Evidence for a covalent intermediate in the *S*-adenosyl-L-methionine-dependent transmethylation reaction catalysed by sirohaem synthase

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CysG, also known as uroporphyrinogen III methylase and sirohaem synthase (CysG; EC 2.1.1.107), is a multifunctional enzyme that is able to transform uroporphyrinogen III into sirohaem via two *S*-adenosyl-L-methionine (AdoMet)-dependent transmethylation reactions, an NAD⁺-dependent dehydrogenation and a ferrochelation. The apparent tight binding of AdoMet to this multifunctional enzyme is investigated. The use of a rapid AdoMet binding assay demonstrates that CysG becomes labelled with both [*methyl*-³H]AdoMet and [*carboxyl*-¹⁴C]AdoMet. Fur-

ther experiments show that the CysG–AdoMet complex is subsequently able to methylate uroporphyrinogen III. CysG remains associated with the labelled constituents of the AdoMet even after denaturation with urea and SDS/PAGE, suggesting that the AdoMet has become covalently linked to the protein. A rapid examination of some of the other transmethylases involved in corrin biosynthesis reveals that they bind the AdoMet in a similar fashion. A multistep transmethylation mechanism is proposed to explain the observed results.

INTRODUCTION

The *cysG* gene encodes a protein that is involved in the final stages of sirohaem biosynthesis in both *Escherichia coli* and *Salmonella typhimurium* [1,2] and has recently been shown to be a multifunctional enzyme (EC 2.1.1.107) [3–5]. The enzyme performs two *S*-adenosyl-L-methionine (AdoMet)-dependent transmethylation reactions at positions 2 and 7 of uroporphyrinogen III [6]. These methyl transfers produce precorrin-2 (dihydrosirohydrochlorin) (Scheme 1), a known intermediate in both the cobalamin and sirohaem biosynthetic pathways. An NAD⁺-dependent dehydrogenation of precorrin-2 yields the isobacteriochlorin sirohydrochlorin, and a subsequent ferrochelation yields sirohaem (Scheme 1) [3–5]. Sirohaem is required by both sulphite and nitrite reductases and mutants associated with the former enzyme are unable to make the amino acid cysteine [1]; hence the prefix *cys* is found affiliated both with genes that encode for the subunits of the enzyme and genes that are required for the biosynthesis of the prosthetic group. CysG is also known to be required for cobalamin biosynthesis in *S. typhimurium* where precorrin-2 acts as the substrate for corrin ring formation [7].

In *E. coli*, sirohaem synthase (CysG) performs all the aforementioned reactions concerned with the transformation of uroporphyrinogen III into sirohaem [3,4]. The protein contains 457 amino acids [8] and the polypeptide chain can be thought of as folded into at least two functional domains (Figure 1) [4]. The N-terminal domain, amino acids 1–201, contains the NAD⁺ binding site (14–41) as well as the catalytic machinery to perform the ferrochelation reaction. The C-terminal domain, amino acids 202–457, contains the AdoMet binding site (218–234) and performs the methyl transfer reaction [3,4,9]. Gene dissection has permitted the expression of the C-terminal domain of the CysG protein as a truncated protein and it has been shown that this truncated protein retains the ability to methylate uroporphyrin-

ogen III in the same way as CysG but is unable to catalyse the macrocyclic oxidation step or the ferrochelation reaction [4]. In this respect the truncated protein is similar to CobA, the uroporphyrinogen methylase involved in corrin biosynthesis from *Pseudomonas denitrificans* [10] and *Bacillus megaterium* [11] and for this reason the domain has been termed CysG^A in contrast with the N-terminal domain, which has been termed CysG^B [4]. In the absence of NAD⁺, both CysG and CysG^A perform an ‘overmethylation’ whereby an extra methyl group is transferred to position 12, producing a trimethylpyrrocorphin with no known physiological function [12,13]. In *S. typhimurium*, CysG is also required for cobalamin biosynthesis as precorrin-2 can be directed along the corrin pathway by methylation at position 20 [7,14]. As *S. typhimurium* belongs to the corrin-producing class of bacteria that make cobalamins anaerobically and insert cobalt at an early stage in the corrin pathway [15,16], CysG may also be responsible for cobalt chelation of precorrin-2 (E. Raux, A. Lanois, F. Levillayer, M. J. Warren, E. Brody, A. Rambach and C. Thermes, unpublished work). The full summary of reactions catalysed by CysG is shown in Scheme 1.

The AdoMet-dependent C-methylation reactions performed by CysG are similar to the other C-methylations carried out during the biosynthesis of vitamin B₁₂ [18–22]. Corrin biosynthesis requires a total of eight peripheral methylations, at C-2, C-7, C-20, C-17, C-12, C-1, C-5 and C-15, whose addition is mediated by six methyltransferases, CysG (or CobA), CobI, CobJ, CobM, CobF and CobL. The discrepancy between the number of methylations (eight) and the number of enzymes (six) is explained by the fact that two of the methyltransferases are bifunctional, CysG (CobA) and CobL [21,22]. The amino acid sequences of the methyltransferases involved in corrin biosynthesis reveal a certain similarity in terms of their primary structure, and evolutionary genetic analysis suggests that they are all derived from a common ancestor [21]. Such structural similarity would also be indicative of a common mechanism. The

Scheme 1 Role played by CysG in tetrapyrrole modification

Reactions catalysed by the multifunctional CysG. The enzyme performs two AdoMet-dependent methyl transfers, initially generating precorrin-1 and subsequently precorrin-2. Sirohaem is generated from precorrin-2 by an NAD^+ -dependent dehydrogenation and a ferrochelation event. In the absence of NAD^+ and at high enzyme concentrations, CysG will 'overmethylate' precorrin-2 to yield a trimethylpyrrocorphin with no known physiological role. CysG may also chelate cobalt at the level of precorrin-2 to give cobalt-precorrin-2 and thus direct the intermediate towards cobalamin biosynthesis.

Figure 1 Domain structure of CysG

CysG can be envisaged as being divided into two functional domains. The C-terminal domain is responsible for the transmethylation reactions and is termed CysG^A, and the N-terminal domain catalyses the NAD^+ -dependent dehydrogenation and ferrochelation reactions and is termed CysG^B. The relative positions of the putative NAD and AdoMet binding sites are shown.

methyltransferases in corrin biosynthesis have been assigned not only on their sequence similarity but also on their ability to bind AdoMet [23]. An AdoMet binding assay, in which *S*-adenosyl[*methyl*- ^3H]methionine is mixed with the protein and

then passed down a small gel-filtration column, has been used to confirm the function of several of the corrin methylases. Co-elution of AdoMet with the protein during gel filtration identifies the protein as a methylase, whereas AdoMet was found to separate from the protein and to be eluted later in the small-molecules fraction in control experiments with BSA [23]. Such tight binding as that observed between the methylases and AdoMet was assumed to be due to a low dissociation constant (K_d). In this paper we provide evidence that the binding is due to a covalent attachment between the AdoMet and the protein.

MATERIALS AND METHODS

Chemicals

Most chemicals, reagents and antibiotics were purchased from the Sigma Chemical Corporation. Sephacryl S-300 and DEAE-Sephacel were obtained from Pharmacia Fine Chemicals. Tryptone, yeast extract and agar were purchased from Difco Laboratories. Mimetic Green-1 affinity chromatography resin

was purchased from Affinity Chromatography Ltd, Cambridge, U.K. S-Adenosyl-L-[methyl-³H]methionine (80 Ci/mmol), S-adenosyl-L-[carboxyl-¹⁴C]methionine (50 mCi/mmol) and Hyperfilm-MP were purchased from Amersham International. Bio-Rad protein assay was obtained from Bio-Rad. Autofluor was purchased from National Diagnostics. Porphobilinogen (PBG) was synthesized from 5-aminolaevulinic acid (5-ALA) from purified 5-ALA dehydratase as previously described [24].

Bacterial strains

E. coli strain TB1 transformed with pCR2552, pMW1 and pMW2 was used for the overexpression of CysG, CbiF and CbiH respectively [6,25]. Strains were grown from 5% inocula for 18 h at 37 °C in LB medium supplemented with ampicillin (50 mg/l).

Enzyme purification

CysG was purified by the method described by Warren et al. [5]. Porphobilinogen deaminase and uroporphyrinogen III synthase were isolated from recombinant *E. coli* strains harbouring plasmids containing the *hemC* and *hemD* genes respectively, as previously described [26]. CbiH and CbiF were purified by the method described by Roessner et al. [25]. Proteins were stored at –20 °C in 50 mM Tris buffer, pH 7.8, with 10 mM 2-mercaptoethanol.

Rapid AdoMet binding assay

Purified protein (1 mg) was incubated with either 0.5 μ Ci of [methyl-³H]AdoMet or [carboxyl-¹⁴C]AdoMet in a final volume of 200 μ l of 50 mM Tris buffer, pH 7.8, and 1 mM 2-mercaptoethanol at 37 °C for 15 min. The specific activity of the AdoMet was adjusted to approx. 50 μ Ci/mmol with unlabelled AdoMet before use. The reaction mixture was applied to a Sephadex G25 column (1 cm $\times$ 15 cm) and fractions (1 ml) were collected. Protein-containing fractions were identified by the Bio-Rad protein assay and the radioactivity of the individual fractions was determined with a Pharmacia Wallac 1410 scintillation counter.

Analysis of AdoMet binding to CysG by fluorography

Labelled protein (20 μ g) was boiled for 2 min in sample disruption buffer and then loaded on to an SDS/12% polyacrylamide gel. After electrophoresis at 40 mA for 30 min the gel was stained with Coomassie Blue and destained in 7% (v/v) acetic acid. The gel was soaked in Autofluor for 1–2 h before drying to enhance the detection of the labelled protein. The gel was exposed to X-ray film at –80 °C.

Generation of uroporphyrinogen III

Uroporphyrinogen III was generated *in situ* by using purified PBG deaminase and uroporphyrinogen III synthase. Generation of 25 μ M uroporphyrinogen III was achieved by incubating 0.1 mM PBG with 10 μ g/ml PBG deaminase and 1 μ g/ml uroporphyrinogen III synthase at 37 °C for 10 min in 50 mM Tris buffer, pH 8.0. For fluorography experiments, methylated CysG (2 mg/ml) was mixed with the appropriate amounts of deaminase and uroporphyrinogen III synthase. The sample was divided into two and uroporphyrinogen III was generated in one of the samples by the addition of PBG. The two samples were

subsequently boiled in disruption buffer and subjected to SDS/PAGE in 12% gels in adjoining lanes.

CD spectra

CD spectra were obtained at the National Chiroptical Spectroscopy Centre (EPSRC) in the Chemistry Department, Birkbeck College, London, U.K. CD spectra were recorded at room temperature over the range 180–260 nm on a Jasco J720 spectropolarimeter with a cell pathlength of 0.02 cm.

RESULTS AND DISCUSSION

Previous work has demonstrated that some of the *Ps. denitrificans* AdoMet-dependent methyltransferases involved in the peripheral methylation of the corrin ring structure of vitamin B₁₂ bind AdoMet very tightly [23]. This was demonstrated by the association of radioactivity derived from S-adenosyl-L-[methyl-³H]methionine with the methyltransferase after separation of an AdoMet/enzyme mixture by gel filtration chromatography. The rapid AdoMet binding assay is a useful technique not only for ascribing the function of ‘unknown’ proteins in the cobalamin biosynthetic operons but also for detection of the methyltransferases during purification. Although this result was interpreted as being due to a high affinity of the enzyme for AdoMet (a low K_d), it could also be due to the covalent attachment of the AdoMet to the protein or methylation of the protein itself. These possibilities were not examined.

The CysG protein was also analysed with the rapid binding assay to see if it demonstrated the same tight binding to AdoMet. The addition of [methyl-³H]AdoMet (0.5 μ Ci, 10 nmol) to a CysG solution, 200 μ l of a 5 mg/ml solution, followed by gel filtration chromatography yielded two radioactive peaks (Figure 2a). These peaks corresponded to AdoMet-associated protein and unbound AdoMet. When the experiment was performed with BSA as a control, only one radioactive peak was observed, corresponding to unbound AdoMet (Figure 2b). The radioactivity associated with fractions 5–7 contained 95% of the applied protein, whereas the radioactivity that was eluted in fractions 11–14 contained no protein. CysG is therefore able to bind AdoMet tightly, in agreement with its role as an AdoMet-dependent methyltransferase. This result is similar to the observations made by Crouzet et al. [23], who used this test with the CobA and CobF methyltransferases from *Ps. denitrificans*. A detailed kinetic analysis of CysG has shown that it has a K_m for AdoMet in the micromolar range, which is similar to the K_m for NAD⁺, another substrate required in the oxidation of precorrin-2 into sirohydrochlorin (S. C. Woodcock and M. J. Warren, unpublished work). However, the latter dinucleotide does not associate with the enzyme on the gel filtration column, indicating that NAD⁺ does not bind as tightly as AdoMet (results not shown). For tight binding, as is apparently observed with AdoMet, a K_d in the nanomolar range would be required. The discrepancy between the K_m and the apparent K_d led us to investigate further the association of AdoMet with CysG.

To test whether the labelled moiety that associated with the CysG fraction on gel filtration was covalently bound to the enzyme, the following two experiments were performed. First, the enzyme was mixed and incubated with [methyl-³H]AdoMet at 37 °C for 10 min and was subsequently treated with 4 M urea. Incubation with urea above a concentration of 4 M with CysG leads to denaturation of the protein, a conclusion deduced from a study of the circular dichroism (CD) spectrum. The native protein shows a CD spectrum consistent with a mixture of helical (8%) and β -strand (36.5%) conformations, whereas there is a

Figure 2 Rapid AdoMet binding assay with BSA and CysG

Graphs showing the elution profile of radioactivity from the rapid AdoMet binding assay performed on a Sephadex G-25 column. Protein was eluted in fractions 5–7 whereas AdoMet was eluted in fractions 11–14. (a) Elution profile observed when a CysG/[methyl- ^3H]AdoMet mixture was analysed; (b) elution profile of a BSA/[methyl- ^3H]AdoMet mixture; (c) elution profile of CysG after mixing with [carboxyl- ^{14}C]AdoMet; (d) comparison of the elution profile of CysG denatured with urea before the addition of [methyl- ^3H]AdoMet ($\square$) and CysG denatured with urea after it had been mixed with [methyl- ^3H]AdoMet ($\circ$). Only the elution profile surrounding fractions 1–9 is shown in this instance to demonstrate the marked difference between them.

Figure 3 CD spectrum of CysG

Comparison of the CD spectra of native CysG (thick line) with that of CysG in 4 M urea (thin line). The native CysG spectrum is characteristic of a protein containing both helical and extended conformations. Addition of 4 M urea leads to a protein with a greatly reduced amount of defined secondary structure and increased random coil conformation. Data acquisition was not possible below 205 nm owing to interference from the urea.

large change in the spectrum when the protein is treated with 4 M urea, associated with the abolition of the major secondary-structure elements (Figure 3). A urea-treated enzyme/[methyl- ^3H]AdoMet mixture was chromatographed on Sephadex G-25. The same elution pattern of two radioactive peaks was observed as previously shown, in that a high proportion of the radioactivity was eluted with the protein-containing fractions 5–7 (Figure 2d). Importantly, if the protein was first denatured with urea before the addition of [methyl- ^3H]AdoMet, none of the label was eluted with the protein-containing fractions but was all accounted for in the unbound fractions 11–14 (Figure 2d). These experiments prove (i) that the tertiary structure of the protein is important for AdoMet binding, and (ii) that the labelling of the protein is due to a specific reaction between CysG and AdoMet. Moreover, the fact that the label remains bound to the protein, even after denaturation, indicates that the association is probably of a covalent nature. Similar AdoMet binding results are also obtained when CysG is treated with 1% (w/v) SDS, although the protein is not denatured to the same extent as by treatment with urea; the CysG/SDS CD spectrum is only slightly altered from the native protein spectrum (results not shown).

The stoichiometry of AdoMet binding was also investigated by increasing the concentration of [methyl- ^3H]AdoMet to CysG until a saturating incorporation of label was observed. This allowed a crude estimate of the binding ratio of AdoMet to protein to be determined and this was found to be about 1 nmol of label per 10 nmol of protein under the conditions tested. Several reasons can be attributed for this apparent low binding ratio (10%) of AdoMet to CysG. In the absence of an accurate active-site titrant, it is not known whether all the protein is fully functional or, as a dimer, whether it demonstrates half-site reactivity; likewise it is also not known what proportion of the enzyme is already associated with unlabelled AdoMet. Taking these arguments into account the observed 10% incorporation of label into the protein must be highly significant.

The second experiment that demonstrates the covalent link between the AdoMet-derived methyl group and CysG comes from the association of the labelled methyl group with the

Figure 4 SDS/PAGE and fluorogram of BSA and CysG after incubation with [*methyl*- ^3H]AdoMet

(a) SDS/polyacrylamide gel of BSA (10 μg) and CysG (10 μg) after both had been mixed with 10 nCi of *S*-adenosyl-L-[*methyl*- ^3H]methionine. Lane 1 contains M_r markers as shown, lane 2 contains BSA and lane 3 contains CysG. A fluorogram taken of this gel clearly shows that only the protein in lane 3 (CysG) is labelled. (b) Fluorogram showing the effect of uroporphyrinogen III on the labelled protein. Lane 1 contains CysG (10 μg) that had been incubated with [*methyl*- ^3H]AdoMet and lane 2 shows the same amount of labelled protein but to which had been added an excess of uroporphyrinogen III.

protein on polyacrylamide gels. Enzyme mixed with [*methyl*- ^3H]AdoMet was boiled in sample disruption buffer and subjected to PAGE. After electrophoresis the gel was dried and analysed by fluorography. The resulting autoradiogram clearly showed a dark band migrating at M_r 50000, indicating that the label had remained associated with the protein even during denaturing electrophoresis (Figure 4a). These two pieces of evidence clearly demonstrate that the protein was being covalently modified with the tritiated component in the AdoMet. The labelling of the CysG was also shown to be time-dependent: increased incubation time at 37 °C with [*methyl*- ^3H]AdoMet and CysG led to a higher incorporation of the label into the protein up to an incubation time of 60 min (results not shown). This was judged from the increased intensity of the labelled bands on the fluorogram.

It was also found that if CysG was preincubated with an excess of unlabelled AdoMet before incubation with [*methyl*- ^3H]AdoMet, no incorporation of label was observed by either gel filtration or fluorography. Conversely if CysG, labelled with [*methyl*- ^3H]AdoMet, was incubated with an excess of unlabelled AdoMet, the protein was found to retain the label. These results suggest that, once attached to the protein, the AdoMet moiety is not readily exchanged. In fact, the labelled protein was found to be stable to both acidic (pH 2) and alkaline (pH 10) environments for at least 1 h and did not dissociate in 1 M hydroxylamine. However, high salt concentrations (up to 1 M NaCl) affected the association of the AdoMet with CysG (results not shown).

The covalent modification of AdoMet methyltransferases has been reported before but in a very different context. Previous modifications of AdoMet-dependent enzymes have been achieved with photolabelling techniques whereby the enzyme-AdoMet complex is irradiated with UV [27,28]. The ensuing free-radical chemistry gives rise to covalently bound adducts of AdoMet at the active site of the proteins. This type of labelling, as reported with the *Eco*RII and *Che*R methyltransferases, is substantially different from the experiments reported here because UV irradiation of CysG-AdoMet complexes leads to a decrease in the incorporation of label into the protein (results not shown). Another protein covalently inactivated by AdoMet is aminocyclopropane carboxylate synthase [29]. This enzyme uses pyridoxal phosphate as a cofactor to generate aminocyclopropane carboxylate from AdoMet. Under certain conditions the enzyme becomes inactivated by the AdoMet-derived aminobutyrate, which covalently modifies the active-site lysine residue. In all these examples the enzymes are irreversibly inactivated and the covalent modification of the protein is not part of the catalytic cycle of the enzymes.

To determine whether CysG is complexed with the complete AdoMet molecule or just the labile methyl group of AdoMet, the binding of [*methyl*- ^3H]AdoMet to CysG was compared with the binding of [*carboxyl*- ^{14}C]AdoMet. When [*carboxyl*- ^{14}C]AdoMet was used in the AdoMet column binding assay two radioactive peaks were again observed (Figure 2c). The [*carboxyl*- ^{14}C]AdoMet behaved in the same way as the [*methyl*- ^3H]AdoMet in that denaturation of the CysG-AdoMet complex with urea did not release the bound label. To prevent the [*carboxyl*- ^{14}C]AdoMet from binding to CysG the protein had to be denatured first with urea (results not shown). These results prove that it is not the transfer of the methyl group from the AdoMet to CysG that is giving rise to the labelled protein. The experiments suggest that the protein is modified with the whole AdoMet molecule, although assays with labelled adenine AdoMet need to be performed to confirm this unambiguously.

The final question raised by this intriguing binding observation is whether the bound AdoMet subsequently transfers its methyl group to the other reaction substrate, uroporphyrinogen III. To answer this question two further experiments were performed. Thus the [*methyl*- ^3H]AdoMet-CysG complex, isolated after gel filtration chromatography, was incubated with uroporphyrinogen III. After incubation, the uroporphyrinogen III was separated from the enzyme by anion-exchange chromatography. Most of the protein was eluted from the anion-exchange resin by 0.5 M NaCl whereas the majority of the tetrapyrrolic material was eluted by 2 M NaCl. The protein fraction contained 33 % of the radioactivity, whereas the uroporphyrinogen III fraction contained 67 %. Most of the label had therefore been transferred to the uroporphyrinogen III. The remainder of the label associated with the protein fraction probably represents unseparated uroporphyrinogen III, as it is difficult to achieve full separation of the protein from the porphyrinogen. Further

Figure 5 Rapid AdoMet binding assay with CbiH

Rapid AdoMet binding profile of *S. typhimurium* CbiH, showing that the CbiH retains the AdoMet after denaturation with 4 M urea (□), whereas denaturation before incubation with AdoMet resulted in greatly reduced binding (○). A very similar profile was obtained with CbiF (results not shown).

convincing evidence that uroporphyrinogen III was able to remove the methyl group from the labelled [*methyl*-³H]AdoMet–protein was obtained from SDS/PAGE and fluorography. Two identical samples containing [*methyl*-³H]AdoMet–CysG as well as small amounts of the enzymes PBG deaminase and uroporphyrinogen III synthase were prepared. Addition of PBG to one of the samples generated uroporphyrinogen III *in situ*. After 10 min incubation at 37 °C the samples were boiled in disruption buffer and subjected to SDS/PAGE; the resulting gel was analysed by fluorography. Visualization of the fluorogram clearly showed that the protein sample to which uroporphyrinogen III had been added had lost the majority of the label (Figure 4b, lane 2), whereas the label was retained in the untreated sample (Figure 4b, lane 1). Together these two experiments demonstrate that uroporphyrinogen III is able to remove the methyl group from the protein and that uroporphyrinogen III is itself the final recipient of the methyl group.

The work of Crouzet et al. [23] has also shown that the *Ps. denitrificans* CobA and CobF transmethyldases of corrin synthesis bind AdoMet very tightly, probably in the same way as described in this paper. To ascertain whether other cobalamin biosynthetic AdoMet-dependent methyltransferases also bind AdoMet covalently, the *S. typhimurium* CbiF and CbiH, which are homologues of the *Ps. denitrificans* CobM and CobJ, were purified from the appropriate recombinant strains [25] and analysed in the rapid AdoMet binding assay. CbiF is the methylase responsible for methylation at C-11, whereas CbiH is responsible for methylation at C-17 and ring contraction. The result of this exercise is presented for CbiH in Figure 5 and shows that CbiH is able to bind the labelled AdoMet and that the label remains associated with the protein even after denaturation with urea. Furthermore denaturation of CbiH with urea before the addition of AdoMet leads to a large reduction in AdoMet binding. This is very similar to the result observed with CysG (Figure 2d). The same result was obtained for CbiF (result not shown). It thus seems likely that many of the B₁₂ methyltransferases use the same multistep methylation process, whereby the methyltransferase first becomes modified with AdoMet before transfer of the methyl group from the complex to the substrate target.

The results presented in this paper provide strong evidence that CysG catalyses the AdoMet-dependent methyltransferase reaction to uroporphyrinogen III in a multistep process. The

Scheme 2 Methylation model for reaction catalysed by CysG

(a) Postulated S_N2 displacement reaction between AdoMet and uroporphyrinogen III. After rearrangement the process is repeated at position 7 to give precorrin-2. (b) Methylation cycle for CysG consistent with the observations made in this study.

protein is first modified by the attachment of AdoMet to form an enzyme intermediate. Interaction with the next substrate, uroporphyrinogen III, produces precorrin-1 and *S*-adenosyl-L-homocysteine by transmethylation of the methyl group from the AdoMet–protein complex to the tetrapyrrole. The enzyme undergoes another round of AdoMet modification which, in turn, catalyses the transformation of precorrin-1 into precorrin-2 (Scheme 2).

The steric course of AdoMet-dependent transmethylation reactions has been studied by using chiral methyl AdoMet and this has shown that the reactions go by overall inversion [30], in agreement with a direct S_N2 displacement of the methyl group from AdoMet. The sulphonium cation in AdoMet makes the molecule thermodynamically unstable and allows it to act as a good alkylating agent. The steric course of the methylations at C-5, C-7 and C-12 of the corrin ring has been investigated and the reaction also shown to go by inversion [31]. The association of the AdoMet with CysG would be consistent with this result as long as the methyl group were not transferred first to the enzyme. This is thus in agreement with our experimental findings.

The work presented in this paper demonstrates that CysG forms a complex association with AdoMet. Radiolabelling experiments demonstrate that the association is of a covalent nature and the AdoMet is only released on incubation with the recipient of the methyl transfer, uroporphyrinogen III. The AdoMet association is not restricted to CysG but is probably found in all the corrin biosynthetic methylases. There are several pertinent questions still to be answered. How is the AdoMet attached to the enzyme, what residue on the protein is important

and to where is the covalent bond made on the AdoMet? Furthermore why is such a tight association required? There thus remain several important mechanistic questions to be addressed in this very interesting family of related AdoMet-dependent methyltransferases.

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