Methyl Transfer from Nitrogen to Cobalt: Model for the B_{12}-Dependent Methyl Transfer Enzymes

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The Co atom can be methylated by reaction of the Co^{I} cobalamin with the $PhNMe_3^+$ ion in aqueous solution at 25 °C at a pH-independent rate (pH 4–10) with the second-order rate constant $k_2 = 2 \times 10^{-3}$ dm^3 mol^{-1} s^{-1}.

Methyl transfer is the simplest nucleophilic substitution reaction, as well as one of the most extensively studied and best understood of all organic reactions.[1] Little is known, however, about the first transfer of a *de novo* synthesised Me group from the N^5 of N–Me–THFA (THFA = tetrahydrofolic acid) to the S of homocysteine to form methionine, for which nature has evolved two families of enzymes.[2] In the B_{12}-dependent enzymes the mechanism involves the alternate formation of Co^{I} and Me–Co corrinoids; in the less efficient B_{12}-independent enzymes there is no evidence for any prosthetic group. N–Me donors able to methylate thiols non-enzymatically and provide a model for the B_{12}-independent pathway include N–Me–py$^+$,[3,4] $PhNMe_3^+$,[4] N^5–Me–THFA (sealed tube at 100 °C for 24 h, 2% yield)[3] and a N^5-dimethylated pterin derivative (70 °C for 24 h, 57% yield), as reported recently by Hilhorst, Chen and Pandit.[5] No methyl transfer from N to Co in a corrinoid has yet been reported in reasonable yield, though Hilhorst reports 'an extremely low conversion' with her pterin derivative.[4] Co^{I}–Cbl does, however, react readily with ethyleneimine to give the Co–$CH_2CH_2NH_3^+$ derivative,[6] demonstrating that alkyl transfer from N to Co can occur provided there is a sufficient driving force. We recently reported a new pathway for the methylation of Co by MeI, involving reaction with a thiol–Co^{II}–corrinoid.[7] We now report the first example of facile transfer of Me from a N atom to a Co^{I} corrinoid.

We have investigated various compounds possessing the N–Me^+ group (whether resulting from protonation or full methylation) to find a protein-free model for Me transfer from a N atom to a Co^{I} corrinoid. Because the Me-donor potential of ring-substituted N–Me-pyridinium ions increases as the basicity of the parent py decreases,[1d] we have focussed primarily on compounds with a basicity as low as that of N^5 in THFA (pK 4.8)[8] and have taken the basicity of a fully methylated compound as comparable to that of the monodemethylated parent. Since imidazoles often behave differently from pyridines (*e.g.* as nucleophiles in ester hydrolysis[9] or H-bond acceptors)[10] we have included an imidazole as well as pyridines and amines/anilines; pK values are taken from ref. 11 except that for **6** from ref. 12. Aqueous solutions (*ca.* 3×10^{-5} mol dm^{-3}) of the very reactive and probably 4-coordinate[13] Co^{I}–Cbl (Cbl = cobalamin) were prepared by reducing deoxygenated solutions of aquo-Cbl (B_{12a}) under nitrogen with $NaBH_4$ in the presence of $Co(NO_3)_2$ as catalyst.[14] Reactions were studied by UV-VIS spectrophotometry in a 1 cm pathlength cell thermostated at 25 °C; such studies are limited to pH > 3 because of the increasing rate of evolution of H_2 bubbles with increasing acidity.[14]

Co^{I}-Cbl reacts with the $PhNMe_3^+$ ion **1** (*cf.* $PhNMe_2$ pK 5.1) as the chloride to give Me–Cbl,† but high concentrations are required. The reactions showed good isobestic points and followed pseudo-first order kinetics. The rate increased with the concentration of **1** (studied up to 1 mol dm^{-3}) but was independent of pH (4–10), corresponding to a second-order rate constant k_2 of 2×10^{-3} dm^3 mol^{-1} s^{-1} ($t_{\frac{1}{2}}$ *ca.* 5 min). By contrast, there was no detectable formation (<10%) of Me–Cbl at pH 4 over 12 h in the presence of 1 mol dm^{-3} solutions of the N–Me-pyridinium ion **2** (*cf.* py pK 5.2) or NMe_4^+ **3** (*cf.* NMe_3 pK 9.8), while the protonated forms of both $PhNMe_2$ **4** (pK 5.1) and PhNHMe **5** (pK 4.85), which could not readily be studied as the neutral forms at pH 8 because of reduced solubility, and of 5-Cl-N-Me-imidazole **6** (pK 5.1)[12] all appear to catalyse the decomposition of Co^{I} back to Co^{II} and/or inhibit reduction to Co^{I}; Co^{I} could be obtained in the presence of 0.1 mol dm^{-3} reagent but slowly produced Co^{II} with no obvious formation of Me–Cbl, while only Co^{II} with no Co^{I} could be observed in the presence of 1 mol dm^{-3} reagent.

Our results show, firstly, that the Co^{I} ion can be methylated by **1**, but the reaction is relatively slow with $k_2 = 2 \times 10^{-3}$ dm^3 mol^{-1} s^{-1}; *cf.* $k_2 = 30$ dm^3 mol^{-1} s^{-1} for MeCl in H_2O.[15] Because of the presence of the aromatic ring in **1**, one cannot exclude the possibility that the mechanism involves an initial partial (or even complete) electron transfer from Co^{I} to the phenyl ring, hence partial radical character in the Me transfer; *cf.* the analogous 'radicaloid' mechanism for the alkylation of carbanions by *N*-alkyl-pyridinium ions.[16] We have earlier proposed such a mechanism for labilising the N–Me bond for transfer to Co^{I},[17] while Hilhorst *et al.* have suggested 'the involvement of an electron transfer step and radical intermediates in transfer to thiols'.[5] Our results also show that neither the mono- nor the di-methylated anilines show any comparable methylating ability. There appears to have been no systematic study of the effect of increasing methylation on either the aniline as Me-acceptor or on the anilinium ion as Me-donor, although a brief report indicates that aniline and *N*-dimethylaniline are methylated by MeI in MeOH at rates which differ by less than 10%.[18] Further work is needed to establish whether the greater methylating ability of **1** over **4** and **5** is due to a lower adverse free energy of desolvation (required to enter the hydrophobic cavity surrounding the Co ion) due to the absence of H-bonding N–H bonds or to an enhanced labilisation of the N–Me bond due to steric crowding and strain induced by full methylation.

The availability of a protein-free model for Me transfer from N to Co^{I} corrinoids, which both have an effective basicity (*cf.* pK 5.1 for **4**) similar to that of N^5 in THFA itself (4.8)[8] as well as an analogous electronic structure (with the –NMe-group as substituent in an aromatic/heterocyclic ring), should further our understanding of the mechanism of reaction of the B_{12}-dependent methyl transferases.

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Footnote

† Me–Cbl was identified as the product by TLC on cellulose using solvent 1 of ref. 19 (sec-BuOH: H_2O 9.5:4) against a known sample prepared from MeI.

References

1 See, for example: (*a*) R. G. Pearson, H. Sobel and J. Songstad, *J. Am. Chem. Soc.*, 1968, **90**, 319; (*b*) C. Lassau and J.-C. Jungers, *Bull. Soc. Chim. France*, 1968, 2678; (*c*) W. J. Albery and M. Kreevoy, *Adv. Phys. Org. Chem.*, 1978, **16**, 87; (*d*) E. M. Arnett and R. Reich, *J. Am. Chem. Soc.*, 1980, **102**, 5892.

2 R. G. Matthews and J. T. Drummond, *Chem. Rev.*, 1990, **90**, 1275.
3 G. N. Schrauzer and R. J. Windgassen, *J. Am. Chem. Soc.*, 1967, **89**, 3607.
4 E. Hilhorst, Dissertation, University of Amsterdam, 1993.
5 E. Hilhorst, T. B. R. A. Chen and U. K. Pandit, *J. Chem. Soc., Chem. Commun.*, 1993, 881.
6 W. Friedrich, H. C. Heinrich, E. Königk and P. Schulze, *Ann. New York Acad. Sci.*, 1964, **112**, 601; H. P. C. Hogenkamp, *Fed. Proc.*, 1966, **25**, 1623.
7 J. M. Pratt, M. S. A. Hamza and G. J. Buist, *J. Chem. Soc., Chem. Commun.*, 1993, 701.
8 R. G. Kallen and W. P. Jencks, *J. Biol. Chem.*, 1966, **241**, 5845.
9 T. C. Bruice and R. Lapinski, *J. Am. Chem. Soc.*, 1958, **80**, 2265.
10 M. H. Abraham, P. P. Duce, D. V. Prior, D. G. Barratt, J. J. Morris and P. J. Taylor, *J. Chem. Soc., Perkin Trans. 2*, 1989, 1355.
11 G. D. Fasman, *Handbook of Biochemistry and Molecular Biology, Physical and Chemical Data*, 3rd edn., CRC Press, Cleveland, OH, 1976, vol. 1.
12 M. S. A. Hamza and J. M. Pratt, *J. Chem. Soc., Dalton Trans.*, 1994, 1367.
13 J. M. Pratt, *Metal Ions in Biol. Systems*, 1993, **29**, 229.
14 S. M. Chemaly, R. A. Hasty and J. M. Pratt, *J. Chem. Soc., Dalton Trans.*, 1983, 2223.
15 G. N. Schrauzer and E. Deutsch, *J. Am. Chem. Soc.*, 1969, **91**, 3341.
16 A. R. Katritzky and G. Musumarra, *Chem. Soc. Revs.*, 1984, **13**, 47.
17 J. M. Pratt, J. Aron, S. M. Chemaly and H. M. Marques, in *The Biological Alkylation of Heavy Elements*, ed. P. J. Craig and F. Glocking, RSC Special Publication no 66, 1988, p. 46.
18 R. G. Pearson, H. Sobel and J. Songstad, *J. Am. Chem. Soc.*, 1968, **90**, 319.
19 R. A. Firth, H. A. O. Hill, J. M. Pratt and R. G. Thorp, *Anal. Biochem.*, 1968, **23**, 429.