $N_1$ nitrogen of biotin is unusually reactive compared with that of desthiobiotin or *O*-heterobiotin which may be due to a transannular interaction between the ureido carbonyl group and the biotin sulfur atom.[23] It thus appears that the ureido $N_1$ nitrogen of biotin, by whatever means, is nucleophilic enough to attack carboxy phosphate in these enzymatic reactions.

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(8) Synthesized by a modification of the procedure in: Webb, M. R. *Methods Enzymol.* **1982**, *87*, 301.
(9) Purified by a modification of the procedure in: Scrutton, M. C.; Fung, C.-H. *Arch. Biochem. Biophys.* **1972**, *150*, 636.
(10) ATP$\gamma$S/$Co^{II}$ reacts at approximately 1/200 the rate of ATP/$Mg^{II}$ under the reaction conditions. The reaction rate of ATP$\gamma$S/$Mg^{II}$ is undetectable with this enzyme, and we assume that the constraints of the active site require the use of a divalent metal ion that can readily coordinate sulfur (see: Jaffe, E. K.; Cohn, M. *J. Biol. Chem.* **1979**, *254*, 10839).
(11) The reaction mixture (30 mL, incubated at 30 °C for 6 h) contained chiral ATP$\gamma$S (20 μmol), pyruvate carboxylase (200 units[9]), $CoSO_4$, (0.5 mM), sodium pyruvate (40 mM), potassium bicarbonate (40 mM), and reduced glutathione (0.66 mM) in Hepes buffer (266 mM, pH 7.8). Acetyl-CoA (0.5 μmol) was added every 30 min.[9]
(12) Purified by ion exchange chromatography on DEAE-Sephadex.[8]
(13) Webb, M. R.; Trentham, D. R. *J. Biol. Chem.* **1980**, *255*, 1775.
(14) Tsai, M.-D. *Biochemistry* **1980**, *19*, 5310.
(15) Green, N. M. *Biochem. J.* **1966**, *101*, 774.
(16) On the basis that normal displacements at phosphorus invert the configuration: Knowles, J. R. *Annu. Rev. Biochem.* **1980**, *49*, 877.
(17) Ashman, L. K.; Keech, D. B. *J. Biol. Chem.* **1975**, *250*, 14.
(18) Pokalis, S. E.; Guchhait, R. B.; Zwergel, E. E.; Lane, M. D.; Cooper, T. G. *J. Biol. Chem.* **1974**, *249*, 6657.
(19) Midelfort, C. F.; Rose, I. A. *J. Biol. Chem.* **1976**, *251*, 5881.
(20) Wimmer, M., private communication.
(21) Tamir, H.; Srinivasan, P. R. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *66*, 547.
(22) Stallings, W. C. *Arch. Biochem. Biophys.* **1977**, *183*, 189.
(23) Fry, D. C.; Fox, T. L.; Lane, M. D.; Mildvan, A. S. *J. Am. Chem. Soc.*, in the press.
(24) Washout of label in the analysis,[8] a somewhat low $^{18}O$ content (71%) in the chiral ATP$\gamma$S used, and the small amount of [$^{16}O$,$^{17}O$,$^{18}O$] thiophosphate analyzed (less than 20 μmol) prevent exact quantitation of the spectrum. The intensities of the second and third peaks in each group clearly indicate, however, that the inorganic thiophosphate analyzed was predominately of the $R_p$ configuration.[13]

## Crystal and Band Electronic Structures of an Organic Salt with the First Three-Dimensional Radical-Cation Donor Network, (BEDT-TTF)$Ag_4(CN)_5$

Urs Geiser, Hau H. Wang, Larry E. Gerdom, Millicent A. Firestone, Lauren M. Sowa, and Jack M. Williams*

*Chemistry and Materials Science and Technology Divisions, Argonne National Laboratory Argonne, Illinois 60439*

Myung-Hwan Whangbo*

*Department of Chemistry, North Carolina State University, Raleigh, North Carolina 27650*

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Most organic conducting salts of π-conjugated donor molecules known so far have a structure consisting of sheetlike networks of electron-donor molecules.[1] Each of these donor sheets, separated by a layer of counteranions, is typically composed of stacks of donor molecules. Thus intermolecular interactions take place within as well as between adjacent donor stacks. Despite such two-dimensional (2D) networks most conducting salts of donor molecules are one-dimensional (1D) metals because interactions between adjacent stacks are typically much weaker than those within each stack.[1,2] This situation can be significantly altered with bis(ethylenedithio)tetrathiafulvalene, $C_{10}S_8H_8$, (BEDT-TTF or simply ET), as shown by Kobayashi et al.[3] for $(ET)_2ClO_4$·

(1) (a) Williams, J. M. *Prog. Inorg. Chem.* **1985**, *33*, 183. (b) *J. Phys. (Les Ulis, Fr.)* **1983**, *44* (*C3*), 767–1714. (c) "Extended Linear Chain Compounds"; Miller, J. S., Ed.; Plenum Press: New York, **1982–1983**; Vol. 1–3. (d) Williams, J. M.; Beno, M. A.; Wang, H. H.; Leung, P. C. W.; Emge, T. J.; Geiser, U.; Carlson, K. D. *Acc. Chem. Res.* **1985**, *18*, 261.
(2) (a) Whangbo, M.-H.; Walsh, W. M., Jr.; Haddon, R. C.; Wudl, F. *Solid State Commun.* **1982**, *43*, 637. (b) Whangbo, M.-H.; Williams, J. M.; Beno, M. A.; Dorfman, J. R. *J. Am. Chem. Soc.* **1983**, *105*, 645. (c) Mori, T.; Kobayashi, A.; Sasaki, Y.; Kobayashi, H. *Chem. Lett.* **1982**, 1923. (d) Grant, P. M. *Phys. Rev. B* **1982**, *26*, 6888.
(3) Kobayashi, H.; Kobayashi, A.; Sasaki, Y.; Saito, G.; Enoki, T.; Inokuchi, H. *J. Am. Chem. Soc.* **1983**, *105*, 297.

0002-7863/85/1507-8305$01.50/0 