Cobalamin-Dependent Methionine Synthase: Probing the Role of the Axial Base in Catalysis of Methyl Transfer between Methyltetrahydrofolate and Exogenous Cob(I)alamin or Cob(I)inami...

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Cobalamin-dependent Methionine Synthase: Probing the Role of the Axial Base in Catalysis of Methyl Transfer between Methyltetrahydrofolate and Exogenous Cob(I)alamin and Cob(I)inamide

Jeanne Sirovatka Dorweiler, Richard G. Finke and Rowena G. Matthews*

Figure A. Initial rate of methyl transfer from CH_3-H_4folate to cob(I)inamide vs initial concentration of cob(I)inamide measured at 37 ° C. The concentration of MetH(2-649) was 6 μM, and the concentration of CH_3-H_4folate was 1 mM. Error bars were calculated from a least squares fit to the initial data points. The rate constant calculated from these individual measurements is 183 M^{-1} s^{-1}.

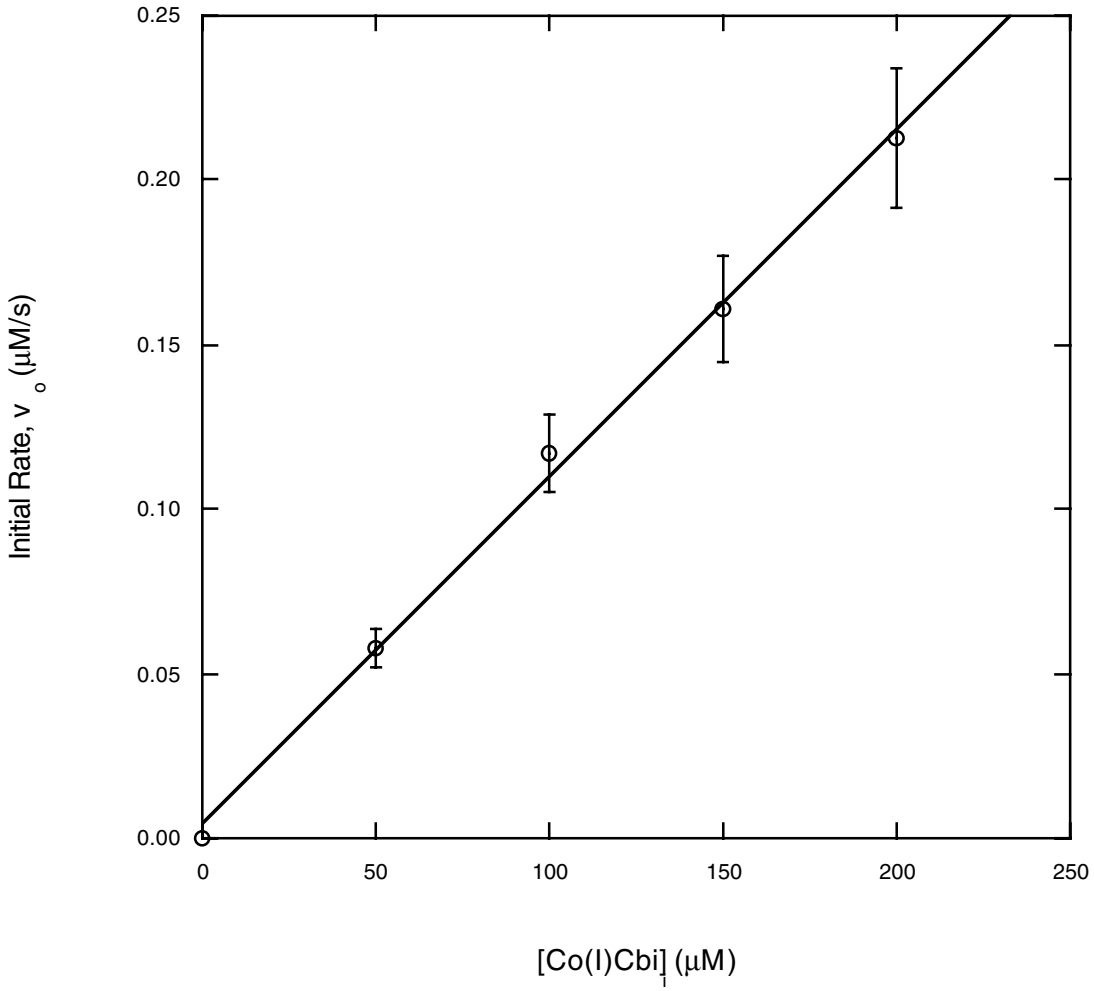

Figure B. Initial rate of methyl transfer from CH_3-H_4folate to cob(I)inamide vs the concentration of MetH(2-649) measured at 37 °C. The initial concentration of cob(I)inamide was 100 μM and the concentration of CH_3-H_4folate was 1 mM. Error bars were calculated from a least squares fit to the initial data points. The constant calculated from these individual measurements is 220 M^{-1} s^{-1}.

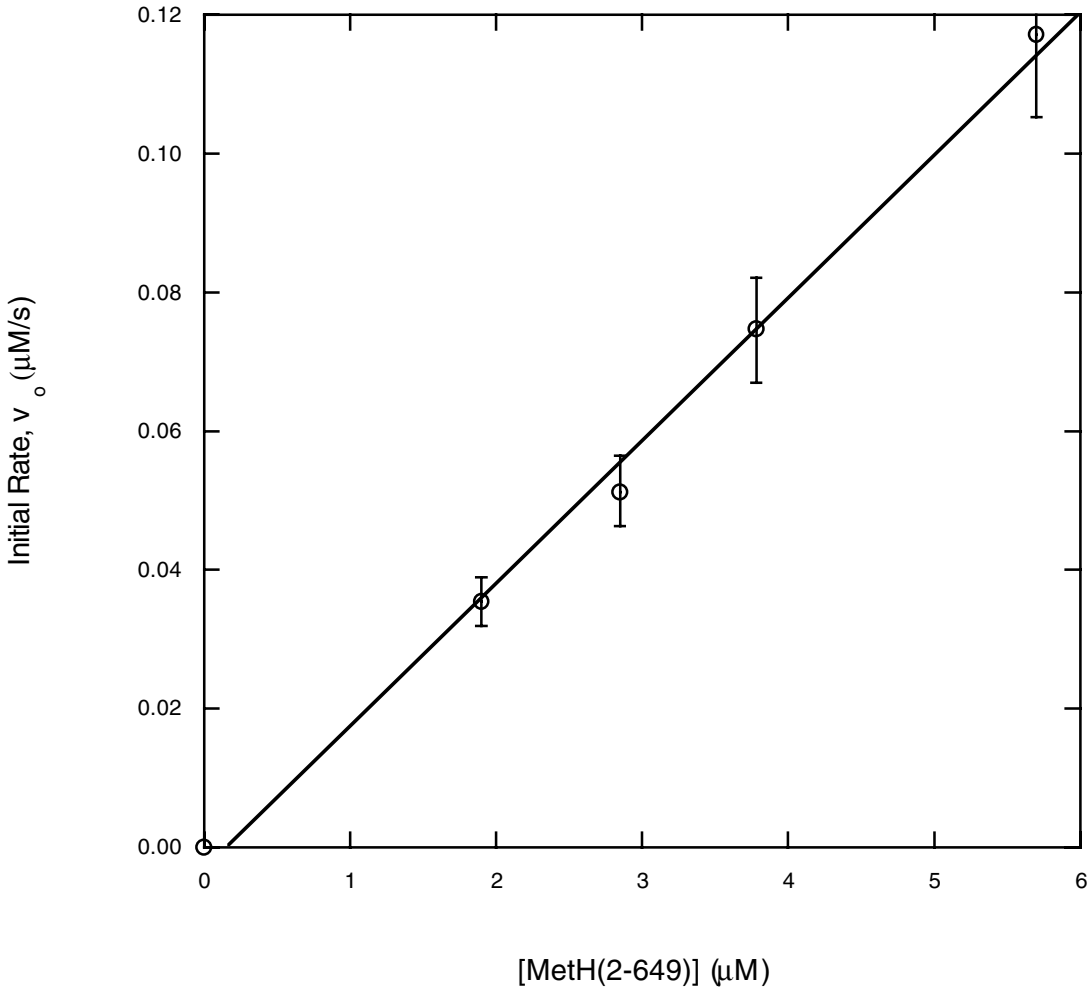

Figure C. Initial rate of methyl transfer from methylcobinamide to H_4folate vs the concentration of methylcobinamide measured at 37 °C. The concentration of MetH(2-649) was 6.5 μM, and the concentration of H_4folate was 4 mM. At methylcobinamide concentrations lower than 200 μM, the reaction produces a mixture of cob(I)inamide and cob(II)inamide, making the reaction kinetics more difficult to follow. Error bars were calculated from a least squares fit to the initial data points.

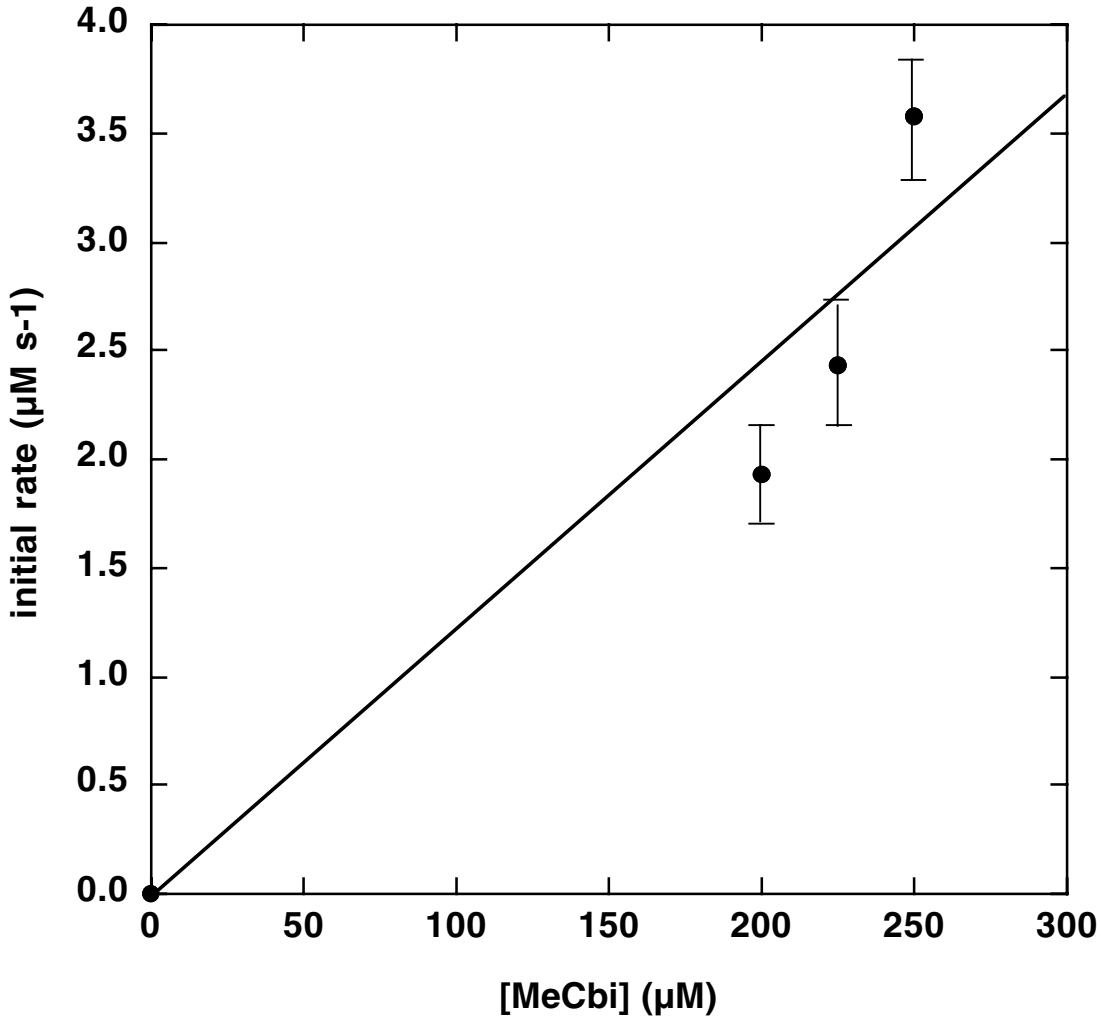

Figure D. Initial rate of methyl transfer from methylcobinamide to H_4folate vs the concentration of MetH(2-649) measured at 37 °C. The initial concentration of methylcobinamide was 200 μM, and the concentration of H_4folate was 4 mM. Error bars were calculated from least squares fitting of the initial data points. The rate constant calculated from the individual data points was 3170 M^{-1} s^{-1}.

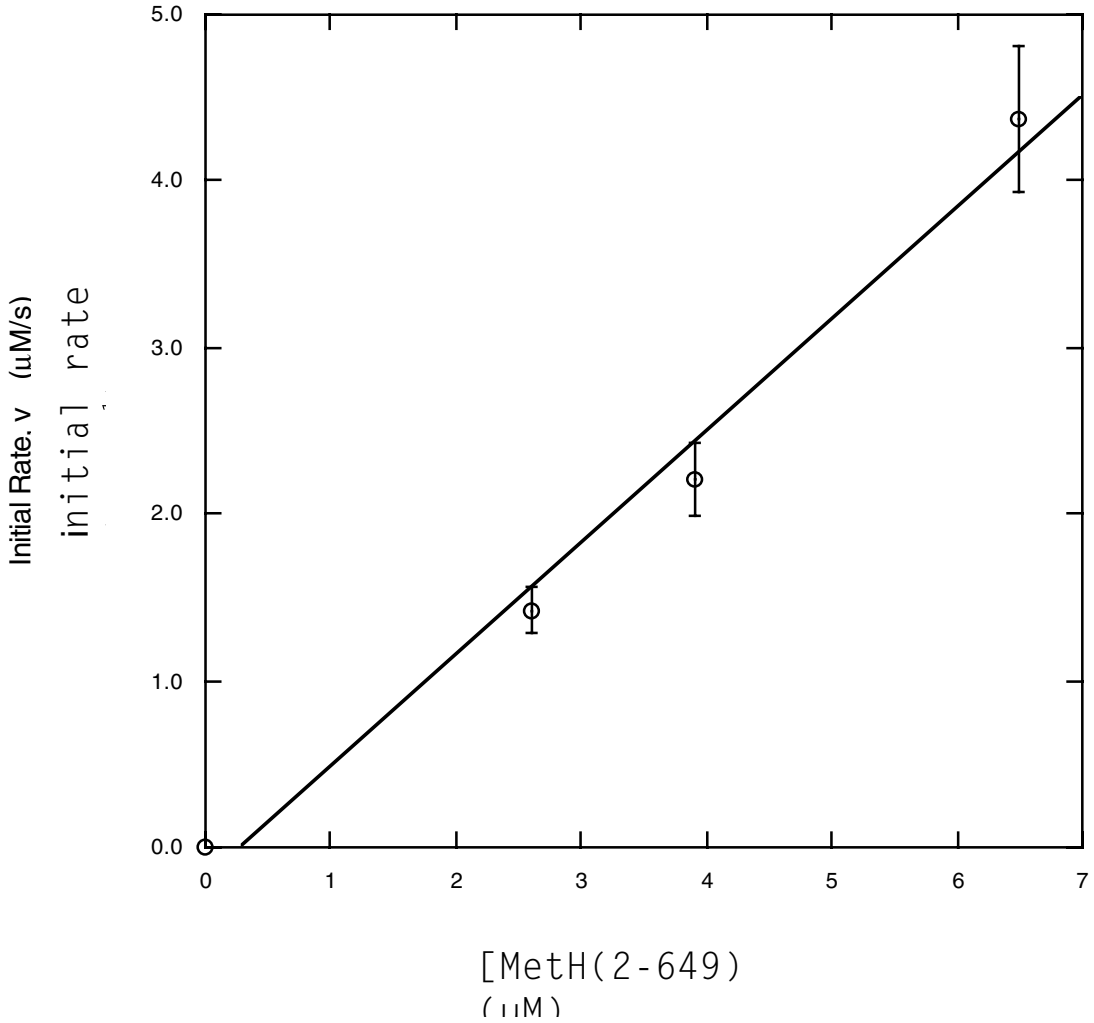

Figure E. Effect of pyridine on methylcobalamin:H_4folate (●) and methylcobinamide:H_4folate (■) methyltransferase activities. Assays were performed as described in Materials and Methods. The initial concentrations in the methylcobalamin:H_4folate methyltransferase assay were: MetH(2-649), 7.4 μM, methylcobalamin, 337 μM, and H_4folate, 4mM. The initial concentrations in the methylcobinamide:H_4folate methyltransferase assays were: MetH(2-649), 6.4 μM; methylcobinamide, 400 μM; and H_4folate, 4 mM.

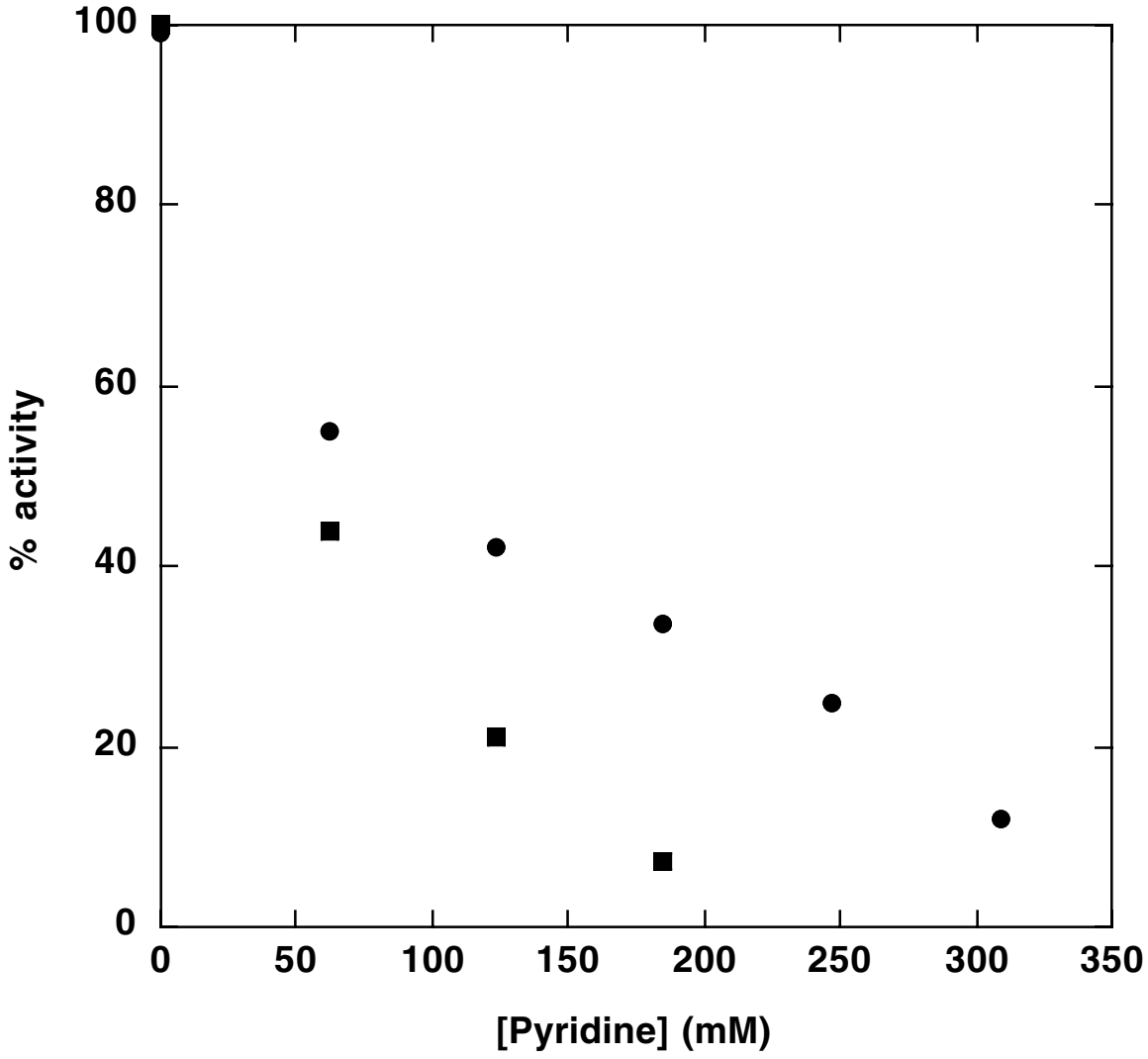

Figure F. Titration of methylcobinamide with pyridine. ~100 μM MeCbi in 50 μM potassium phosphate buffer, pH 7.2 at 37 ° C was titrated with aliquots of neat pyridine. Spectra were measured in the presence of 0, 25, 50 100, 200 and 400 mM pyridine. The calculated association constant for this titration is 6.2 ± 0.8 M^{-1}.

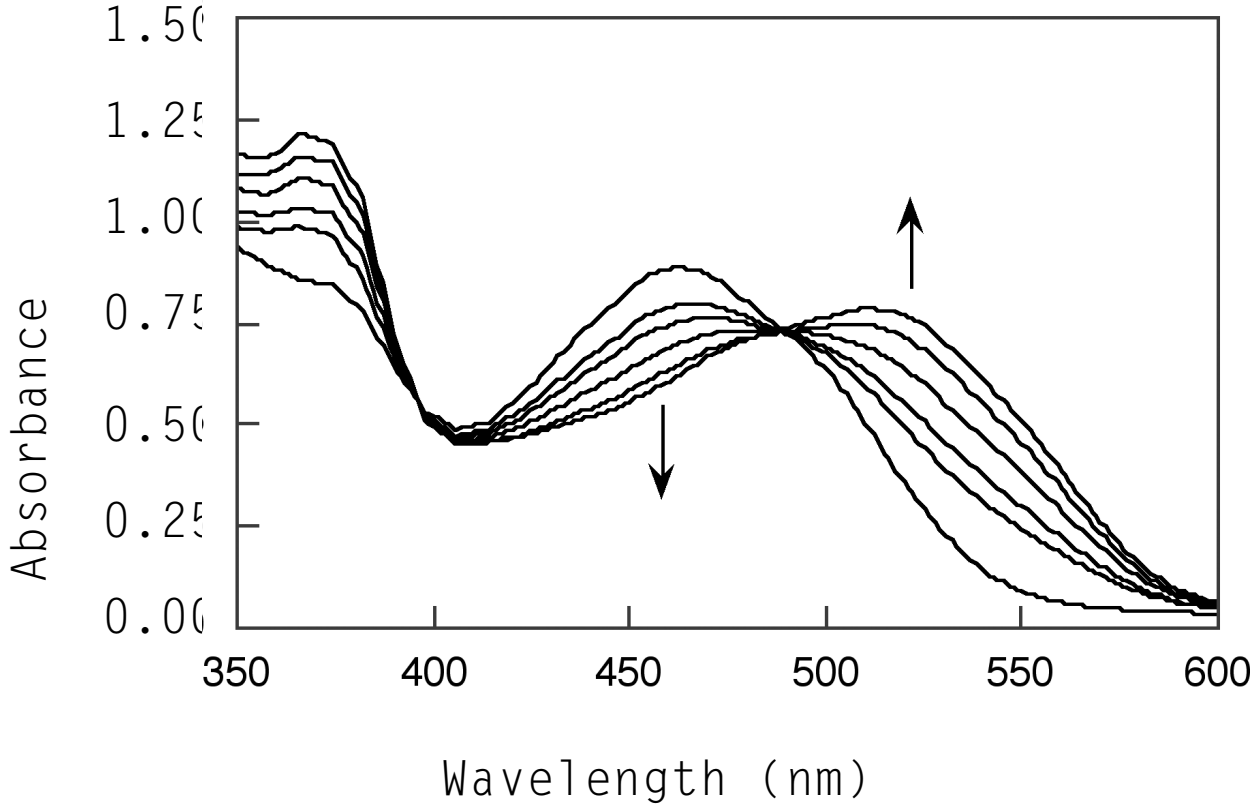

Figure G. Corrected methylcobinamide:H_4folate methyltransferase rate constants measured in the presence of pyridine vs. the percent base-on methylcobinamide measured under the same conditions. The uncorrected data for the effect of pyridine on the methylcobinamide:H_4folate methyltransferase assay are taken from Figure E, and corrected for the percent inhibition seen in the methylcobalamin:H_4folate methyltransferase assay data also shown in Figure E. These corrected second order rate constants are plotted against the percent base-on methylcobinamide calculated from the association constant measured in the experiment shown in Figure F.

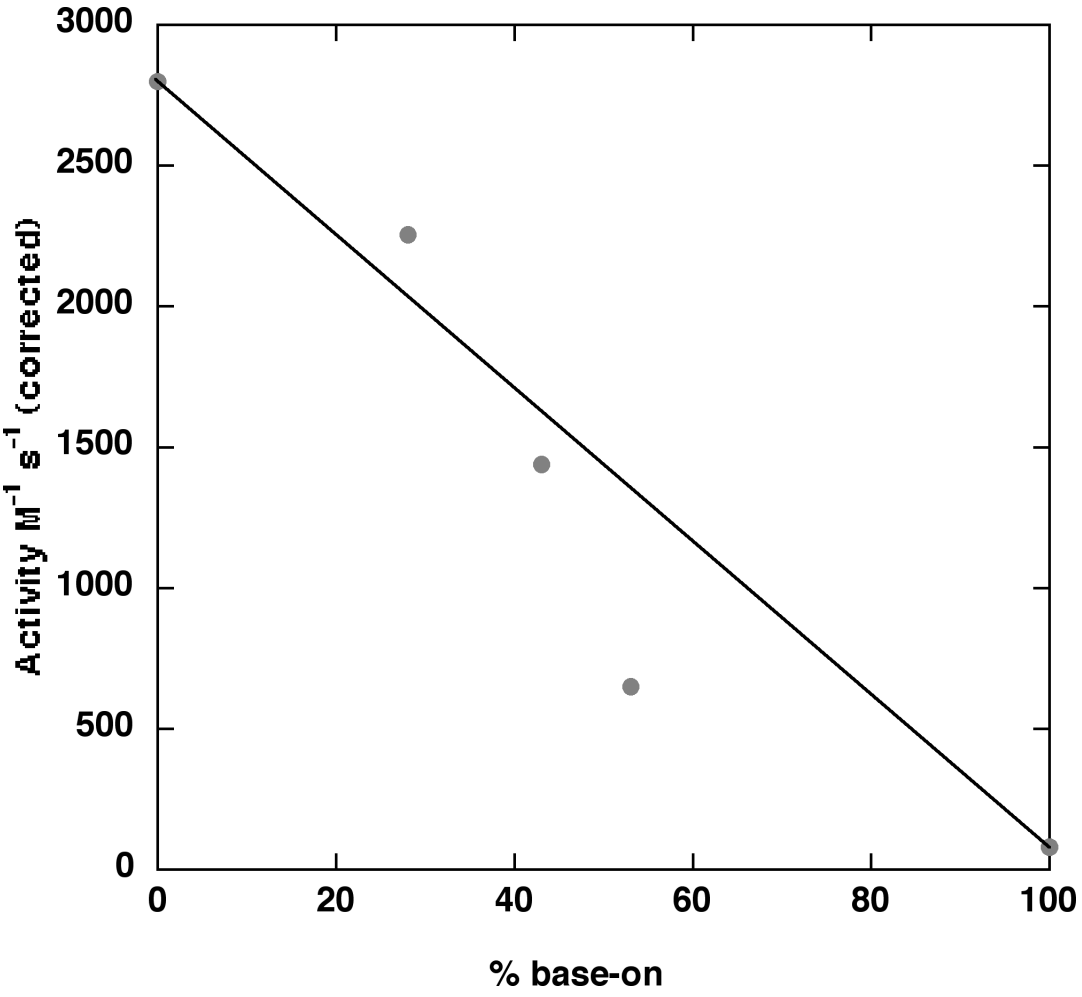

Figure H. The observed rate constant for methyl transfer from $PhNMe_3^+Cl^-$ to cob(I)alamin vs the concentration of $PhNMe_3^+Cl^-$. Assays were conducted in 50 mM potassium phosphate buffer, pH 7.2 and 25 ° C. The initial concentration of cob(I)alamin was 100 μM, and values for k_{obs} were determined by dividing the observed initial rate of reaction by the initial concentration of cob(I)alamin. Error bars were calculated from least squares fitting of the initial data points. The second order rate constant calculated from the individual data points is 1.06×10^{-3} $M^{-1} s^{-1}$.

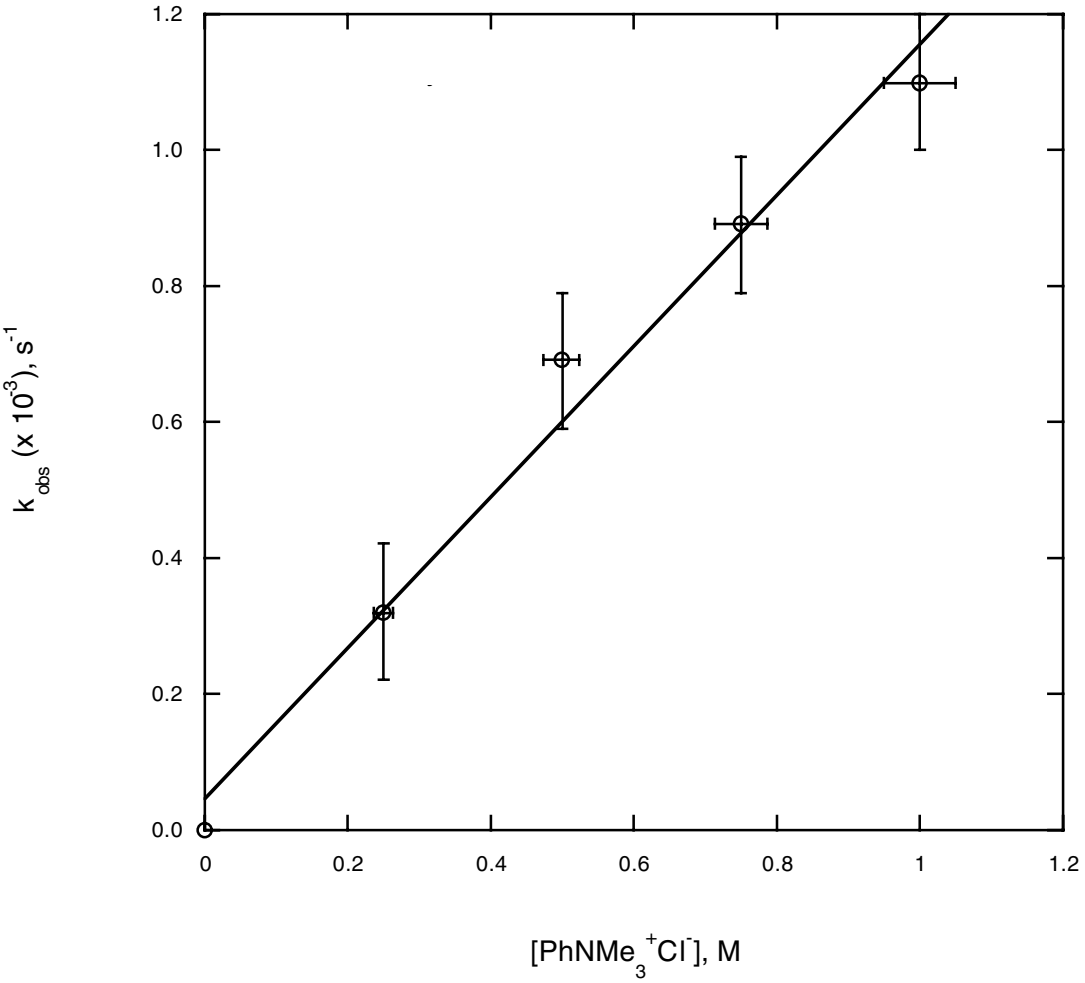

Figure I. The observed rate constant for methyl transfer from $PhNMe_3^+Cl^-$ to cob(I)inamide vs the concentration of Ph $NMe_3^+Cl^-$. The initial concentration of cob(I)inamide was 100 μM, and and values for k_{obs} were determined by dividing the observed initial rate of reaction by the initial concentration of cob(I)inamide. Error bars were calculated from least squares fitting of the initial data points. The second order rate constant calculated from the individual values for k_{obs} was 0.94 x 10^{-3} M^{-1} s^{-1}.