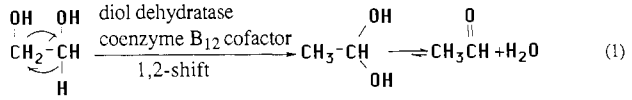
Volume 28
Number 6
March 22, 1989

Inorganic Chemistry

Communications

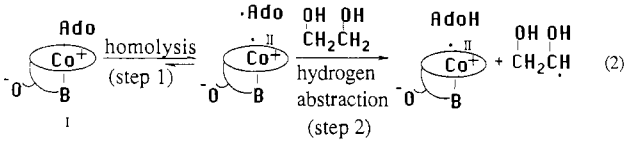
Coenzyme B_{12} Dependent Diol Dehydratase Model Studies: Chemical Evidence against Co(B_{12}) Participation in the Rearrangement Step

Providing chemical evidence for or against proposed elementary steps in the B_{12}-dependent enzymic rearrangement reactions,[1] such as that for diol dehydratase[2] (eq 1), has been the focus of considerable interest and effort.[3-5] Recently, we demonstrated the



(1)

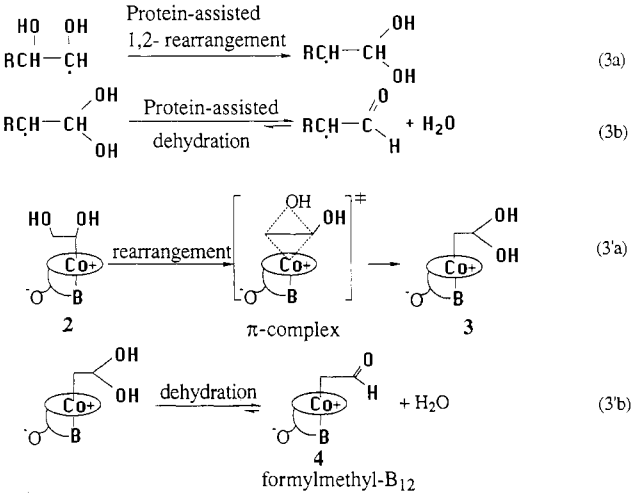
reversible *thermal* homolysis of the Co–C bond of adenosylcobalamin[6] (ado-B_{12} or coenzyme B_{12}, **1**; see eq 2, step 1). This



(2)

in turn has allowed us to initiate studies probing the Ado• + substrate C–H hydrogen atom abstraction step[7] (eq 2, step 2) and its poorly understood kinetic isotope effect measurements.[8] The mechanism of the third step, the rearrangement step, has been the focus of a number of previous studies,[3-5] including our own work,[5] but remains unsettled and is the focus of the present work.

Although the bulk of the chemical[3-5] and enzymic stereochemical[9,10] evidence favors the cobalt nonparticipation, enzyme-bound radical rearrangement pathway (eq 3a,b), an alternative mechanism that persists[11] is the Co(B_{12})-participation, π-complex pathway[4] (eq 3'a,b). A recent communication in-



volving a Rh(OEP) (OEP = octaethylporphyrin) reaction[11] (eq 4b; synthesis eq 4a) was offered as evidence for the Co-partici-

(1) (a) B_{12}; Wiley-Interscience: New York, 1982; Vols. 1 and 2. (b) *Vitamin* B_{12}. *Proceedings of the Third European Symposium on Vitamin* B_{12} *and Intrinsic Factor*; Zagalak, B., Friedrich, W., Eds.; Walter de Gruyter: New York, 1979. (c) *Recl.: J. R. Neth. Chem. Soc.* **1987**, *106*, 342–351 (Proceedings of the 3rd International Conference on Bioinorganic Chemistry).

(2) (a) Toraya, T.; Fukui, S. In B_{12}; Dolphin, D., Ed.; Wiley-Interscience: New York, 1982; Vol. 2, Chapter 9. (b) McGee, D. E.; Richards, J. H. *Biochemistry* **1981**, *20*, 4293. (c) Tanizawa, K.; Nakajima, N.; Toraya, T.; Tanaka, H.; Soda, K. *Z. Naturforsch.* **1987**, *42C*, 353.

(3) Selected lead references to model studies of the rearrangement step of diol dehydratase are provided below as well as in ref 4, 5, and 22. A complete list of work prior to 1983 is available (ref 12–16 of ref 5a). (a) Dixon, R. D.; Golding, B. T.; Mwesigye-Kibende, S.; Ramakrishna Rao, D. N. *Phil. Trans. R. Soc. London B* **1985**, *311*, 531 and references therein. (b) Müller, P.; Rêtey, J. *J. Chem. Soc., Chem. Commun.* **1983**, 1342. (c) Salem, L.; Eisenstein, O.; Anh, N. T.; Burgi, H. B.; Devaquet, A.; Segal, G.; Veillard, A. *Nouv. J. Chim.* **1977**, *1*, 335. (d) Russell, J. J.; Rzepa, H. S.; Widdowson, D. A. *J. Chem. Soc., Chem. Commun.* **1983**, 625.

(4) (a) Silverman, R. B.; Dolphin, D. *J. Am. Chem. Soc.* **1976**, *98*, 4633. (b) Silverman, R. B.; Dolphin, D. *Ibid.* **1976**, *98*, 4626; **1974**, *96*, 7094; **1973**, *95*, 1686; **1975**, *97*, 2924. (c) Silverman, R. B.; Dolphin, D. *J. Organomet. Chem.* **1975**, *101*, C14. (d) Silverman, R. B.; Dolphin, D. *J. Am. Chem. Soc.* **1972**, *94*, 4028. (e) Silverman, R. B.; Dolphin, D.; Carty, T. J.; Krodel, E. K.; Abeles, R. H. *Ibid.* **1974**, *96*, 7096. (f) Vickrey, T. M.; Katz, R. N.; Schrauzer, G. N. *Ibid.* **1975**, *97*, 7248. (g) Schrauzer, G. N.; Michaely, W. J.; Holland, R. J. *Ibid.* **1973**, *95*, 2024. (h) Michaely, W. J.; Schrauzer, G. N. *Ibid.* **1973**, *95*, 5771.

(5) (a) Finke, R. G.; McKenna, W. P.; Schiraldi, D. A.; Smith, B. L.; Pierpont, C. *J. Am. Chem. Soc.* **1983**, *105*, 7592. (b) Finke, R. G.; Schiraldi, D. A. *J. Am. Chem. Soc.* **1983**, *105*, 7605. (c) Finke, R. G.; McKenna, W. *J. Chem. Soc., Chem. Commun.* **1980**, 460.

(6) (a) Finke, R. G.; Hay, B. P. *Inorg. Chem.* **1984**, *23*, 3041; **1985**, *24*, 1278. (b) Hay, B. P.; Finke, R. G. *J. Am. Chem. Soc.* **1986**, *108*, 4820. (c) Hay, B. P.; Finke, R. G. *Polyhedron* **1988**, *7*, 1469. (d) Clean thermal homolysis of the benzimidazole base-free derivative of Ado-B_{12}, adocobinamide, has also been accomplished: Hay, B. P.; Finke, R. G. *J. Am. Chem. Soc.* **1987**, *109*, 8012.

(7) (a) The H-transfer steps are fairly well (but not completely)[7b] established on the basis of elegant enzyme labeling studies,[1] but there is surprisingly little *direct* evidence for a discrete Ado• radical. A largely concerted Ado-B_{12} Co---C cleavage and substrate S---H (or other –X–H)[7b] hydrogen abstraction is another process that merits consideration.[8d] (b) Very recent enzymic studies provide strong evidence for the participation of a second H-transfer site (–XH), as suggested in 1982 by Cleland,[7c] in diol dehydratase,[7d] ribonucleotide reductase,[7e] and ethanolamine ammonia lyase.[7f] (c) Cleland, W. W. *Crit. Rev. Biochem.* **1982**, *13*, 385–428. See also the acknowledgments to W. W. Cleland.[7e,f] (d) McGee, D. E. Ph.D. Thesis, California Institute of Technology, 1983. (e) Stubbe, J. *Biochemistry* **1988**, *27*, 3893 and references therein. (f) O'Brien, R. J.; Fox, J. A.; Kopczynski, M. G.; Babior, B. M. *J. Biol. Chem.* **1985**, *260*, 16131.

(8) For the isotope effect studies in the diol dehydratase system, see: (a) Moore, K. W.; Bachovchin, W. W.; Gunter, J. B.; Richards, J. H. *Biochemistry* **1979**, *18*, 2776. (b) Frey, P. A.; Karabatsos, G. L.; Abeles, R. H. *Biochem. Biophys. Res. Commun.* **1965**, *18*, 551. (c) Essenberg, M. K.; Frey, P. A.; Abeles, R. H. *J. Am. Chem. Soc.* **1971**, *93*, 1242. (d) Wang, Y.; Lin, Y.; Koenig, T.; Finke, R. G. Experiments in progress.

(9) (a) Rêtey, J.; Umani-Ronchi, A.; Arigoni, D. *Experentia* **1966**, *22*, 72. (b) Rêtey, J.; Umani-Ronchi, A.; Seibl. J.; Arigoni, D. *Ibid.* **1966**, *22*, 502. (c) Rêtey, J. In *Stereochemistry*; Tamm, Ch., Ed.; Elsevier Biomedical Press: Amsterdam, 1982; Chapter 6.

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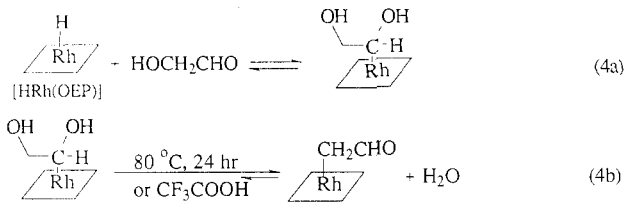


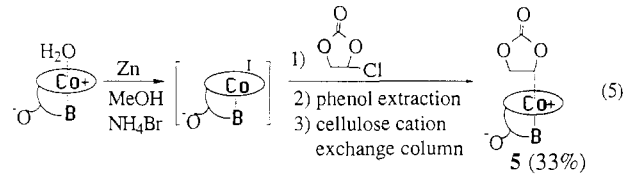
(4a)

(4b)

pation pathway (see Scheme I therein[11]), despite important differences when compared to the B_{12} cofactor.[11] Differences worth noting include the Co vs Rh (and corrin vs OEP) differences, the ≥0.5-V more positive Co(corrin) vs Rh(OEP) $E_{1/2}$ reduction potential,[12] and the enzyme's physiological temperature, pH, and turnover rate of ca. 10^2/s vs the more extreme conditions of eq 4 (either 80 °C and 24 h (i.e. $\gg 10^{-7}$ times slower than the enzyme) or strong acid (CF_3COOH) catalyst). The key conceptual difference between Co nonparticipation (eq 3) and Co participation (eq 3′) is the *protein vs B_{12}-cofactor control*, respectively, of the rearrangement step.

Herein we provide perhaps the most direct chemical model test to date of the cobalt-participation mechanism. We have prepared a carbonate-protected form of the putative,[5a] B_{12}-bound 1,2-diol intermediate, **2**, and then deprotected it and evaluated the resulting products. Is (formylmethyl)cobalamin (**4**), the key product expected from the cobalt participation (eq 3′), formed or not?

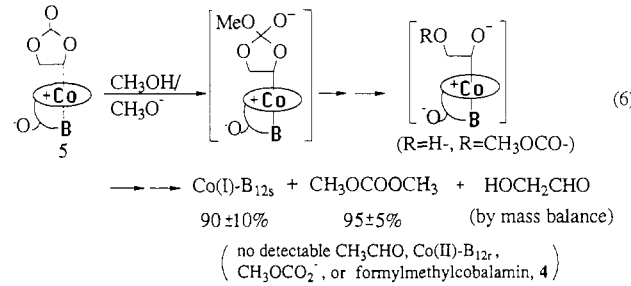
The synthesis and purification of the carbonate-protected intermediate, (2-oxo-1,3-dioxolan-4-yl)cobalamin (**5**) proceeded smoothly as expected[5,13] and as shown in eq 5.[14] FAB-MS



(5)

confirms the molecular weight of **5** (calc $[M + H]^+$ m/z for $C_{65}H_{91}O_{17}N_{13}PCoH^+$ 1416.57, found m/z 1416.6), and ^{13}C NMR and IR spectroscopies confirm the presence of the carbonate group (^{13}C NMR, D_2O vs MeOH external standard, δ 157 ppm, –OC(O)O–; IR, MeOH, $\nu_{C=O}$ region, 1765 and 1800 cm^{-1} (due to Fermi resonance)[5b], on a large background of B_{12} amide carbonyls, 1650 cm^{-1}). HPLC and ^1H NMR spectroscopy (Figure A, supplementary material) demonstrate that **5** consists of a mixture of two pure diastereomers, as expected, since B_{12} is chiral and chloroethylene carbonate is racemic.[15]

The key experiment, the deprotection of **5**, proved most interesting (eq 6). The products of methanolysis are 90 ± 10%

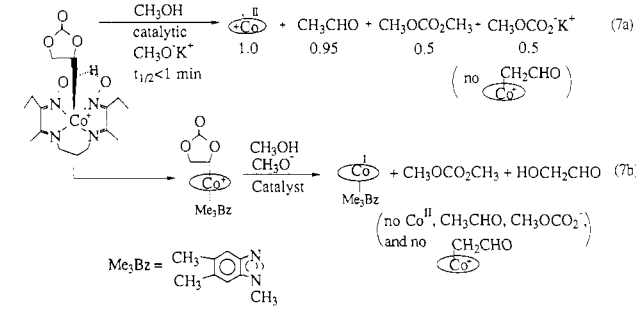


(6)

of Co(I)-B_{12s} (λ_{max} = 389 nm), 95 ± 5% $CH_3OCOOCH_3$ (IR $\nu_{C=O}$ = 1755 cm^{-1}, $\epsilon = (4.6 \pm 0.1) \times 10^2$ cm^{-1} M^{-1}) and (by mass balance) $HOCH_2CHO$ (eq 6).[16] Note that no trace of the cobalt-participation product, (formylmethyl)cobalamin (**4**) is observed. In a crucial control experiment, authentic and independently prepared (formylmethyl)cobalamin[17] (4) was shown by UV–vis spectroscopy to be stable to MeO^-/MeOH (and to pH 10 aqueous borate buffer, vide infra), *unequivocally ruling out **4** as an intermediate in the reaction in eq 6. This, in turn, unequivocally rules out any and all cobalt-participation mechanisms that yield formylmethylcobalamin (e.g. eq 6)*. This is a significant finding, as all cobalt-participation mechanisms we or others[11] have been able to write should yield at least some (formylmethyl)cobalamin. (Control experiments,[14b] our earlier work,[5] and the well-established organic chemistry of carbonate carbonyl groups support the presence of the two bracketed intermediates shown in eq 6; details will be reported in a subsequent full paper.)

Since the enzyme operates optimally in pH 10 aqueous solution (the effective pH at its active site is unknown, however), we have also examined the deprotection of **5** in pH 10 borate buffer. The products are, similarly, Co(I)-B_{12s} (λ_{max} = 389 nm), CO_3^{2-} (detected as $BaCO_3$), and $HOCH_2CHO$ (by mass balance), but again no (formylmethyl)cobalamin.

An important feature of the deprotection of **5** (eq 6) is that the products are exactly and only those predicted for base-on B_{12} on the basis of our earlier B_{12}-model work (eq 7).[5b] The Costa



(7a)

(7b)

$Co[C_2(DO)(DOH)_{pn}]$ B_{12} model (eq 7) was picked because its Co^{III}/Co^{II} and Co^{II}/Co^{I} redox potentials closely match those of

(10) Finke, R. G.; Schiraldi, D. A.; Mayer, B. J. *Coord. Chem. Rev.* **1984**, *54*, 1.

(11) (a) Wayland, B. B.; Van Voorhees, S. L.; Del Rossi, K. L. *J. Am. Chem. Soc.* **1987**, *109*, 6513. (b) Note that the stereospecificity seen in Golding et al.'s elegant Co(III)-π-complex studies means that this type of π-complex alone cannot explain the partial to complete racemization seen in the enzymic studies.[10,23] Golding, B. T.; Holland, H. L.; Horn, U.; Sakrikar, S. *Angew. Chem., Int. Ed. Engl.* **1970**, *9*, 959.

(12) The Rh^{II}/Rh^{I} reduction potential of −1.4 V (SCE) at 25 °C is an estimated, upper (positive) limit. It is obtained from one of the few available Rh(porphyrin) $E_{1/2}$ measurements (they are complicated by Rh(II) dimerization), $E_{1/2}$ = −1.63 V SCE at −78 °C for Rh(TPP)-$[NH(CH_3)_2]_2Cl$,[12a,12b] and by assuming a $\delta E_{1/2}/\delta T$ of 3 mV/°C (the maximum value from tables provided in ref 12c,d; a more typical $\delta E_{1/2}/\delta T$ is 1 mV/°C). The difference between a relatively electron-donating *octaethylporphyrin* vs relatively electron-withdrawing *tetraphenyp*orphyrin should also shift the Rh(OEP) value negative of the −1.4-V limit. Note that the formally dinegative octaethylporphyrin ligand (vs uninegative Co(corrin)) is one important factor contributing to the more negative Rh(OEP) $E_{1/2}$ value. The greater electronegativity of Co *vs* that of Rh is another. (a) Kadish, K. M.; Yao, C.-L.; Anderson, J. E.; Cocolios, P. *Inorg. Chem.* **1985**, *24*, 4515. (b) Kadish, K. M. *Prog. Inorg. Chem.* **1986**, *34*, 435. (c) deBethune, A. J.; Licht, T. S.; Swendeman, N. *J. Electrochem. Soc.* **1959**, *106*, 616. (d) Van Duyne, R. P.; Reilley, C. N. *Anal. Chem.* **1972**, *44*, 142.

(13) This synthesis and study is patterned after our successful work with the carbonate-protecting group and the B_{12}-model complex $Co[C_2(DO)(DOH)_{pn}]$ (see eq 7).[5] Detailed mechanistic studies show, for example, that initial attack of MeO^- occurs, as expected, at the carbonate carbonyl carbon.[5]

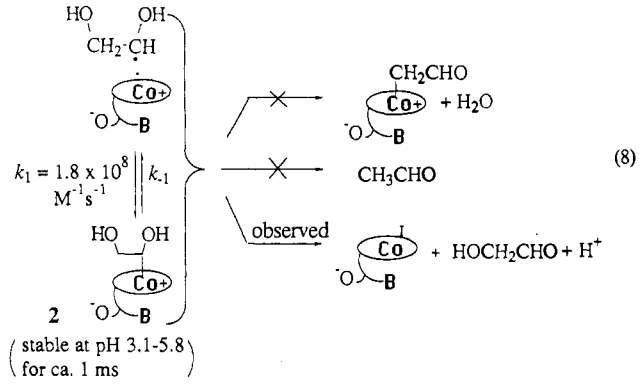
(14) (a) As shown in eq 5 and as detailed elsewhere,[14b] 250 mg of B_{12a} in methanol was reduced with Zn to Co(I)-B_{12s} under an inert (N_2) atmosphere. After the addition of 50 equiv of freshly distilled alkylating reagent, chloroethylene carbonate, and after the reaction changed color (from black to red, about 10–15 min, under dim light), the solution was desalted via the phenol extraction method.[14c] Pure **5** was separated from the reaction mixture by using a cellulose cation-exchange column (CM-23 (Whatman)) in 33% yield. (b) Wang, Y. Ph.D. Thesis, University of Oregon, Aug 1988. (c) Dolphin, D. In *Methods in Enzymology*; Academic Press: New York, 1971; Vol. XVIII, p 34.

(15) HPLC, two peaks at 43 and 58 min (52% and 48% relative intensity, respectively), C_{18} reversed-phase column, isocratic 25% MeOH/75% 0.01 M pH 7 aqueous phosphate buffer. The ^1H NMR spectrum (Figure A, supplementary material) exhibits, for example, (only) two $H_{C\text{-}10}$ peaks (6.75 and 6.72 ppm) and two $H_{C\text{-}20}$ peaks (0.67 and 0.73 ppm).

(16) The exceedingly air-sensitive Co(I)-B_{12s} was detected in a 0.1-mm path length cell (i.e. at higher, 4×10^{-3} M B_{12s}, concentration) by its characteristic λ_{max} at 389 nm. $CH_3OCOOCH_3$ was quantified by IR spectroscopy (1755 cm^{-1}). No attempt was made to determine the yield of glycolaldehyde, as it has been detected previously (eq 7b),[5] and control experiments indicate it is unstable under the methanolysis conditions.[5]

B_{12}.[18] Since an axial 1,5,6-trimethylbenzimidazole base shifts the Costa model Co^{II}/Co^{I} redox potential 170 mV positive (more easily reduced) thereby changing the products from Co(II) to Co(I) (eq 7b), and since base-on B_{12} yields similar products (eq 6), it seems likely that the redox potentials are a key to the observed cobalt (and rhodium, vide infra) reactivity. *The rhodium octaethylporphyrin reaction (eq 4) is a very poor cobalt corrin (B_{12}) model in this regard.* The Co^{II}/Co^{I} $E_{1/2}$ of B_{12} ($Co^{II}_{base\text{-}on}$ to $Co^{I}_{base\text{-}off}$) is −0.85 V (SCE),[19] while the Rh^{II}/Rh^{I} $E_{1/2}$ of Rh(OEP) is at least 0.5 V more difficult to reduce, $E_{1/2}$ negative of −1.4 V (SCE).[12] This offers a highly suggestive explanation for why the observed path for cobalt (i.e. the formation of nonenzymic redox side products (eq 6)) does not occur for the Rh(OEP) "B_{12} model"—it is not available.[20,21]

An alternative explanation is that acidic (eq 4b) rather than basic (eq 6) conditions are required for the cobalt-participation, π-complex mechanism (i.e. that our model and its basic conditions (eq 6 and 7) do not mimic the enzyme active site). However, Meyerstein's data[22] effectively extend our results to pH 3.1—with identical findings and conclusions. Meyerstein, who used pulse radiolysis to generate $^{\bullet}CH(OH)CH_2OH$ in the presence of Co(II)-B_{12r}, saw only the same redox side reactions (e.g. Co(I)-B_{12s} formation) at pH 3.1 and 5.8 (eq 8).[22] Thus, although acidic



(8)

conditions are well-known to favor Co(III)-π-complex formation from (β-hydroxyalkyl)cobalamins,[4,11] the *kinetically preferred* pathway when an *α-hydroxy* group is present is not Co(III)-π-complex formation but rather the redox side reaction, *even at pH 3.1*.[22] Furthermore, it is possible to provide strong arguments against the specific Co(III)-π-complex mechanism recently proposed[11] on the basis of five independent types of literature evidence.[23]

In summary, all of the available enzymic and chemical model evidence argues that cobalt participation in the rearrangement step of diol dehydratase *is not only unnecessary, it leads to a redox side reaction.* The protein, and not the B_{12} cofactor, appears to be controlling the rearrangement step(s) and their stereochemistry. Furthermore, the enzymic stereochemical studies[9,10] *demand* some nonstereospecific pathway other than a (stereospecific)[11b] cobalt-participation pathway (eq 3′a,b). To date, only the participation of protein-bound radicals[3a,5,10] (eq 3a,b) can explain *all* of the available chemical model,[17b] enzyme stereochemical, and

(17) (a) Preparing authentic (formylmethyl)cobalamin involved resolving the controversy[4a,f] concerning its preparation and identity; details[14b] will be described elsewhere.[17c] (b) Yet another piece of evidence against cobalt participation comes from the study of (formylmethyl)cobalamin (**4**). Neither we[17c] nor others[4] have been able to demonstrate the Co–C homolysis of **4** as required by cobalt-participation mechanisms, $Co\text{–}CH_2CHO$ (**4**) → Co(II)-B_{12r} + $^{\bullet}CH_2CHO$. Instead, Co–C heterolysis occurs.[17c] (c) Wang, Y.; Finke, R. G. Manuscript in preparation.

(18) Elliott, C. M.; Hershenhart, E.; Finke, R. G.; Smith, B. L. *J. Am. Chem. Soc.* **1981**, *103*, 5558.

(19) (a) De Tacconi, N. R.; Lexa, D.; Savêant, J. M. *J. Am. Chem. Soc.* **1979**, *101*, 467. (b) Lexa, D.; Savêant, J. M. *J. Am. Chem. Soc.* **1976**, *98*, 2652. (c) Although the $E_{1/2}$ values for protein-bound B_{12} are almost surely somewhat different from the solution $E_{1/2}$ values, no evidence or precedent exists to suggest that they will be changed ≥0.5 V, much less in the direction needed to align the Co(corrin) $E_{1/2}$ values with those of Rh(OEP).

(20) (a) Moreover, $E_{1/2}$ of a typical α-hydroxy radical, for example $(CH_3)_2C(OH)^{\bullet}$, $E_{1/2}$ = ca. −1.3 V (SCE),[20b] shows that it can reduce Co(II)-B_{12r} *but not* Rh^{II}*(OEP)*, providing confirming evidence for this statement. (The more reducing $(CH_3)_2CO^{\bullet-}$, $E_{1/2}$ negative of −2.1 V (SCE), should be able to reduce even Rh^{II}(OEP).) (b) Lilie, V. J.; Beck, G.; Henglein, A. *Ber. Bunsenges. Phys. Chem.* **1971**, *75*, 458.

(21) (a) Consistent with this is Schrauzer's early experiment[4g] providing negative evidence for the formation of **2** via a reaction analogous to the Rh(OEP) reaction:

$$Co(I)\text{-}B_{12s} + H^+ + HOCH_2CHO \not\rightarrow \mathbf{2}\ \text{(not observed)}$$

pH 4.7–12 was tried

The failure of this reaction at pHs >4.7 is not surprising as the pK_a of H–Co(III)-B_{12a} is estimated as ca. 1.[21b] While this suggests that one should repeat this work at lower pH values, the oxidation of Co(I)-B_{12s} by H^+ and the acid sensitivity of any (formylmethyl)cobalamin or CH_3CHO produced will mitigate against the success of such studies. (b) Lexa, D.; Saveant, J.-M. *Acc. Chem. Res.* **1983**, *16*, 235.

(22) (a) Elroi, H.; Meyerstein, D. *J. Am. Chem. Soc.* **1978**, *100*, 5540. (b) Mulac, W. A.; Meyerstein, D. *Ibid.* **1982**, *104*, 4124.

(23) It is necessary here, in response to a reviewer's comments, to address why the *cobalt corrin* (B_{12}) mechanism that others proposed (see Scheme I in ref 11) can be ruled out, unless unprecedented enzymic effects are operative, on the basis of at least five independent types of literature evidence. Three key steps in that mechanism are $Co\text{–}CH(OH)CH_2OH$ formation, its π-complex rearrangement to $Co\text{–}CH_2CH(OH)_2$, and the avoidance of a (too stable) $Co\text{–}CH_2CHO$ (formylmethyl)cobalamin intermediate by the hypothetical step $Co\text{–}CH_2CH(OH)_2 \rightarrow Co^{\bullet} + {}^{\bullet}CH_2CH(OH)_2$ (see steps iii–v of Scheme I in ref 11, reproduced below).

$$Co^{II\bullet} + {}^{\bullet}CH(OH)CH_2OH \rightleftarrows Co\text{–}CH(OH)CH_2OH \quad \text{(iii)}$$

$$Co\text{–}CH(OH)CH_2(OH) \rightleftarrows Co\text{–}CH_2CH(OH)_2 \quad \text{(iv)}$$

$$Co\text{–}CH_2CH(OH)_2 \rightleftarrows Co^{II\bullet} + {}^{\bullet}CH_2CH(OH)_2 \quad \text{(v)}$$

First, and as previously summarized elsewhere,[5a,10] all the enzymic evidence argues that step iii never occurs. In fact, all ESR-detected—*and kinetically competent*—Co(II)-B_{12r} and substrate radical are >10 Å apart.[5a,10] Second, Meyerstein's[22] and our earlier (and our present) chemical precedent[5] shows that, from pH 10 to pH 3, neither steps iii nor iv occur; rather, the kinetically preferred pathway is a nonenzymic redox side reaction to Co(I) + $HOCH_2CHO$. Third, careful analysis shows that the proposed step v (proposed to avoid the two-stable $Co\text{–}CH_2CHO$ intermediate) is extremely unlikely. It consists of a reaction, step v, *uphill (in solution) by ca. 30 kcal/mol* (the $Co\text{–}CH_2R$ BDE),[6] that must be faster than the exergonic[24] dehydration $Co\text{–}CH_2(OH)_2 \rightleftarrows Co\text{–}CH_2CHO + H_2O$. Not only is the required 10^{12}–10^{20} enzymic rate acceleration of step v essentially unprecedented[6] (it is unprecedented if one notes that Co–C cleavage of alkylcobalamins lacking the adenine moiety of coenzyme B_{12} are accelerated only ca. 10× when bound to diol dehydratase, see: Toraya, T.; Ishida, A. *Biochemistry* **1988**, *27*, 7677), but it begs the question of "Why would the enzyme evolve to use such an energetically improbable mechanism?" The only possible gain is catalysis of the rearrangement step (step iv), but the enzyme can much more easily accomplish this via nearly thermoneutral, well-precedented,[5,10] and fast (ca. 10^6 sec^{-1}) radical fragmentation/readdition reactions (over nine precedents exist here[5,10]). Moreover, a chemically unprecedented but protein-assisted OH migration is also plausible. Fourth, the enzymic stereochemical evidence of apparent partial racemization (propanediol) or complete racemization (labeled ethylene glycol) is the opposite of the complete stereospecificity expected on the basis of established Co(III)-π-complex chemistry.[11b] This forces proponents of the π-complex mechanism to explain the stereochemistry (as done elsewhere)[10] via the (enzyme-bound) $^{\bullet}CH(OH)CH_2OH$ and $^{\bullet}CH_2CH(OH)_2$ radicals (i.e. the radicals in steps iii and v). This in turn strongly suggests that since the protein already controls the substrate stereochemistry, it likely also controls the rearrangement step. The lack of consideration of the enzymic stereochemical results has caused proponents of the π-complex mechanism to miss this important point.[11] Fifth, there is still no acceptable chemical precedent for the proposed *cobalt-corrin* assisted rearrangement, step iv. Instead, a redox side reaction occurs anytime Co(II)• and $^{\bullet}CH(OH)CH_2OH$ come in contact.[11]

(24) (a) In fact and despite reports to the contrary, the proposed hydrate form, $Co\text{–}CH_2CH(OH)_2$, of (formylmethyl)cobalamin, $Co\text{–}CH_2CHO$,[14b] has not been directly observed. A number of papers[14b,24b] unequivocally demonstrate that hyperconjugation, $Co\text{–}CH_2CHO \leftrightarrow Co^{+}CH_2{=}CHO^{-}$, dominates the electronic structure and physical properties (e.g. IR, NMR, carbonyl polarity) of species with β-carbonyl groups like (formylmethyl)cobalamin. This stabilizes (formylmethyl)cobalamin, shifting the dehydration equilibria (eq 3′b) to the right so that the hydrated form has only been inferred via oxygen-exhange experiments.[24c] (b) For lead references see: Masuda, H.; Taga, T.; Sugimoto, H.; Mori, M. *J. Organomet. Chem.* **1984**, *273*, 385. Brown, K. L.; Zahonyi-Budo, E. *J. Am. Chem. Soc.* **1982**, *104*, 4117. Brown, K. L.; Ramamurthy, S. *Organometallics* **1982**, *1*, 413. (c) Curzon, E. H.; Golding, B. T.; Wong, A. K. *J. Chem. Soc., Chem. Commun.* **1982**, 63.

other evidence.[5,10] However, the chemical composition (e.g. both acidic and basic diol substrate binding sites?) and the structure of the active site, and thus further mechanistic insights, will require an X-ray diffraction structural analysis for at least one of the B_{12}-dependent enzymes.[25] Such crystallographic results will also test the one assumption on which this work, and the interpretations herein, are based, the assumption that the enzyme chemistry differs in degree, *but not in kind*, vs a *close* solution mimic.[26]

(25) The crystallization and preliminary diffraction data of an inactive form of methylmalonyl-CoA mutase has been published recently: Marsh, N.; Leadlay, P. F.; Evans, P. R. *J. Mol. Biol.* **1988**, *200*, 421.
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Acknowledgment. We wish to thank the NIH for their financial support (Grant DK-26214).

Supplementary Material Available: Figure A, showing a ^1H NMR spectrum of **5** (1 page). Ordering information is given on any current masthead page.

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Received September 29, 1988

Articles

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Non-Metal Redox Kinetics: Hypochlorite and Hypochlorous Acid Reactions with Sulfite

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Received July 6, 1988

Pulsed-accelerated-flow spectroscopy is used in the ultraviolet region to measure pseudo-first-order rate constants in the range 5000–107 000 s^{-1} for the reactions of excess SO_3^{2-} with HOCl and OCl^- in the presence of HCO_3^-/CO_3^{2-} buffer. The rate constant for the reaction of HOCl and SO_3^{2-} is so large (7.6×10^8 M^{-1} s^{-1}, 25.0° C, $\mu = 0.5$) that the rate of proton transfer from water or from HCO_3^- to OCl^- can limit the reaction velocity. A mechanism with Cl^+ transfer to sulfur is proposed via a reactive intermediate, $HOClSO_3^{2-}$, that decomposes by itself or with HCO_3^- assistance to form $ClSO_3^-$. The subsequent hydrolysis of $ClSO_3^-$ to give Cl^- and SO_4^{2-} is slow by comparison (270 s^{-1}). The contribution of the HOCl path is small only above 0.05 M OH^-, where the rate becomes base-independent due to the reaction between OCl^- and SO_3^{2-} (2.3×10^4 M^{-1} s^{-1}, 25.0 °C, $\mu = 0.5$). This reaction may proceed by oxygen atom transfer; the rate constant is a factor of 3.3×10^4 smaller than the Cl^+-transfer rate constant for HOCl and SO_3^{2-}.

Introduction

The oxidation of sulfite ion by hypochlorite is fast and has a simple stoichiometry (eq 1). A rate constant for this reaction

$$OCl^- + SO_3^{2-} \xrightarrow{k_1} Cl^- + SO_4^{2-} \quad (1)$$

was initially measured by Lister and Rosenblum[1] 25 years ago by use of a continuous-flow mixer mounted on a trolley that moved the observation tube through the light path of a spectrophotometer. The value they obtained for k_1 was 1.1×10^4 M^{-1} s^{-1} (30.0 °C, $\mu = 0.62$), which was independent of base concentration from 0.05 to 0.20 M NaOH. Although they discussed the reaction as an example of an oxygen atom transfer process, there is no direct evidence that it occurs by this path.

Halperin and Taube[2] measured ^{18}O exchange for the reactions of sulfite with chlorate and with chlorite and concluded that oxygen atom transfer occurred in both cases. They ruled out a mechanism that involved intermediates with S–Cl bonds for these reactions. However, their results with hypochlorous acid and sulfite showed no evidence of oxygen atom transfer from the oxidizing agent to sulfite. This reaction was carried out in 0.2 M HCl, and the experiment was regarded as inconclusive because of rapid oxygen exchange between HOCl and H_2O via the Cl_2 equilibrium. They suggested two possible modes of reaction: one by oxygen atom transfer (where eq 1 is the elementary reaction) and the other via formation of chlorosulfate followed by its rapid hydrolysis. They did not reach any conclusions about which mechanism was predominant. The high concentration of HCl used in their study complicated the chemistry because of the formation of HOCl, Cl_2, SO_3H^-, and SO_2.

In recent work Yiin and Margerum[3] showed by stopped-flow indicator experiments that chlorosulfate is indeed an intermediate in the reaction of hypochlorous acid with sulfite (eq 2 and 3). The rate constant (k_h) for the hydrolysis of $ClSO_3^-$ at 25.0 °C is 270 s^{-1}.

$$HOCl + SO_3^{2-} \rightarrow OH^- + ClSO_3^- \quad (2)$$

$$ClSO_3^- + H_2O \xrightarrow{k_h} SO_4^{2-} + Cl^- + 2H^+ \quad (3)$$

Srivastava et al.[4] used a flow thermal method to study the OCl^- and SO_3^{2-} reaction. They reported a k_1 value of 6.75×10^3 M^{-1} s^{-1} at 30 °C and did not control the ionic strength, which varied from 0.008 to 0.036. They stated that the reaction rate did not change over the pH range 9.4–11.5. This is contrary to our results, because we observe an enormous increase in rate below pH 12. It may be that their measuring device was not capable of following faster reactions or that they were observing the hydrolysis of $ClSO_3^-$ rather than its formation. Although their discussion[4] suggests that HOCl rather than OCl^- is the reactant, this disagrees with their own lack of a pH effect.

Walker[5] used stopped-flow spectroscopy to estimate a rate constant of 2.5×10^4 M^{-1} s^{-1} for the reaction of OCl^- and SO_3^{2-} at 25.0 °C, $\mu = 0.50$. He examined a wider range of OH^- concentrations (0.25–0.005 M NaOH) than had been tested by Lister[1] and observed a large increase in the rate below 0.03 M [OH^-]. Below pH 11.5, the reactions are too fast to measure by conventional stopped-flow instruments. However, the pulsed-accelerated-flow (PAF) method[6,7] has been used to measure first-order rate constants as large as 124 000 s^{-1}. If a sufficiently large absorbance change is used, even larger first-order constants (180 000 s^{-1}) can be measured.[8] Until recently, PAF instru-

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