Methylation of zinc bound thiolates; a model for cobalamine independent methionine synthase

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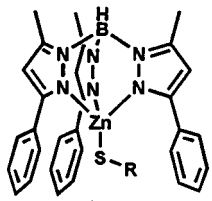
Pyrazolylborate–zinc–thiolate complexes react under mild conditions with methyl iodide, dimethylsulfate and trimethylsulfonium iodide, liberating the corresponding methyl thioethers; the driving force for these reactions lies in the high nucleophilicity of the zinc-bound thiolates and the low donor quality of thioethers toward zinc.

Methylation of thiols is an essential biological process,[1] being required *inter alia* for the biosynthesis of methionine or during DNA repair by the Ada protein. It is becoming evident that zinc plays an important rôle in this process,[2] being involved catalytically in Ada,[3] cobalamine independent methionine synthase,[4] or methanol–CoM–methyltransferase.[5] A common source of the methyl group is methyltetrahydrofolate, in the form of a methylammonium cation. Frequently methylcobalamine is the methyl group transfer agent, but in the world of plants which lacks vitamin B_{12} the methylating enzyme catalyzes the direct alkyl transfer from the methyl source to the thiol.

Model studies involving zinc complexes were published by Walker and Lippard[6] [reactions of $Zn(SPh)_4^{2-}$, $LZn(SPh)_3^-$ or $L_2Zn(SPh)_2$ with trimethyl phosphate] and by Darensbourg and coworkers[7] [reactions of a solvated tetradentate-N_2S_2 zinc complex with iodomethane and dibromopropane]. In the former case it was concluded that the thiolate dissociates from zinc prior to alkylation, and in the latter case the intermediate replacement of a thiolate ligand by a solvent molecule could not be ruled out. We now present evidence for the intramolecular alkylation of zinc bound thiolates and show how the ligand environment of zinc as well as the nature of the thiolates and the methylating agents affect the group transfer reactions.

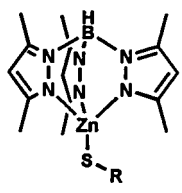
Based on the proposal[8] that in cobalamine independent methionine synthase the reacting thiol homocysteine is activated as a protein–zinc–thiolate in a neutral or monoanionic L_3Zn–SR complex, we chose again substituted pyrazolylborates Tp* to mimic the protein L_3 environment, thereby ensuring that the Tp*Zn thiolates are uncharged and that the zinc ion is encapsulated by the 3-substituents of the Tp* ligands. Complexes **1** and **2** were used as model compounds. Such complexes form spontaneously from the corresponding Tp*Zn–OH complexes (our 'enzyme models')[9] and thiols at neutral pH,[10] as verified here for **1a** and **1b**. **2a** and **2b**, whose corresponding $Tp^{Me,Me}Zn$–OH complex is unstable, were prepared from $Tp^{Me,Me}Zn$–Cl and the sodium thiolates.†



L^1Zn–SR

1a: R = Et

1b: R = CH_2Ph



L^2Zn–SR

2a: R = Et

2b: R = CH_2Ph

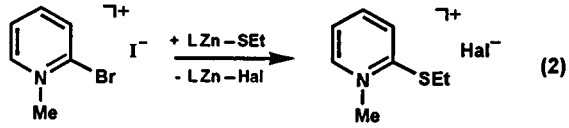
All four complexes **1** and **2** reacted with methyl iodide in a 1:1 ratio in chloroform at room temperature according to eqn. (1).‡ There can be no doubt that the methylations occur at

$$LZn-SR + MeX \longrightarrow LZn-X + MeSR \qquad (1)$$

the zinc bound thiolates, the main pieces of evidence being the non-polar reaction conditions and the fact that cationic Tp*Zn•L complexes can exist only in the presence of very good donor ligands and only in the absence of even weakly coordinating anions.[11] It was verified that the free thiols do not react with methyl iodide under the given reaction conditions. Furthermore, the intermediate existence of free thiolates was deemed unlikely by an exchange experiment: replacement of SEt^- in **1a** by SCH_2Ph^- to form **1b**‡ is about 20 times slower than methylation of SEt in **1a** under the same conditions. Preliminary kinetic data indicate that, as expected, the reactions are bimolecular. Surprisingly, the reactions of **1a** and **1b** are about ten times faster than those of **2a** and **2b**. This indicates that the higher hydrophobicity around zinc in complexes **1** due to encapsulation by the phenyl groups outweighs their lower accessibility due to steric hindrance by the same phenyl groups as a rate-determining factor. An important part of the driving force for these reactions must be ascribed to the poor donor quality of the resulting thioethers toward zinc. It is extremely unlikely that they would form the above-mentioned cationic Tp*Zn–L complexes, and we are actually not aware of any structurally characterized zinc complex with a monodentate thioether ligand.

Methylation reactions according to eqn. (1) were also achieved with dimethylsulfate in chloroform.‡ Although dimethylsulfate is known to be a stronger methylating agent than methyl iodide, it did not react faster here. This is further evidence for the intramolecular nature of these methylations: in a four-center Zn–S/C–X transition state of a bimolecular reaction there is more driving force to proceed *via* a soft–soft interaction (Zn–I) than *via* a soft–hard interaction (Zn–OSO_2OMe). In line with this the related Tp*Zn–OSO_2Me complexes[11] were found to be difficult to handle. $Tp^{Ph,Me}Zn$–OSO_2OMe has so far been characterized only in solution, and instead of $Tp^{Me,Me}Zn$–OSO_2OMe the complex $(Tp^{Me,Me})_2Zn$ was isolated, which is a known dismutation product of unstable $Tp^{Me,Me}Zn$–X complexes.[12]

In an attempt to apply more 'natural' methylating agents, complex **1a** was treated with trimethylsulfonium iodide as a model for the biological methyl donor *S*-adenosyl methionine[4] and with *N*-methylpyridinium iodide as a model for methyltetrahydrofolate. These reactions required higher temperatures and the more polar solvent acetonitrile for the ionic reagents. A clean methylation was achieved with trimethylsulfonium iodide, leaving $Tp^{Ph,Me}Zn$–I. The *N*-methylpyridinium reagent did not transfer a methyl group. When the more electrophilic reagent 2-bromo-*N*-methylpyridinium iodide was used its bromide substituent was replaced by the ethylthio group according to eqn. (2). Thus the zinc bound thiolate of **1a** is a strong enough nucleophile to attack halopyridinium systems. In



this case the remaining zinc species was a mixture of $Tp^{Ph,Me}Zn$–Br and $Tp^{Ph,Me}Zn$–I.‡

The methylation reactions reported here are the closest representations of their biological models yet, specifically of cobalamine independent methionine synthase. The precursors of the Tp*Zn thiolate complexes, the Tp*Zn hydroxide complexes which exist at neutral pH and which may represent the resting enzymes, incorporate the thiols spontaneously with liberation of H_2O, *i.e.* without pH effects. The thiolate complexes react with neutral and ionic methylation reagents in a sterically restricted situation reminiscent of that in the enzymes. An important factor driving the reactions must be the high nucleophilicity of the zinc bound thiolate groups, as we previously found for the zinc bound hydroxide in analogous Tp*Zn–OH complexes.[9,13] Coupled with the low donor strength of thioethers toward zinc, again analogous to the leaving tendency of H_2O from $[Tp^*Zn–OH_2]^+$, this makes the methylation reactions facile and rapid.

At this stage of the investigation we see two challenges. One concerns the choice of alkylating agents which should be closer relatives of methyltetrahydrofolate or *S*-adenosyl methionine. The other concerns the testing of a mechanistic implication of the intramolecular alkylations by methyl iodide. A four-center transition state Zn–O/E–O has been found likely for hydrolytic cleavages of $R_nE(O)$–X substrates by Tp*Zn–OH.[14] If an analogous Zn–S/C–I transition state is implied for the reactions between Tp*Zn–SR and CH_3–I, then this corresponds to front-side attack of the nucleophile at the C-I unit which means retention of configuration at carbon. A mechanistic investigation is indicated which should answer this question as well as that of possible ionic intermediates of the methylation reactions.

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Notes and references

† The constitution of Tp*Zn–SR complexes has been established by structure determinations of $Tp^{tBu,Me}$Zn-SEt[9a] and $Tp^{Cum,Me}$Zn–SPh.[10c] The new species **1** and **2** were characterized by analyses, spectra and a structure determination of **2a**.

‡ On the millimolar scale the methylations by methyl iodide take 1–2 h for **1a**, **b** and about 1 day for **2a**, **b**. The quantitative nature of the methylation reactions and the identity of the resulting thioethers were ascertained by NMR. In those cases where Tp*Zn–Hal or Tp^*_2Zn complexes resulted these were isolated by crystallization and identified by comparing their spectra with data from the literature. The reaction between **1a** and an excess of $[N(PPh_3)_2][SCH_2Ph]$ leads to *ca*. 10% of **1b** after 3 days and takes weeks to reach equilibrium.

1 R. G. Matthews and J. T. Drummond, *Chem. Rev.*, 1990, **90**, 1275.
2 R. G. Matthews and C. W. Goulding, *Curr. Opin. Chem. Biol.*, 1997, **1**, 332.
3 L. C. Myers, M. P. Terranova, A. E. Ferentz, G. Wagner and G. L. Verdine, *Science*, 1993, **261**, 1164.
4 J. C. Gonzales, K. Peariso, J. E. Penner-Hahn and R. G. Matthews, *Biochemistry*, 1996, **35**, 12 228.
5 K. Sauer and R. K. Thauer, *Eur. J. Biochem.*, 1997, **249**, 280.
6 J. J. Wilker and S. J. Lippard, *Inorg. Chem.*, 1997, **36**, 969.
7 C. A. Grapperhaus, T. Tuntulani, J. H. Reibenspies and M. Y. Darensbourg, *Inorg. Chem.*, 1998, **37**, 4052.
8 K. Peariso, C. W. Goulding, S. Huang, R. G. Matthews and J. E. Penner-Hahn, *J. Am. Chem. Soc.*, 1998, **120**, 8410.
9 (*a*) R. Alsfasser, M. Ruf, S. Trofimenko and H. Vahrenkamp, *Chem. Ber.*, 1993, **126**, 703; (*b*) M. Ruf, K. Weis and H. Vahrenkamp, *J. Chem. Soc., Chem. Commun.*, 1994, 135; (*c*) K. Weis and H. Vahrenkamp, *Inorg. Chem.*, 1997, **36**, 5589.
10 (*a*) M. Ruf and H. Vahrenkamp, *Inorg. Chem.*,1996, **35**, 6571; (*b*) M. Ruf, R. Burth, K. Weis and H. Vahrenkamp, *Chem. Ber.*, 1996, **129**, 1251; (*c*) R. Burth and H. Vahrenkamp, *Z. Anorg. Allg. Chem.*, 1998, **624**, 381.
11 T. Brandsch, F. A. Schell, K. Weis, M. Ruf, B. Müller and H. Vahrenkamp, *Chem. Ber.*, 1997, **130**, 283.
12 A. Looney, R. Han, I. B. Gorell, M. Cornebise, K. Yoon, G. Parkin and A. L. Rheingold, *Organometallics*, 1995, **14**, 274.
13 (*a*) M. Ruf and H. Vahrenkamp, *Chem. Ber.*, 1996, **129**, 1025; (*b*) K. Weis, M. Rombach, M. Ruf and H. Vahrenkamp, *Eur. J. Inorg. Chem.*, 1998, 263.
14 M. Rombach, C. Maurer, K. Weis, E. Keller and H. Vahrenkamp, *Chem. Eur. J.*, in the press.

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