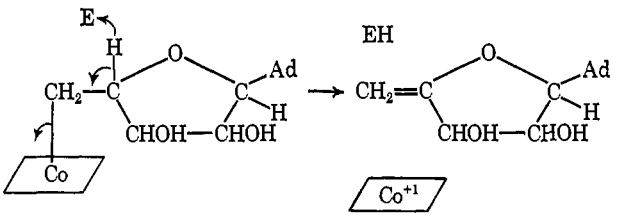
Table I. Specific Activities of Vitamin B_{12} Coenzyme, Residual Substrate, and Product During the Conversion of D-1,2-Propanediol-1-^{3}H to Propionaldehyde-2-^{3}H[a]

Time, sec	Specific activity (cpm/μmol) × 10^{-5}		
	B_{12}-coenzyme	Residual substrate	Product
0	1.8	4.0	
5	75	4.3	0.27
10	88	4.4	0.22
30	130	5.9	0.26
60	160	10.3	0.22

[a] The D-1,2-propanediol was stereospecifically labeled with tritium in the C-1 hydrogen which is transferred to C-2 (B. Zagalak, P. A. Frey, G. L. Karabatsos, and R. H. Abeles, *J. Biol. Chem.*, **241**, 3028 (1966)). The experiment was carried out at 10°. A separate reaction mixture containing 98 units of diol dehydrase, 4 μmol of potassium phosphate buffer, pH 8.0, 3.2×10^{-3} μmol of B_{12} coenzyme, and 29.8 μmol of substrate in a total volume of 0.55 ml was used for each point. The reaction was started by adding the substrate in 0.10-ml volume, and, after the appropriate time, 0.1 ml of 20% TCA was added to stop the reaction. A 0.05-ml aliquot was immediately withdrawn, diluted with water, and assayed colorimetrically for its propionaldehyde content. A separate 0.10-ml aliquot was withdrawn, diluted into 1.0 ml of a solution containing 0.24 *M* propionaldehyde carrier in 0.2 *M* potassium phosphate, pH 8.0, treated with 1–2 mg of charcoal, and centrifuged. Propionaldehyde was isolated from the supernatant fluid as the dimethone derivative, which was assayed radiochemically to determine the specific activity of propionaldehyde. B_{12}-coenzyme was isolated from the remainder of the reaction mixture, and its specific activity was determined according to procedures described previously (P. A. Frey, M. K. Essenberg, and R. H. Abeles, *ibid.*, **242**, 5369 (1967)). The specific activity of residual D-1,2-propanediol-1-^{3}H was calculated from the known initial specific activity and data on the amounts of propionaldehyde and propionaldehyde-2-^{3}H produced at each time.

cess. In an experiment, which has previously been published,[5] it was shown that when propionaldehyde-2-^{3}H and the enzyme-bound coenzyme equilibrate, tritium is not concentrated in the coenzyme. Propionaldehyde-2-^{3}H was added to diol dehydrase–vitamin B_{12} coenzyme complex. Aliquots of the coenzyme were isolated periodically and examined for tritium content. The specific activity of the coenzyme after 1.5 hr of incubation was equal to that of the aldehyde. This shows that no unusual equilibrium isotope effect favoring tritium in B_{12} coenzyme occurs. It must therefore be concluded that the incorporation of tritium into the coenzyme which occurs when 1,2-propanediol-1-^{3}H is converted to propionaldehyde cannot occur through exchange with the enzyme-bound propionaldehyde, and that the mechanism proposed by Schrauzer and Sibert for labeling of the coenzyme is inconsistent with the experimental results obtained with diol dehydrase. Alternative mechanisms for the exchange of tritium between substrate and coenzyme have been proposed.[6,7]

A mechanism for the activation of the enzyme-bound coenzyme was also proposed,[2] in which a basic group on the enzyme abstracts a hydrogen from the C-4′ position of the enzyme-bound coenzyme to produce vitamin $B_{12(s)}$ and dehydroadenosine. According to this mechanism ^{3}H should be introduced into the C-4′ position of the coenzyme when the reaction is carried out in H_2O-^{3}H. To test the exchangeability of the C-4′ hydrogen, DL-1,2-propanediol (2.4 mmol), diol dehydrase (255 units), B_{12} coenzyme (1.2×10^{-2} μmol), and dibasic potassium phosphate (2.5 μmol) were incubated in a total volume of 1.5 ml in H_2O-^{3}H (1.3×10^6 cpm/μg-atom) at 37° for 10 min. At this point about 1400 μmol of diol had reacted so that at least 10^5 turnovers had taken place. The coenzyme was then isolated and purified by procedures previously described.[6] The specific activity of the purified coenzyme was less than 5 cpm/μg. If one carbon-bound hydrogen of B_{12} coenzyme had equilibrated with the solvent during the course of the enzymatic reaction, and if no equilibrium isotope effects had occurred, the specific activity of the coenzyme would be 1.2×10^3 cpm/μg. This experiment therefore does not suport the proposed mechanism of activation of the coenzyme. Since enzyme reactions are known in which proton transfers occur without exchange with the solvent protons,[8] the experiment does not eliminate this activation mechanism. We feel, however, that mechanisms for which experimental support exists are more attractive than those for which no support exists.



The mechanism of action of vitamin B_{12} coenzyme proposed by Schrauzer and Sibert is based on model experiments with cobaloximes and cobalamines. It should be pointed out that no aspect of this mechanism is borne out by experiments with the enzyme, and that some features, as shown by this communication, are directly contradicted by experiments with the enzyme. Although model systems are invaluable in the study of enzyme reactions, it is essential that they be related to the mechanism of the enzymatic reaction. Relevance is established by the ability of the model system to explain a maximum number of experimental facts pertaining to the enzyme system. Coincidental formation of similar reaction products is in itself not sufficient to to guarantee a relevant model system.

(5) P. A. Frey, S. S. Kerwar, and R. H. Abeles, *Biochem. Biophys. Res. Commun.*, **29**, 873 (1967).
(6) P. A. Frey, M. K. Essenberg, and R. H. Abeles, *J. Biol. Chem.*, **242**, 5369 (1967).
(7) W. W. Miller and J. H. Richards, *J. Amer. Chem. Soc.*, **91**, 1498 (1969).
(8) I. A. Rose, *Annu. Rev. Biochem.*, **35**, 51 (1966).

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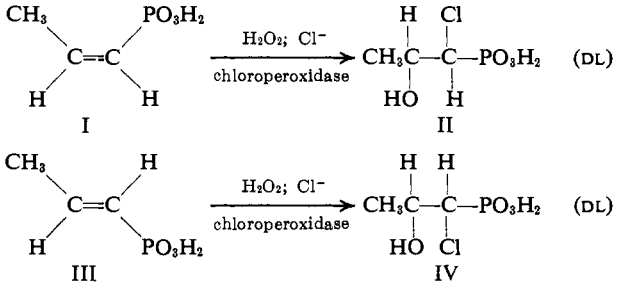
Enzymatic Formation of Chiral Structures in Racemic Form

Sir:

Enzymatic syntheses of chiral molecules from achiral substrates proceed in a stereospecific and asymmetric

manner. To our knowledge, there is no exception in the literature[1] to this rule.

However, we wish to describe an instance of an *enzymatic synthesis of a chiral molecule in its racemic form from an achiral substrate.*



Hydrogen peroxide (0.055 *M*, 21 ml) was added over a period of 8 hr to a solution of *cis*-propenylphosphonic acid (I)[2] (1 mmol), potassium chloride (2 mmol), and 25 mg of chloroperoxidase preparation[3] in 20 ml of potassium phosphate buffer (pH 2.75). The solution was periodically assayed for enzymatic activity (Morris and Hager[3]) and when, after 6 hr, this diminished to 0.1 of the original value, an additional 28 mg of enzyme was added. The solution was concentrated *in vacuo* to 15 ml and passed through 30 ml of Dowex 50-X8 (H^+ form). The acidic eluate was concentrated *in vacuo* to a syrup which was then treated in methanol with diazomethane and concentrated again, and the residue chromatographed on a 100-g silica gel H dry column using a chloroform–isopropyl alcohol mixture (92:8, v/v) as solvent eluent. From the effluent was isolated 80 mg of *threo*-dimethyl 1-chloro-2-hydroxypropylphosphonate (dimethyl ester of II); $[\alpha]_D$ 0° (*c* 2, methanol).[4]

(1) M. Dixon and E. C. Webb, "Enzymes," 2nd ed, Academic, New York, N. Y., 1964, 205. The stereospecificity of reactions catalyzed by (isolated) enzymes is to be contrasted with transformations in cell systems which, in some cases, yield products with less than 100% optical purity, thus indicating stereoselective instead of stereospecific reaction; *e.g.*, fermenting yeast reduces ketones with variable and substrate-dependent (12–90%) stereoselectivity [R. MacLeod, H. Prosser, L. Fikentscher, J. Lanyi, and H. S. Mosher, *Biochemistry*, **3**, 838 (1964)]. However, the fact that actively fermenting yeast is capable of reducing ketones which are not reduced by the purified yeast ADH–DPNH enzyme (see MacLeod, *et al.*, p 844) suggests that the overlapping effect of more than one enzyme may be responsible for the lack of stereospecificity. For instance, yeast contains separate L-lactate and D-lactate dehydrogenases (J. Westley, "Enzymic Catalysis," Harper and Row, New York, N. Y., 1969, p 66).

(2) N. N. Girotra and N. L. Wendler, *Tetrahedron Lett.*, 4647 (1969).

(3) Prepared by Drs. R. F. White, T. A. Jacob, and F. W. Bollinger of these laboratories, employing the method of D. R. Morris and L. P. Hager, *J. Biol. Chem.*, **241**, 1763 (1966). The enzyme purification was carried to the second ethanol precipitate (29 units/mg). (Crystalline chloroperoxidase contains 1600 units/mg; see Morris and Hager.) We also wish to thank Professor L. P. Hager, University of Illinois, Urbana, Ill., for the gift of a similar enzyme preparation.

(4) Chromatography was monitored by tlc (silica gel plates, 92:8 $CHCl_3$–*i*-C_3H_7OH); all the product-containing fractions were combined before checking for optical activity, thus excluding the possibility of losing optically active material by inadvertent separation. None of the *erythro* isomer (dimethyl ester of IV) was detected in this product by gas–liquid chromatography [QF-1 (20%) 1/4 in. × 10 ft at 180°; injection at 230°]. In an artificial mixture of 0.5% *erythro* and 99.5% *threo* (both of nonenzymatic origin) the presence of the *erythro* isomer was detectable, retention times 13.8 and 16.2 min, respectively. The infrared and pmr spectra of product were identical with those of authentic material prepared from I by reaction with *t*-butyl hypochlorite followed by esterification with diazomethane.[2] Authentic (+)-II[2] with diazomethane gave the corresponding (+) antimer, $[\alpha]_D$ 10.3° (*c* 2, methanol); $[\alpha]_{250}$ 83° (*c* 6, acetonitrile). The optical activity of an artificial mixture of the latter compound with (nonenzymatically made) racemate (0.3% of active ester mixed with 99.7% racemate, Σc 6.3, in acetonitrile at 250 nm) was clearly detectable (Cary 60 spectropolarimeter). Under similar conditions (*c* 6.26, acetonitrile, 250 nm) the enzymatically prepared material was found optically inactive. For the ORD measurements, we thank Dr. J. J. Wittick of these laboratories.

Enzymatic hydroxychlorination of *trans*-propenylphosphonic acid (III) was performed in a similar way, to give *erythro*-dimethyl 1-chloro-2-hydroxypropylphosphonate[5] (dimethyl ester of IV), also optically inactive.

Subjecting optically active *threo*-1-chloro-2-hydroxypropylphosphonic acid[6] to the action of the same enzymatic system, the isolated *threo*-dimethyl 1-chloro-2-hydroxypropylphosphonate displayed full optical activity, thus excluding the possibility of subsequent racemization of an initially formed optically active chlorohydrin. According to the chlorodimedon assay (see Morris and Hager, ref 3), our enzyme preparation lost its activity on exposure for a few minutes to a pH 8.01 phosphate buffer. In another control—with omission of the enzyme—I was found to be unreactive with the H_2O_2–Cl^- system at pH 3.

Chloroperoxidase has been found to be an active catalyst for halogenation of several classes of compounds, *e.g.*, cyclic β-diketones,[7a] β-keto acids,[7b] anisole,[7c] and unsaturated steroids.[7d] However, a stereochemical definition of the reaction is impossible in these cases because the products are either achiral or, as in the case of 9(11)-dehydrosteroids,[7d] the configurations of the products are influenced by asymmetric induction.

According to Hager,[8] the mechanism of chloroperoxidase action involves an enzyme–halogenium ion complex,[7c] the reaction of which, with the acceptor molecule, yields the halogenated product with regeneration of the free enzyme. Surprisingly, this intimate involvement of the enzyme in the halogen transfer does not assure the asymmetric course of the reaction.

(5) This product gave identical infrared and pmr spectra with those of an authentic preparation, prepared from *trans*-propenylphosphonic acid, by the method under ref 2. CH_3–CH in this compound is a doublet at 1.42 ppm (TMS, $CDCl_3$), J = 6 cps; the methyl protons in the *threo* isomer appear as double doublets at 1.36 ppm, J = 6.5 and 1.5 cps.

(6) See ref 2.

(7) (a) L. P. Hager, D. R. Morris, F. S. Brown, and H. Eberwein, *J. Biol. Chem.*, **241**, 1769 (1966); (b) P. D. Shaw and L. P. Hager, *ibid.*, **236**, 1626 (1961); (c) F. S. Brown and L. P. Hager, *J. Amer. Chem. Soc.*, **89**, 719 (1967); (d) S. L. Neidleman and S. D. Levine, *Tetrahedron Lett.*, 4057 (1968).

(8) L. P. Hager, "Mechanism of the Peroxidase Halogenation Reaction," Abstracts of Papers, V, Symposium, Membrane Function and Electron Transfer to Oxygen, Jan 20–24, 1969, University of Florida, Fla.

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Nature of the Skeletal Change in a Metal-Catalyzed Diene Rearrangement

Sir:

The skeletal rearrangements of 1,4-dienes by a nickel-based homogeneous catalyst[1] are particularly interesting from a mechanistic standpoint. The observed transformations require the accomplishment of five fundamental changes common to many transition metal catalyzed olefin oligomerization reactions,[2] namely C–H bond cleavage and formation, C–C π bond cleavage and formation, and C–C σ bond formation. In addition they require a sixth process, much less common in homogeneous catalysis, the fission of a C–C σ bond.

(1) (a) R. G. Miller, *J. Amer. Chem. Soc.*, **89**, 2785 (1967); (b) R. G. Miller and P. A. Pinke, *ibid.*, **90**, 4500 (1968).