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Facile α/β Diastereomerism in Organocobalt Corrinoids. Studies of the Interconversion of Diastereomers by Thermolysis, Photolysis, and Cobalt-to-Cobalt Alkyl Group Transfer[1]

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Nonalkylated cobinamides in the Co^{III}, Co^{II}, and Co^{I} oxidation states have been shown to catalyze the interconversion of α- and β-methylcobinamide (CH_3Cbi) by direct cobalt-to-cobalt methyl group transfer. In every case, the final product composition, starting from either diastereomer, is ca. 95% β-CH_3Cbi and 5% α-CH_3Cbi, the same product distribution obtained by reductive alkylation of combinamide by CH_3I. It is consequently concluded that this is the equilibrium product distribution for the CH_3Cbi's and that α-CH_3Cbi and β-CH_3Cbi come to equilibrium during synthesis by reductive alkylation. For all other alkylcobinamides (RCbi's, R = CH_3CH_2, CF_3CH_2, $NCCH_2$, CF_2H, and CF_3), isomerization could not be observed upon treatment with cobinamide in any oxidation state and these cobalt-to-cobalt alkyl group transfers ($R \neq CH_3$), if they occur at all, are shown to be several orders of magnitude slower than methyl group transfer. The previously reported facile photoinduced isomerization of α- and β-RCbi's is shown to be completely inhibited by a relatively low concentration of the radical trap 4-hydroxy-2,2,6,6-tetramethylpiperidinyloxy (4-HTEMPO), suggesting that this isomerization requires escape of the alkyl radical and cob(II)inamide from the solvent cage followed by recombination. The proposed mechanism is thus diffusion limited and suggests that the observed ratio of diastereomers, 2-3:1, β/α, regardless of R, represents the kinetically controlled distribution of products for the prototypical RCbi synthesis reaction, the diffusional combination of R$^\bullet$ and cob(II)inamide. A thermally-induced isomerization has also been demonstrated for R = CH_3, CH_3CH_2, CF_3CH_2, $NCCH_2$, and $CH_3CH_2OCH_2CH_2$ at 70 °C, which leads to a final equilibrium distribution of products in which the β diastereomer predominates by >10:1 regardless of R. Radical trapping experiments suggest that thermally-induced isomerization probably results from thermal Co-C bond homolysis for R = CH_3CH_2 and CF_3CH_2, but that thermally-induced isomerization of the CH_3Cbi's probably occurs via cobalt-to-cobalt transfer due to unavoidable trace contamination with nonalkylated cobinamide. It is concluded that reductive alkylation of cobinamide by RX leads to a steady state product distribution which is under neither thermodynamic nor kinetic control, except for R = CH_3, for which the diastereomers come to equilibrium via cobalt-to-cobalt methyl group transfer to either cob(I)inamide or cob(II)inamide.

Introduction

In recent publications,[2,3] we have reported that the reductive alkylation of cobalt(III) cobinamides[4] with alkyl halides results in the formation of pairs of diastereomeric α- and β-RCbi's,[1] as earlier described for methylcobalt corrinoids by Friedrich and co-workers.[5-10] Somewhat surprisingly, the relative proportions of the diastereomers vary widely with R. Thus, reduction of Factor B[11] with Zn/acid followed by treatment with CH_3I leads to a high yield of alkylated product, only a trace of which (4%) is the α diastereomer, and treatment with CH_3CH_2Br yields only the β diastereomer, β-CH_3CH_2Cbi.[3] In contrast, when CF_3CH_2I, $ROCH_2CH_2Br$'s, or $NCCH_2Br$ is the alkylating agent, 38 to 87% of the alkylated product is the α diastereomer.[2,3,12] In the extreme, alkylation with CF_3I, which produces mixtures of CF_3Cbi's and CF_2HCbi's due to reductive defluorination of the former under reaction conditions,[3,13] gives product mixtures which are <8% β diastereomer.[3] These results are surprising since it is generally assumed that the β ("upper") face of cobinamide, with its upward projecting a, c, and g acetamide side chains, is less sterically crowded than the α ("lower") face which is bracketed by the downward projecting b, d, and e propionamides and the secondary amide f side chain. Thus, either kinetic or thermodynamic control of the diastereomeric outcome of reductive alkylation would be expected to favor the β diastereomer on steric grounds.

An analogous result is obtained when H_2OCbl[1] is reductively alkylated at low pH, where the axial nucleotide of the β-RCbl product is uncoordinated and protonated.[2,3,12,14-16] In contrast, at neutral pH, where the axial nucleotide of the product β-RCbl remains coordinated, only β-RCbl's are formed. A study of the pH dependence of the diastereomeric outcome of the reductive alkylation of H_2OCbl with CF_3CH_2I, $NCCH_2Br$, and CF_3I, showed that at low pH, the diastereomeric composition of the product mixtures was similar to that obtained by reductive alkylation of cobinamides.[14] As the pH was increased, the proportion of the α diastereomer decreased until the product was nearly all β diastereomer at neutral pH. This study suggested that the pH dependence of the diastereomeric composition of the product was determined by the pK_a for the base-on/base-off reaction of the β-RCbl product,[15,16] rather than by any acid-base behavior of the reactants. This, in turn, suggested that the products are under thermodynamic control and that some mechanism exists which allows equilibration of the diastereomers under synthesis conditions.

One possibility for diastereomer interconversion under synthetic conditions is cobalt-to-cobalt alkyl group transfer. Such group transfer reactions have been shown to occur for methylcobalt corrinoids,[17-19] where, for instance, the methyl group of β-CH_3Cbl can be transferred to diaquacob(III)inamide ($(H_2O)_2$Cbi[1]),[17] cob(II)inamide,[19] or cob(I)inamide.[19] Such methyl group transfer reactions to cobalt(I), cobalt(II), and cobalt(III) complexes have also been shown to occur among vitamin B_{12} model complexes[20-24]

(1) In α-alkylcobalt corrinoids, the organic ligand occupied the "lower" axial ligand position, while in β-alkylcobalt corrinoids, the organic ligand is in the "upper" position. Abbreviations: α-RCbi = α-alkylcob(III)inamide, β-RCbi = β-alkylcob(III)inamide, α-RCbl = α-alkylcob(III)alamin, β-RCbl = β-alkylcob(III)alamin, $(H_2O)_2$Cbi = diaquacob(III)inamide, H_2OCbl = aquacob(III)alamin, CNCbl = cyanocobalamin, TEMPO = 2,2,6,6-tetramethylpiperidinyl-1-oxy, 4-HTEMPO = 4-hydroxy-2,2,6,6-tetramethylpiperidinyl-1-oxy, CAPS = 3-(cyclohexylamino)propanesulfonic acid.

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including cobalt bis(dioximato) complexes,[20,23,24] cobalt tetraaza macrocyclic complexes,[24] and between such model cobalt complexes and cobalt corrinoids.[24] While cobalt-to-cobalt alkyl group transfers in cobalt corrinoids have only been demonstrated for methyl group transfer,[17-19] Dodd et al.[23] have demonstrated alkyl group transfer from alkylcobalt bis(dioximato) complexes to cobalt(II) and cobalt(I) bis(dioximato) complexes for a wide variety of alkyl groups, although the rate constants for such transfer reactions decrease markedly with increasing steric bulk of the organic ligand.

We now describe the results of experiments designed to determine if cobalt-to-cobalt alkyl group transfer reactions can lead to isomerization of α- and β-RCbi's. In addition, we report the observation of a thermally induced isomerization of α- and β-RCbi's for several R groups and further studies of the recently reported[25] photoinduced isomerization. As detailed below, with the exception of R = CH_3, which apparently reaches equilibrium due to cobalt-to-cobalt alkyl group transfer during synthesis, none of these pathways can be responsible for diastereomer interconversion under synthesis conditions.

Experimental Section

CNCbl[1] was from Sigma, and 4-HTEMPO[1] was from Aldrich. Factor B[11] was prepared by a modification[15] of the method of Renz.[26] $(H_2O)_2$Cbi was prepared by reduction of Factor B to cob(II)inamide with zinc in 10% acetic acid[14] followed by cannula transfer into an aerobic 0.1 M HCl solution for reoxidation. This method of reoxidation minimizes the formation of stable yellow corrinoids (xanthocorrinoids).[27,28] Final purification was effected by semipreparative HPLC.[29,30] Alkylcobinamides were prepared by reductive alkylation with appropriate alkyl halides in zinc/acetic acid as described previously,[2,3,12,30] and the diastereomers were separated by semipreparative HPLC. In cases where one of the two diastereomers is a minor product (β-CF_2HCH_2Cbi, α-CH_3CH_2Cbi) or is not obtained by reductive alkylation (α-CH_3CH_2Cbi), this diastereomer was obtained by anaerobic photoinduced isomerization of the major diastereomer, as described previously.[25]

RCbi's were quantitated by UV–visible spectroscopy after conversion to dicyanocobinamide (ϵ_{368} = 3.04 × 10^4 M^{-1} cm^{-1})[31] by photolysis in excess cyanide. The progress of isomerization reactions was followed by analytical HPLC. Reaction products were quantitated by using the averaged values of chromatogram integrations obtained by UV detection at 254 and 350 nm after correction for differing molar absorptivities as described previously.[14] Argon purged through a vanadium oxide gas train was used throughout.

For alkyl group transfer reactions, cob(II)inamide was generated by controlled potential reduction of $(H_2O)_2$Cbi at a mercury pool electrode at −0.80 V under anaerobic conditions in pH 7.0 phosphate buffer. After cessation of the current, the potential was switched off and the solution was transferred by cannula into an anaerobic solution of the appropriate RCbi. Alternatively, $(H_2O)_2$Cbi was reduced to cob(II)inamide with zinc/pH 7.0 phosphate for 30 min and then transferred through a cannula with a glass wool plug into a solution of RCbi. For group transfer reactions to cob(I)inamide, $(H_2O)_2$Cbi was reduced by controlled potential reduction at −1.3 V in 0.05 M CAPS[1] buffer, pH 9.5, for 2 h. After cessation of current flow, the potential was turned off and the solution was transferred by cannula into an anaerobic solution of the appropriate RCbi.

Photoinduced isomerization reactions were conducted by irradiation with a 3-V tungsten lamp as described previously.[25] Thermally-induced isomerizations at 70 °C were carried out in pH 7.0 phosphate buffered solutions in small glass vials continuously purged with argon and incubated in a 70 °C water bath. Thermally induced isomerizations at 135 °C were conducted in sealed glass ampules, the contents of which were made anaerobic by three consecutive freeze/pump/thaw cycles under argon, and sealed under vacuum. The ampules were incubated at 135 °C and removed periodically for analysis by HPLC.

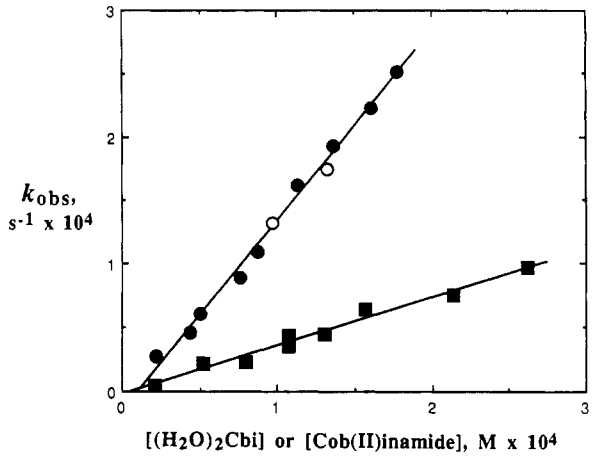

Figure 1. Plots of the observed first-order rate constants, k_{obs}, for the isomerization of α-CH_3Cbi catalyzed by cob(III)inamide or cob(II)inamide vs the concentration of cobinamide catalyst. (■) cob(III)inamide-catalyzed isomerization (eqs 1–3). The solid line is a linear regression line, slope 0.378 ± 0.021 M^{-1} s^{-1}, intercept = −(2.02 ± 2.98) × 10^{-6} s^{-1}, r^2 = 0.980. (○, ●), cob(II)inamide-catalyzed isomerization (eq 4) where cob(II)inamide was generated by controlled potential reduction at −0.8 V (solid symbols) or by reduction with zinc/pH 7.0 phosphate (open symbols). The solid line is a linear regression line, slope 1.51 ± 0.05 M^{-1} s^{-1}, intercept = −(1.80 ± 0.48) × 10^{-5} s^{-1}, r^2 = 0.991.

Results

Isomerization by Cobalt-to-Cobalt Alkyl Group Transfer. When a solution of 2.1 × 10^{-4} M α-CH_3Cbi was treated with equimolar $(H_2O)_2$Cbi at 25 °C in pH 7.0 phosphate buffer, a relatively slow ($t_{1/2}$ = 136 min) isomerization ensued which led to a final product mixture which was 94% β-CH_3Cbi. The progress of this reaction, as monitored by HPLC, was first-order as determined by the linearity of plots of ln ($f_\alpha - f_\alpha^\infty$) vs time, where f_α is the fraction of methylcobinamide as the α diastereomer at time t, and f_α^∞ is the fraction of methylcobinamide as the α diastereomer after the cessation of changes in the product composition. The slopes of such plots provided values of the observed rate constant, k_{obs}, for cob(III)inamide-catalyzed isomerization. The average composition of the final reaction mixture was 6 ± 3% α-CH_3Cbi. Similarly, when β-CH_3Cbi was treated with $(H_2O)_2$Cbi, a small amount (6.5 ± 0.9%) of β-CH_3Cbi was converted to α-CH_3Cbi. The small accumulation of the α diastereomer starting from β-CH_3Cbi prevented determination of observed rate constants. The similarity of the composition of the final reaction mixtures starting from either diastereomer suggests that equilibrium is attained and that the β diastereomer is thermodynamically favored by ca. 20:1. The average value of f_α^∞ from all observations of cob(III)inamide-catalyzed isomerization (N = 9) was 6 ± 3% α-CH_3Cbi. We note that reductive alkylation of Factor B with CH_3I provides a product mixture of similar composition (4% α-CH_3Cbi).[3]

Figure 1 shows the effect of variation of the concentration of $(H_2O)_2$Cbi on the observed, first-order rate constants for isomerization. The apparently linear dependence of k_{obs} on [$(H_2O)_2$Cbi] suggests the simple kinetic scheme of eq 1, for which the rate law of eqs 2 and 3 holds. Thus, the slope of the plot

$$\alpha\text{-}CH_3Cbi + (H_2O)_2Cbi \underset{k_{-2}^{Co^{III}}}{\overset{k_2^{Co^{III}}}{\rightleftharpoons}} \beta\text{-}CH_3Cbi + (H_2O)_2Cbi \quad (1)$$

$$\ln (f_\alpha - f_\alpha^\infty) = -(k_{-2}^{Co^{III}} + k_2^{Co^{III}})[(H_2O)_2Cbi]t + \ln (k_2^{Co^{II}}/(k_2^{Co^{II}} + k_{-2}^{Co^{III}})) \quad (2)$$

$$k_{obs} = -(k_{-2}^{Co^{III}} + k_2^{Co^{III}})[(H_2O)_2Cbi] \quad (3)$$

of k_{obs} vs [$(H_2O)_2$Cbi] (Figure 1, 0.378 ± 0.021 M^{-1} s^{-1}) is equal to the sum of the forward and reverse rate constants for transfer

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of the CH_3 group to $(H_2O)_2$Cbi. Using a value of 16.5 for the equilibrium constant, the individual rate constants are $k_2^{Co^{III}} \sim 0.36\ M^{-1}\ s^{-1}$ and $k_{-2}^{Co^{III}} \sim 0.022\ M^{-1}\ s^{-1}$.

All attempts to demonstrate similar alkyl group transfers to cob(III)inamide for other RCbi's failed. Thus, for R = CH_3CH_2, $CNCH_2$, CF_3CH_2, CF_3, and CF_2H, incubation of either the α or β diastereomer ((1.0–2.0) × 10^{-4} M) with $(H_2O)_2$Cbi ((1.0–3.0) × 10^{-4} M) for 5 days at 25 °C failed to produce any isomerization. Assuming a 2% HPLC detection limit[3] and an equilibrium diastereomer ratio similar to that for the CH_3Cbi's (ca. 20:1, β:α, vide infra), the observed first-order rate constants for cob(III)inamide-catalyzed isomerization could be estimated to be $<1.8 \times 10^{-8}\ s^{-1}$. This, in turn, leads to an estimate of $<1.8 \times 10^{-4}\ M^{-1}\ s^{-1}$ for the sum of $k_2^{Co^{III}}$ and $k_{-2}^{Co^{III}}$ for these R; i.e., they are at least (2.15×10^3)-fold less reactive than the CH_3Cbi's.

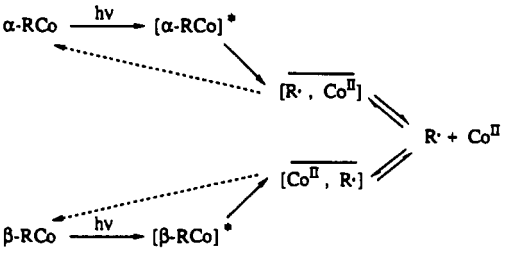
Similarly, cob(II)inamide, generated by controlled potential reduction at −0.8 V[32] in pH 7.0 phosphate buffer or by reduction with zinc/pH 7.0 phosphate, catalyzed the isomerization of α- and β-CH_3Cbi's. As was the case for cob(III)inamide, the composition of the final reaction mixture (7.1 ± 2.4% α-CH_3Cbi starting from the β diastereomer, 6.9 ± 1.2% α-CH_3Cbi starting from the α diastereomer) was independent of the starting diastereomer and was essentially identical to that obtained by reductive alkylation of Factor B with CH_3I.[3] Plots of ln $(f_\alpha - f_\alpha^\infty)$ vs time were again linear and their slopes provided values of the observed rate constant, k_{obs}, although, again, the small extent of conversion of β-CH_3Cbi to the α diastereomer prevented determination of k_{obs} for that direction. The dependence of k_{obs} on the concentration of cob(II)inamide (Figure 1) was also linear, suggesting the simple scheme of eq. 4. From the slope of this plot and the rate law analogous to eq 2, the value of $k_2^{Co^{II}} + k_{-2}^{Co^{II}}$ was estimated to be $1.51 \pm 0.05\ M^{-1}\ s^{-1}$. By use of a value of 13.1 for the equilibrium constant (from the average end point composition), values of $k_2^{Co^{II}} \sim 1.4\ M^{-1}\ s^{-1}$ and $k_{-2}^{Co^{II}} \sim 0.11\ M^{-1}\ s^{-1}$ could be estimated. Thus, the CH_3Cbi's are about 4-fold more reactive towards methyl group transfer with cob(II)inamide than with cob(III)inamide.

$$\alpha\text{-}CH_3Cbi + \text{cob(II)inamide} \underset{k_{-2}^{Co^{II}}}{\overset{k_2^{Co^{II}}}{\rightleftharpoons}} \beta\text{-}CH_3Cbi + \text{cob(II)inamide} \quad (4)$$

As was the case with cob(III)inamide, none of the other α- or β-RCbi's (R = CH_3CH_2, $CNCH_2$, CF_3CH_2, CF_3, and CF_2H, at (1.3–8.3) × 10^{-5} M) showed any tendency to isomerize when treated with (1.2–3.0) × 10^{-4} M cob(II)inamide for 4 to 5 days. Again, assuming 2% HPLC detection limit[3] and an equilibrium composition of ca. 20:1, β:α, upper limits of (1.6-5.0) × $10^{-4}\ M^{-1}\ s^{-1}$ could be set for $k_2^{Co^{II}} + k_{-2}^{Co^{II}}$ for all R ≠ CH_3. Thus, these RCbi's are at least ((3.0–9.4) × 10^3)-fold less reactive toward cob(II)inamide than the CH_3Cbi's.

Cob(I)inamide, generated by controlled potential reduction at −1.3 V in pH 9.5 CAPS[1] buffer, also catalyzed the interconversion of α- and β-CH_3Cbi. Once again, the composition of the final reaction mixture was independent of the starting diastereomer (5.3 ± 2.1% α-CH_3Cbi starting from the α diastereomer and 5.2 ± 1.7% α-CH_3Cbi starting from the β diastereomer) and was the same as that obtained from cob(III)inamide- and cob(II)inamide-catalyzed isomerization and by reductive alkylation with CH_3I. In the case of cob(I)inamide, the isomerization was extremely rapid. Thus, when 1.0×10^{-4} M α-CH_3Cbi was treated with equimolar cob(I)inamide, the final product composition was obtained as soon as the first sample could be taken (≤30 s). Unfortunately, due to the extreme lability of cob(I)inamide to oxidation by traces of oxygen, it was not possible to work at lower concentrations of cob(I)inamide in order to estimate the rate constant for this isomerization. Assuming that the process is first order in α-CH_3Cbi, a lower limit of 0.14 s^{-1} can be set on k_{obs}. If, in analogy to the cob(III)inamide- (eqs 1–3) and cob(II)inamide-catalyzed isomerizations (eq 4), the cob(I)inamide-catalyzed isomerization is also first order in cob(I)inamide, then a lower limit of $1.4 \times 10^3\ M^{-1}\ s^{-1}$ can be set for $k_2^{Co^I} + k_{-2}^{Co^I}$. In contrast, all of the other α- and β-RCbi's (except for α- and β-$NCCH_2$Cbi which underwent rapid dealkylation in the presence of cob(I)inamide) remained unisomerized when treated with 2.5 × 10^{-4} M cob(I)inamide for 20–50 min. Again, assuming a 2% detection limit, an upper limit of 6.7×10^{-6} to $1.9 \times 10^{-5}\ s^{-1}$ can be set for k_{obs}, and consequently, an upper limit of (2.7–7.6) × $10^{-2}\ M^{-1}\ s^{-}$ can be set for $k_2^{Co^I} + k_{-2}^{Co^I}$. Thus, the cob(I)inamide-catalyzed isomerization of these RCbi's (R = CH_3CH_2, CF_3CH_2, CF_3, and CF_2H) must be at least ((1.9–5.2) × 10^4)-fold slower than that of the CH_3Cbi's.

Scheme I



Isomerization by Anaerobic Photolysis. We have recently reported[25] that low level visible irradiation of anaerobic solutions of α- and β-RCbi's leads to facile interconversion of the diastereomers. In every case studied (R = CH_3, CH_3CH_2, CF_3CH_2, CF_2H, and $NCCH_2$), the final product contained 25–30% α diastereomer and the composition of the final product mixture was independent of the diastereomeric nature of the starting material, although the α diastereomers were considerably more reactive than the β diastereomers. As photolysis of alkylcobalt corrinoids is well known to result in the photohomolysis of the Co–C bond,[33–38] this photoinduced isomerization evidently involves recombination of R· and cob(II)inamide radicals. Alelyunas et al.[39] had previously demonstrated a similar photoinduced isomerization of α-(2-oxo-1,3-dioxolan-4-yl)cobalamin upon anaerobic irradiation under neutral conditions. In this case, since cob(II)alamin is base-on at neutral pH ($pK_{base\text{-}off}$ = 3.10),[14] isomerization goes to completion toward the β diastereomer, as also observed by us for α-CH_3Cbl and α-CH_3CH_2Cbl.[25] Alelyunas et al.[39] further demonstrated that at relatively low concentrations (0.01 M) of the radical trap TEMPO,[1] photolysis of α-(2-oxo-1,3-dioxolan-4-yl)cobalamin led only to dealkylation and not to isomerization. As such low concentrations of TEMPO are expected only to trap free, as opposed to solvent caged, radicals, this observation suggested that photoinduced isomerization requires escape of R· and cob(II)alamin from the solvent cage followed by diffusional recombination with base-on cob(II)alamin to form the β diastereomer.

We have now found similar evidence for the necessity of cage escape in the photoinduced isomerization of α- and β-RCbi's. When a 1.0×10^{-4} M anaerobic solution of β-CH_3Cbi was irradiated in the presence of the radical trap, 4-HTEMPO (1.0×10^{-3} M), no α-CH_3Cbi was produced. Instead, a relatively rapid dealkylation occurred with a first order rate constant of $3.7 \times 10^{-3}\ s^{-1}$. At this very low concentration of radical trap, trapping within the cage is very unlikely, and this observation suggests that cage escape is indeed required for isomerization to occur. Thus, photoinduced isomerization seems likely to proceed as indicated in Scheme I, where the two different caged radical pairs represent one with an α orientation ($[R^\cdot, Co^{II}]_\alpha$) and one with a β orientation ($[Co^{II}, R^\cdot]_\beta$). As indicated in this scheme, photoinduced isom-

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Table I. Rate Constants and Product Compositions for the Thermal Isomerization of Alkylcobinamides[a]

RCbi	f_α^{∞} [b]	$10^5 k_{obs}$,[c] s^{-1}	% dealkylation
α-CH_3Cbi	0.070	18.2 ± 0.6	<10
β-CH_3Cbi	0.074	*d*	<5
α-CH_3CH_2Cbi	0.040	20.2 ± 0.3	30
β-CH_3CH_2Cbi	0.035	*d*	30
α-CF_3CH_2Cbi	0.060	0.701 ± 0.020	<5
β-CF_3CH_2Cbi	0.050	*d*	20
α-$NCCH_2$Cbi	0.075	1.67 ± 0.10	<5
β-$NCCH_2$Cbi	0.060	*d*	<15
α-$CH_3CH_2OCH_2CH_2$Cbi	0.060	1.56 ± 0.05	25
β-$CH_3CH_2OCH_2CH_2$Cbi	0.020	*d*	<5
α-CF_2HCbi[e]	<0.25[f]	~3.9	

[a] In pH 7.0 phosphate buffered anaerobic aqueous solution at 70 °C except as noted. [b] Fraction of the final product mixture as the α diastereomer. [c] Observed first-order rate constant for approach to the final product composition obtained from the slopes of semilogarithmic plots of the decrease in the fraction of the product as the α diastereomer with time (Figure 2). [d] Not determinable due to the small amount of isomerization which occurs from the β diastereomer. [e] At 135 °C in sealed ampules. [f] Not followed to completion due to decomposition at this temperature.

erization is inherently nonequilibrium due to the irreversibility of the conversion of the electronically excited states of the RCbi's to the caged pairs. Thus photoinduced isomerization does not lead to equilibration, but to a photoinduced steady state in which the β diastereomers predominate by about 2–3 to 1.

Isomerism by Anaerobic Thermolysis. As previously observed by Friedrich and co-workers[5-8] for various methylcobalt corrinoids, heating of α-CH_3Cbi in anaerobic solution leads to extensive isomerization to the β diastereomer. Thus, at 70 °C, α-CH_3Cbi was converted in a first-order process (Figure 2) with a half-time of 64 min to a mixture containing 93% β-CH_3Cbi and 7% α-CH_3Cbi with very little net dealkylation (Table I). A similar product mixture was obtained when β-CH_3Cbi was heated anaerobically at 70 °C (Table I), although the small extent of conversion of β-CH_3Cbi to the α diastereomer prevented determination of the rate constant. This final product composition was essentially identical to that obtained when either α- or β-CH_3Cbi was reacted with cob(III)inamide, cob(II)inamide, and cob(I)inamide, and also to the ratio of diastereomers obtained by reductive alkylation of Factor B with CH_3I.[3]

A similar thermally-induced isomerization could also be demonstrated at 70 °C for other RCbi's including R = CH_3CH_2 (Figure 2), CF_3CH_2, $NCCH_2$, and $CH_3CH_2OCH_2CH_2$ (Table I). In each case, the α diastereomer underwent a first-order isomerization to a final product mixture which was >90% β diastereomer, and the β diastereomer also approached the same final composition. These final product ratios were distinctly different from those obtained by reductive alkylation of Factor B with the appropriate alkyl halides,[2,3,12] even at similar temperatures.[14] The rate constants for the first-order approach to the final product composition varied by a factor of 50 across the series of complexes. In most cases, little dealkylation accompanied isomerization, although as much as 30% dealkylation was observed for α-CH_3CH_2Cbi and α-$CH_3CH_2OCH_2CH_2$Cbi by the time the final product mixture was attained. CF_3Cbi's and CF_2HCbi's failed to undergo isomerization at 70 °C. However, when α-CF_2HCbi was heated in anaerobic solution to 135 °C (in sealed ampules), a relatively slow isomerization ensued which could not be followed to completion due to decomposition, but clearly formed a product mixture in which the β-diastereomer predominated (Table I). No isomerization could be detected for α-CF_3Cbi even at 135 °C, at which temperature it underwent a slow dealkylation.

Since isomerization of α-CH_3Cbi by methyl group transfer to $(H_2O)_2$Cbi had already been demonstrated at 25 °C, the possibility that the observed thermal isomerization of CH_3Cbi's was due to methyl group transfer to nonalkylated corrinoids present as unavoidable trace contaminants was investigated. When α-CH_3Cbi was incubated anaerobically at 70 °C in the presence of 0.2 equiv of $(H_2O)_2$Cbi, the isomerization reaction was 9-fold faster ($t_{1/2}$ = 7 min). In contrast, 0.2 equiv of $(H_2O)_2$Cbi did not increase the rate of isomerization of either α-CH_3CH_2Cbi or α-CF_3CH_2Cbi. In addition, 6.0×10^{-3} M 4-HTEMPO prevented the isomerization of α-CH_3CH_2Cbi (1.0×10^{-4} M) at 70 °C, and instead led to complete dealkylation with a half-time of about 200 min. Similarly, in the presence of 6.0×10^{-3} M 4-HTEMPO, α-CF_3CH_2Cbi (2.0×10^{-4} M) underwent complete decomposition at 70 °C with a half-time of 1800 min, and no isomerization could be detected. In contrast, 6.0×10^{-3} M 4-HTEMPO did not prevent the isomerization of α-CH_3Cbi (3.0×10^{-5} M), which cleanly isomerized to >90% β-CH_3Cbi.

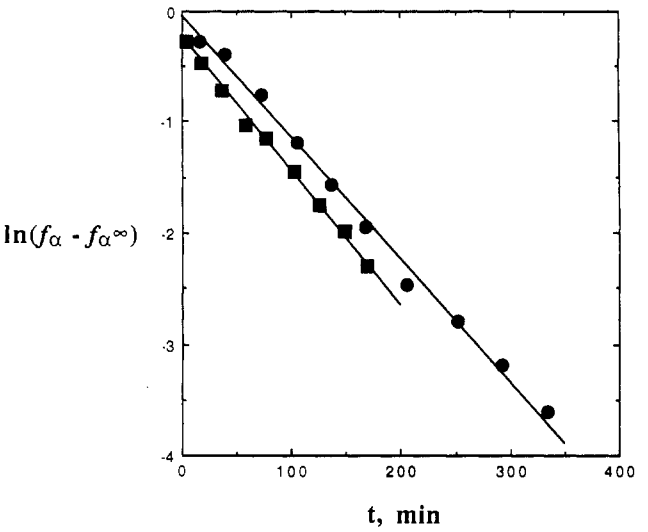


Figure 2. Representative first-order plots of ln ($f_\alpha - f_\alpha^{\infty}$) vs time, where f_α is the fraction of RCbi as the α diastereomer at time *t*, and f_α^{∞} is the fraction of RCbi as the α diastereomer at the completion of time-dependent changes, for the thermally-induced isomerization of α-CH_3Cbi and α-CH_3CH_2Cbi. The solid lines are linear regression lines. (●) α-CH_3Cbi, slope = −0.00109 ± 0.00003 min^{-1}, intercept = −0.058 ≙ 0.065, r^2 = 0.991; (■) α-CH_3CH_2Cbi, slope = −0.0121 ± 0.002 min^{-1}, intercept = −0.228 ± 0.017, r^2 = 0.998.

Discussion

Methyl group transfer from methylcobalt chelates to Co^{III} chelates has previously been observed in cobalt corrinoids[17] and in several cobalt chelate model systems.[21,23] In the case of cobalt dioximato systems, this reaction was estimated to be several orders of magnitude slower than the transfer of methyl groups to cobalt(II) chelates,[23] but unavoidable trace contamination of preparations with Co^{II} species prevented accurate rate measurements. Fanchiang et al.[17] have stuided the methyl group transfer from β-CH_3Cbl to $(H_2O)_2$Cbi as observed by ^1H, ^{13}C (using β-$^{13}CH_3$Cbl), and ^{31}P NMR spectroscopy and reported kinetic results using $(1-2) \times 10^{-3}$ M β-CH_3Cbl and $(1-10) \times 10^{-3}$ M $(H_2O)_2$Cbi. These authors found that this reaction went to completion to form β-CH_3Cbi[40] and that the reaction was first order in β-CH_3Cbl in the presence of excess $(H_2O)_2$Cbi. The kinetics were not affected by added O_2, N_2O, or H_2O_2. They also reported evidence from ^1H and ^{31}P NMR observations of the formation of a complex between β-CH_3Cbl (1.07×10^{-3} M) and $(H_2O)_2$Cbi (1.02×10^{-2} M). On the basis of these observations, Fanchiang et al.[17] postulated a mechanism which involved a one-electron transfer within such a complex, to form a cob(II)inamide-β-$CH_3Cbl^{\bullet+}$ complex followed by rapid $CH_3^{\bullet}$ transfer within the complex to form the observed products.

Our current results (Figure 1) show that such CH_3 group transfer to cob(III)inamide does result in interconversion of the

(40) Presumably, a small amount (~5%) of α-CH_3Cbi should also be produced, but would be difficult to see in NMR spectra. In the presence of 1×10^{-2} M pyridine, β-$^{13}CH_3$Cbi (1×10^{-3} M) reacted with 2×10^{-3} M $(H_2O)_2$Cbi to form two products, one ($\delta_{^{13}C}$ = −0.02 ppm, 72%) assigned to α-(py)-β-(CH_3)Cbi and one ($\delta_{^{13}C}$ = 0.40 ppm, 28%) assigned to α-(CH_3)-β-(py)Cbi (chemical shifts relative to external TMS).[17] For comparison, the ^{13}C chemical shifts of β-$^{13}CH_3$Cbi and α-$^{13}CH_3$Cbi are 1.67 and 3.19 ppm, respectively (relative to external TSP).[3]

α- and β-CH_3Cbi's. However, no kinetic evidence was found for prior complexation of the CH_3Cbi's with $(H_2O)_2$Cbi. This may be due to the fact that in our studies, monitored by HPLC, the concentration of both reactants was at least an order of magnitude lower than those employed by Fanchiang et al.[17] Thus, while complexation may indeed occur during the cob(III)inamide-catalyzed isomerization of α- and β-CH_3Cbi's, the concentration of the complex may be too low to permit observation of the anticipated "saturation" kinetics under the conditions used.

While Dodd et al.[23] were unable to accurately determine the kinetics of CH_3 transfer from methylcobalt bis(dioximato) complexes to cobalt(III) cobaloxime due to the complication of trace contamination with Co^{II} chelates, this is unlikely to represent a problem with the cobalt corrinoids due to the oxygen lability of cob(II)inamide and the fact that our measurements were carried out in aerobic solution. Thus, in the cobalt corrinoids, CH_3Cbi's are only about 4-fold less reactive toward methyl group transfer to cob(III)inamide than to cob(II)inamide. While the methyl group transfer reaction to $(H_2O)_2$Cbi was relatively facile, no alkyl group transfer could be demonstrated to $(H_2O)_2$Cbi for any other RCbi, even after as long as 120 h of reaction.

The transfer of alkyl groups from alkylcobalt complexes to cobalt(II) and cobalt(I) reagents has been more thoroughly studied. Kräutler and co-workers[18,19] have determined the equilibrium constants for transfer of CH_3 from β-CH_3Cbl to heptamethyl cob(II)yrinate, cob(II)inamide, and cob(I)inamide. Equilibrium and kinetic studies of the transfer of methyl groups from methylcobalt chelates to cobalt(II) and cobalt(I) dioximato,[20-24] tetraaza macrocyclic,[24] and Schiff's base and other model chelates[21,22,24] have also been reported. In addition, alkyl group transfers to cobalt(II) complexes for R ≠ Ch_3 have also been reported in dioximato[22,23] and other model systems[22] and to cobalt(I) dioximato complexes.[23] Such group transfer reactions can be considered to occur either via bimolecular substition reactions[21-23] or via electron transfers through saturated carbon bridges.[24] In the case of cobalt dioximato complexes, Dodd et al.[23] have shown that alkyl group transfer reactions to cobalt(II) and cobalt(I) complexes occur with inversion of configuration at the α carbon of the alkyl ligand by use of stereospecifically deuterated [1,2-$^2H_2$]-2-phenylethylcobalt complexes and have characterized these reactions as bimolecular homolytic and nucleophilic displacements, respectively.

Dodd et al.[23] have also reported an extensive series of rate constants for the transfer of various alkyl groups from alkylcobalt dioximato complexes to cobalt(I) and cobalt(II) dioximato reagents. Interestingly, rate constants for such transfers to cobalt(I) and cobalt(II) reagents were quite similar, but for each reaction, the rate constants varied widely (~10^6-fold) with the steric size of the alkyl group. Thus, methylcobalt complexes were at least 170- to 440-fold more reactive than ethylcobalt complexes[41] and (8×10^3)- to (6×10^4)-fold more reactive than secondary alkylcobalt complexes in either reaction. These results qualitatively agree with those obtained here, i.e., methyl transfer from the CH_3Cbi's to cob(III)inamide was at least (2.15×10^3)-fold faster, and methyl group transfer to cob(II)inamide was at least (3×10^3)-fold faster than the transfer of any other alkyl group, such that transfer could not be observed for any alkyl group other than CH_3.

In contrast to the cobalt dioximato system, methyl group transfer from α-CH_3Cbi to cob(I)inamide was very much faster than transfer to cob(II)inamide. Thus, at accessible concentrations of cob(I)inamide (≥1.0×10^{-4} M), the methyl group transfer reaction was complete as fast as the first sample could be taken for HPLC analysis (≤30 s). However, all of the other RCbi's, with the exception of the $NCCH_2$Cbi's which underwent rapid dealkylation in the presence of cob(I)inamide, failed to undergo any isomerization when treated with cob(I)inamide and were at least (1.9×10^4)-fold less reactive than the CH_3Cbi's.

Table II. Comparison of the Diastereomeric Composition of the RCbi's Obtained from Reductive Alkylation and Photoinduced and Thermally-Induced Isomerization

	f_α[a]		
RCbi	reductive[b] alkylation	photoinduced isomerization[c]	thermal isomerization[d]
CH_3CH_2Cbi	<0.02[e]	0.27	0.04
CH_3Cbi	0.04	0.25	0.07
$EtOCH_2CH_2$Cbi	0.20		0.04
$NCCH_2$Cbi	0.73	0.28	0.07
CF_3CH_2Cbi	0.87	0.31	0.05
CF_3Cbi	0.93	*f*	*f*
CF_2HCbi	0.98[g]	0.25	<0.25[h]

[a] f_α is the fraction of RCbi product as the α diastereomer. [b] Reductive alkylation of Factor B in Zn/acid with RX (refs 2 and 3). For R = $NCCH_2$, CF_3CH_2, and CF_3, f_α has been shown to be independent of temperature over the range 0–75 °C (ref 8). [c] Final product composition upon irradiation of an anaerobic aqueous solution, pH 7.0 (ref 19). [d] Final product composition upon heating an anaerobic aqueous solution, pH 7.0, at 70 °C, except as noted (this work). [e] Undetectable. [f] Undergoes decomposition only; no isomerization can be detected. [g] Obtained as a secondary product during the reductive alkylation with CF_3I due to reductive defluorination of the CF_3Cbi's (refs 3 and 7). [h] At 135 °C. Decomposition prevented following the reaction to completion.

The most important observation from these alkyl group transfer reactions, aside from the inertness of alkyl groups other than CH_3, is the fact that methyl group transfer from *either* α-CH_3Cbi or β-CH_3Cbi to cobinamide in any of the three oxidation states leads to final product mixtures which have the same composition, i.e., ~5% α-CH_3Cbi and ~95% β-CH_3Cbi. Since the same product distribution is approached starting from either diastereomer in each of the three reactions, this composition evidently represents the equilibrium mixture. As the same product distribution is obtained by reductive alkylation of cobinamide with CH_3I, the formation of the diastereomeric CH_3Cbi's during reductive alkylation must lead to an equilibrium mixture of diastereomers. Clearly, under these synthesis conditions,[2,3,42] CH_3 transfer to cob(II)inamide or cob(I)inamide is sufficiently rapid to permit equilibration by either of these routes.[43] However, for R ≠ CH_3, transfer to cob(II)inamide is far to slow to permit equilibration of the diastereomers and transfer to cob(I)inamide is probably too slow as well. In addition, as discussed below, the results of thermally-induced isomerization reactions strongly suggest that synthesis reaction mixtures are not at equilibrium for R ≠ CH_3.

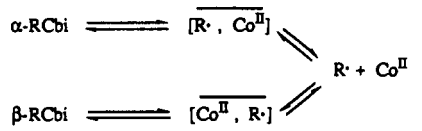
Table II shows a comparison of the diastereomeric product composition of RCbi's from synthesis by reductive alkylation with RX, from anaerobic photoinduced isomerization from either diastereomer, and from thermally-induced isomerization from either diastereomer. The radical trapping experiments described above strongly suggest that photoinduced isomerization proceeds via formation of free radicals as shown in Scheme I. The product distribution from this reaction (25–31% α diastereomer) is largely independent of the R group and shows a preference (ca. 2.5:1) for the β face of cobinamide. These results are in stark contrast to those obtained from reductive alkylation, where the α diastereomer accounts for from <2% (i.e., undetectable) to >95% of the product, depending on R. If it is correct that photoinduced isomerization is under diffusion control (i.e., the highest energy point on the reaction profile is a diffusional barrier), then the ratio of products obtained (ca. 2.5:1, β/α) represents the kinetically

(41) The reactions of the methylcobalt complexes (unlike those for other R's) were too fast to permit accurate determination of the rate constants by the methods used. Thus lower limits of 44 and 17 M^{-1} s^{-1} were set for the transfer of CH_3 from methyl(pyridine)bis(dimethylglyoximato)cobalt(III) to pyridinebis(cyclohexylglyoximato)cobalt(II) and pyridinebis(cyclohexylglyoximato)cobalt(I), respectively.[23]

(42) Under typical synthesis conditions,[2,3] the concentration of cobinamide is 3.6×10^{-3} to 1.0×10^{-2} M.

(43) The nature of the species being alkylated (i.e., cob(II)inamide or cob(I)inamide) in zinc/acid reductants is unclear, although cob(II)inamide is the only species detected spectrophotometrically.[13] Assuming that the first trace of CH_3Cbi product reacted with ($3.6–10.0$) $\times 10^{-3}$ M cob(II)inamide,[42] equilibrium would occur with a half-time of 46 s to 2.1 min. For reaction with cob(I)inamide at the same concentrations, the half-time would be <1 s.

Scheme II



controlled product ratio for a prototypical alkylcobinamide synthesis reaction, i.e., the diffusional combination of R· and cob(II)inamide. This reaction would then show the expected preference for the β face due to the differential side chain steric congestion of the α and β faces. The very different stereochemical outcome of reductive alkylation would then suggest that the products are not under kinetic control under these conditions.

Thermal Co–C bond homolysis to form an alkyl radical and a cobalt(II) comples is well known to occur for a number of alkylcobalt corrinoids including 5′-deoxyadenosylcobalt corrinoids,[44-47] neopentyl- and benzylcobalt corrinoids,[30,48-53] and methylcobalamin,[54,55] as well as for a number of alkylcobalt model chelates.[56-63] It thus seemed likely to us that, in analogy to the facile photoinduced isomerization of α- and β-RCbi's, thermally-induced Co–C bond homolysis could also lead to isomerization. As shown in Table I, this is in the case for most of the RCbi's investigated here, which undergo isomerization at readily measurable rates at the relatively mild temperature of 70 °C. In contrast, 5′-deoxyadenosylcobalamin decomposes with a half-time of 17 h at 85 °C,[45] and its cobinamide analog is even more inert.[46] Thermal isomerization of CH_3CH_2Cbi's at 70 °C is not surprising since base-on β-CH_3CH_2Cbl is known to be quite thermally labile,[64] undergoing significant decomposition in aerobic solution at temperatures as low as 60 °C.[16]

However, there has been considerable controversy regarding the nature of the Co–C bond cleavage in the thermolysis of alkylcobalt complexes with β hydrogens, which often undergo apparent eliminations to give olefinic products. While such reactions have been claimed to be concerted eliminations of hydridocobalt species,[36,65-68] they have also been characterized as Co–C bond homolyses followed by β-hydrogen transfer from the alkyl radical to cobalt(II) species to form the observed products.[50,53,56,58,61,69,70] Recently, this latter mode of cleavage has been convincingly demonstrated for (1-phenylethyl)cobaloximes by radical trapping experiments,[61] evidence for thermal Co–C homolysis in the decomposition of 2-hydroxycyclopentylcobalamin has been presented,[71] and thermolysis and photolysis of alkylcobaloximes (including those with β hydrogens) have been shown to generate identical primary products.[72] It thus begins to appear likely that for virtually all organocobalt thermolyses, the primary step involves Co–C bond homolysis.

Our radical trapping experiments with 4-HTEMPO for RCbi thermolysis seem to confirm this conclusion. The complete inhibition of the isomerization of α-CH_3CH_2Cbi and α-CF_3CH_2Cbi by 4-HTEMPO strongly suggests the primary involvement of radicals in this process. The fact that such inhibition occurs at relatively low[39] concentrations of trap (6.0×10^{-3} M) suggests that radical trapping occurs outside of the solvent cage and that thermally-induced α/β isomerization, in analogy to photoinduced isomerization (Scheme I), occurs as shown in Scheme II, the important difference being that the thermal process is reversible and the products are in thermal equilibrium.

The situation for the CH_3Cbi's is clearly different. Martin and Finke have shown that β-CH_3Cbl has a very high bond dissociation energy[54] and only undergoes significant Co–C bond homolysis at 120 °C and above ($t_{1/2}$ = 10.75 h in ethylene glycol at 120 °C).[55] The known effect of coordination of the axial nucleotide on the thermal homolysis of 5′-deoxyadenosylcobalamin[46] and on other organocobalamins[30,48,49,51,52] suggests that β-CH_3Cbi will be even more inert. Thus, thermal isomerization of α- and β-CH_3Cbi at 70 °C cannot be occurring via the radical mechanism of Scheme II and, instead, is likely to be due to cobalt-to-cobalt methyl group transfer to nonalkylated corrinoids present as unavoidable trace contaminants. Support for this conclusion comes from the fact that 4-HTEMPO (6.0×10^{-3} M) does not prevent this thermal isomerization and the fact that added $(H_2O)_2Cbi$ (0.2 equiv) increases the rate of thermally-induced isomerization of α-CH_3Cbi by 9-fold. This suggests that at 70 °C in the absence of added $(H_2O)_2Cbi$, thermally-induced isomerization of β-CH_3Cbi could occur at the observed rate due to contamination with as little as 2.2% nonalkylated material. In contrast, the thermally-induced isomerization of α-CH_3CH_2Cbi and α-CF_3CH_2Cbi was not enhanced by added $(H_2O)_2Cbi$ (0.2 equiv), demonstrating that alkyl group transfer to cob(III)inamide does not occur even at this elevated temperature.

The fact that similar final product ratios are obtained during thermally-induced isomerization of the RCbi's starting from either diastereomer suggests that this process comes to equilibrium, as is necessary if the suggested mechanism for this processs (Scheme II) is correct. In every case (except for α-CF_2HCbi, for which the final composition could not be observed due to decomposition), the β diastereomer predominates by >10:1 at equilibrium and this composition is relatively insensitive to R. Thus, the apparent equilibrium position of all of the α/β isomerizations favors the less sterically crowded β face by some 1.6 to 2.0 kcal at 70 °C. These results are again in stark contrast to those obtained from reductive alkylation (Table II). Although such syntheses are generally conducted at room temperature (or at 0 °C), the product composition for representative reductive alkylations (R = $NCCH_2$, CF_3CH_2, and CF_3) has been shown to be independent of temperature over the range 0–75 °C.[14] This strongly suggests that the diastereomeric products of reductive alkylation of cobinamide with alkyl halides (with the exception of R = CH_3) are *not* under thermodynamic control. If it is indeed correct that the reductive alkylation products are under neither thermodynamic nor kinetic control, then the products are in a nonequilibrium steady state. Although alkylcobalt corrinoids are known to be labile toward reductive dealkylation under synthesis conditions,[13] the composition of the reaction mixture has been shown not to be effected by

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differential lability of the diastereomers.[14] This then requires that under reductive alkylation conditions, some reaction other than cobalt-to-cobalt alkyl group transfer allows the interconversion of the diastereomers but does not come to equilibrium. Studies to demonstrate conclusively the applicability of Schemes I and II to the isomerization processes discussed here, as well as to further probe the steric effects on these isomerizations, are currently in progress.

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Rates of Substitution by Sulfur Nucleophiles in *cis*-Diamminebis(guanosine)platinum(II) Chloride

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The rates of displacement of guanosine from *cis*-$[(NH_3)_2Pt(Guo)_2]^{2+}$ by six sulfur-containing nucleophiles have been measured at several temperatures and several concentrations of nucleophile by using ^{13}C NMR spectroscopy. *cis*-Diamminebis(guanosine)platinum(II) chloride [(**1**)Cl] reacts with the nucleophiles sarcosine-*N*-carbodithioate, dimethyldithiocarbamate, diethyldithiocarbamate, thiourea, 1-methyl-2-thiourea, and 1-ethyl-2-thiourea to form products in which a guanosine is removed in the initial step and additional ligands may be removed in subsequent steps if the nucleophile is added in excess. The rate of guanosine displacement in the first step of these reactions was found to be slower than that for the corresponding reaction with cyanide; the calculated enthalpies and entropies of activation are consistent with these findings, also. Other sulfur-containing nucleophiles investigated which did not have a significant effect on the displacement of guanosine from **1** over a 24-h period include NaSCN, $Na_2S_2O_3$, L-methionine, thiobarbituric acid, DMSO, glutathione, L-cysteine, and thiocarbohydrazide.

Introduction

The reaction of *cis*-diamminedichloroplatinum(II) (*cis*-DDP or cisplatin) with DNA, in which the platinum complex can bind both mono- and bifunctionally at the N-7 position to form an intrastrand cross-link between two adjacent guanine bases in the DNA helix, is generally accepted to be the basis for its antineoplastic activity.[1] Previous reports have determined the exact mode of binding, including the dihedral angles and orientation of the guanines, from X-ray crystallographic data and from NMR spectroscopic studies.[2] The use of cisplatin as an antitumor drug is somewhat limited by its concentration-dependent nephrotoxicity[3] and a variety of other side effects.[4] The control of these adverse effects via the administration of compounds which possess nucleophilic sulfur atoms, such as thiosulfate,[5] diethyldithiocarbamate,[6] glutathione,[7] WR-2721,[8] and the like has been reported in some detail. These compounds not only can react directly with cisplatin but also may react with platinum bound to DNA, in which case they would have a tendency to reduce the antineoplastic activity of the cisplatin. The present study was undertaken to obtain rate data on the reactions of sulfur-containing nucleophiles with the model compound *cis*-$[(NH_3)_2Pt(Guo)_2]^{2+}$ (**1**). Previous studies have found that such model compounds mirror many of the more significant reactions of platinum with DNA.[9] In order to displace a nucleoside from a platinum complex, a nucleophile capable of competing with the nucleoside for the coordination site on the platinum center must be present. The nucleophiles selected for these studies incorporated structural features of or are identical to compounds that (1) have been used to control the adverse effects of cisplatin in vivo, (2) have been used experimentally in vitro to remove platinum from DNA, or (3) are important nucleophiles within the cell. With these criteria in mind, the following nucleophiles were examined: NaSCN, $Na_2S_2O_3$, L-methionine, thiobarbituric acid, DMSO, glutathione (GSH), L-cysteine, thiocarbohydrazide, cyanide, sarcosine-*N*-carbodithioate (Sar-DTC), thiourea (Tu), diethyldithiocarbamate (DiEt-DTC), dimethyldithiocarbamate (DiMe-DTC), 1-methyl-2-thiourea (MeTu), and 1-ethyl-2-thiourea (EtTu). Of these nucleophiles, only the last seven provided data acceptable for kinetic analysis. The first compound investigated was cyanide since it has been shown to be the most effective at removing

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