FEBS 20940 FEBS Letters 436 (1998) 401–402

His^{84} rather than His^{35} is the active site histidine in the corrinoid protein MtrA of the energy conserving methyltransferase complex from *Methanobacterium thermoautotrophicum*

Karin Sauer, Rudolf K. Thauer*

Max-Planck-Institut für terrestrische Mikrobiologie and Laboratorium für Mikrobiologie, Fachbereich Biologie der Philipps-Universität Marburg, Karl-von-Frisch-Straße, D-35043 Marburg, Germany

Received 4 September 1998

Abstract The energy conserving corrinoid containing MtrA-H complex from *Methanobacterium thermoautotrophicum* is composed of eight different subunits of which MtrA harbors the corrinoid prosthetic group, the corrinoid being bound in the base-off/His-on configuration. Based on sequence comparisons it was recently proposed that His^{35} of MtrA is the active site histidine. We report here that His^{84} rather than His^{35} is the axial ligand to the cobamide in MtrA.

© 1998 Federation of European Biochemical Societies.

Key words: Corrinoid protein; B_{12} binding motif; N^5-Methyltetrahydromethanopterin:coenzyme M methyltransferase; Methanogenic archaeon

1. Introduction

The energy conserving methyltransferase complex from methanogenic archaea catalyzes the exergonic formation of methyl-coenzyme M and tetrahydromethanopterin from N^5-methyltetrahydromethanopterin and coenzyme M ($\Delta G°'$ $= -30$ kJ/mol) and couples this reaction with the electrogenic translocation of sodium ions over the cytoplasmic membrane, in which the complex is integrated [1]. The complex is composed of eight different subunits designated MtrA–H and contains per mol 1 mol 5-hydroxybenzimidazolylcobamide [2]. The 23 kDa subunit MtrA harbors the corrinoid prosthetic group. EPR spectroscopic evidence indicates that the cobamide is bound to MtrA in the base-off/His-on configuration [3].

Corrinoid proteins with the corrinoid bound in the base-off/His-on configuration generally show the consensus sequence DxHxxG-x_{41-42}-SxL-x_{24-28}-GG (Fig. 1) [4–6]. The histidine in the sequence is the lower ligand to the cobalt of the protein bound corrinoid. The consensus sequence, in a modified form, was recently proposed to be present also in MtrA from *Methanosarcina mazei, Methanococcus jannaschii*, *Methanopyrus kandleri*, and *Methanobacterium thermoautotrophicum* [7] (Fig. 1). It was concluded that the conserved histidine in the modified consensus sequence is the histidine to which the cobalt of the cobamide in MtrA is bound. In MtrA of *M. thermoautotrophicum* this conserved histidine is His^{35}.

The conclusion that His^{35} of MtrA is involved in cobamide binding conflicts with a recent report that His^{84} of MtrA from *M. thermoautotrophicum* was identified by site-directed mutagenesis to be the active site histidine [11]. We have therefore reinvestigated the problem by analyzing a His^{35}Lys mutant.

2. Materials and methods

The gene *mtrA*, in the truncated form *mtrA* 1, was mutated and heterologously expressed in *Escherichia coli* as described [11]. The His^{35}Lys mutation was confirmed by sequencing. The overproduced MtrA 1 apoprotein was purified and reconstituted to the corrinoid holoprotein with cob(II)alamine [3]. From the holoprotein EPR spectra were recorded at 77 K using a Bruker EMX EPR spectrometer equipped with a Bruker ER 041 XG microwave bridge (X-band) and a Bruker NMR gauss meter EMX 035. The data were analyzed with the EPR software WIN-EPR. The purified MtrA protein was subjected to Edman degradation which was kindly performed by Dr. D. Linder (Biochemisches Institut, Justus-Liebig-Universität, Gießen).

3. Results and discussion

An interaction of a histidine residue via one of its nitrogens with the cobalt in cobalamine can be determined by EPR spectroscopy [12–15]. The corrinoid in the cob(II) oxidation state shows an EPR spectrum with the g_z signal split into eight equally spaced lines due to interaction of the electron with the cobalt(II) nucleus (nucleus spin 7/2). When the cobalt(II) is axially ligated by a histidine the eight lines are further split into triplets signaling the interaction of the electron with the ^{14}N in the axial ligand (nucleus spin = 1). When the axial ligand is a ^{15}N-labeled histidine the EPR spectrum will exhibit a doublet hyperfine splitting due to the interaction of the electron with the ^{15}N in the axial ligand (nucleus spin = 0.5).

MtrA of *M. thermoautotrophicum* contains in its sequence three histidines, His^{35}, His^{84} and His^{95} [2]. To determine whether His^{35} interacts with the cobalt of the protein bound corrinoid the His^{35} was mutated to Lys. The His^{35}Lys mutated MtrA apoprotein was isolated from *E. coli* cells grown on minimal medium containing (A) $(^{14}NH_4)_2SO_4$ (8 mM), (B) $(^{15}NH_4)_2SO_4$ (8 mM) or (C) $(^{15}NH_4)_2SO_4$ (8 mM) and [^{14}N]histidine (5 mM) and the holoprotein was reconstituted from the apoprotein and [^{14}N]cobalamine. The EPR spectra of the holoprotein in the cob(II)alamine oxidation state thus obtained are shown in Fig. 2. The spectrum shows triplet hyperfine splitting in experiments A and C and doublet hyperfine splitting in experiment B, indicating that a nitrogen of histidine interacts with cobalt(II) of the cobalamine in the MtrA holoprotein His^{35}Lys mutant.

It had to be ascertained that His^{35} was really mutated to Lys in the heterologously produced MtrA 1 protein. The mu-

*Corresponding author. Fax: (49) (6421) 178209.
E-mail: thauer@mailer.uni-marburg.de

0014-5793/98/$19.00 © 1998 Federation of European Biochemical Societies. All rights reserved.
PII: S0014-5793(98)01180-6

A. Consensus sequence of the B_{12} binding motif

--DxH xxG -x_{41-42}- SxL -x_{24-28}- GG-- **MetH, MtaC, MtmC, MttC, MtsB, MutS, MutB**

B. Sequence in MtrA proposed to be a modified B_{12} binding motif

--GxH^{35}xxG -x_{41}- SxV -x_{26}- GA -- **MtrA from *M. mazei***

--GxH^{37}xxE -x_{41}- SxV -x_{24}- GA -- **MtrA from *M. jannaschii***

--GxH^{35}xxE -x_{44}- AxV -x_{25}- GA -- **MtrA from *M. kandleri***

--AxH^{35}xxD -x_{40}- SxV -x_{25}- GA -- **MtrA from *M. thermoautotrophicum***

Fig. 1. Consensus sequence of the B_{12} binding motif [6] and the sequence in MtrA proposed to be a modified B_{12} binding motif [7]. A: MetH, cobalamine-dependent methionine synthase; MtaC, MtmC, MttC and MtsB, corrinoid proteins involved in methyl transfer from methanol, monomethylamine, trimethylamine and methylthiols, respectively, to coenzyme M; MutS, glutamate mutase; and MutB, methylmalonyl-CoA mutase. For sequences see [4,5,8–10]. In methyltransferases but not in mutases the two amino acids between the active site histidine and the glycine are conserved: HDIG. In some cases serine is replaced by threonine. The aspartate in the B_{12} binding motif has a function in the protonation of the active site histidine in the catalytic cycle [6]. B: MtrA, corrinoid harboring 23 kDa subunit of the N^5-methyltetrahydromethanopterin:coenzyme M methyltransferase complex from methanogenic archaea. For sequences see [7].

tated protein was therefore subjected to Edman degradation. The sequence revealed that His^{35} was indeed replaced by a Lys:

```
-VEKKSPAEGWPVVNGDYIVGDPESPVAATTLASKIEDIP   MtrA1 (His35Lys)
VVEKKSPAEGWPVVNGDYIVGDPESPVAATTLASHIEDIP   MtrA1
```

The results show that in MtrA His^{35} is not the active site histidine as suggested by the alignment shown in Fig. 1. His^{95} had previously been excluded [11]. Thus His^{84} has to be the active site histidine. This is substantiated by the finding that MtrA apoprotein mutated in His^{84} no longer reconstituted with cobalamine to the holoprotein [11].

His^{84} is not part of a sequence with the general corrinoid binding motif indicating that binding of B_{12} in MtrA must be different from that in other corrinoid proteins with B_{12} bound in the base-off/His-on configuration. This may reflect that the MtrA-H complex differs from all other corrinoid proteins in catalyzing a reaction coupled with energy conservation via electrogenic sodium ion translocation [16].[8–11] in figs

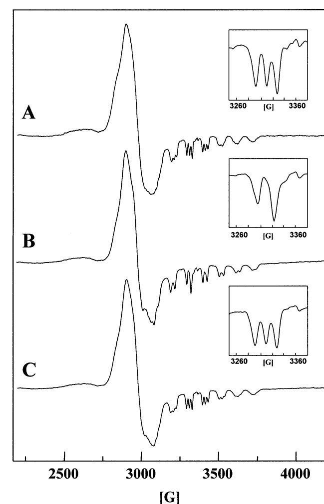

Fig. 2. EPR spectrum of mutated MtrA1 (His^{35}Lys). The holoprotein was reconstituted from the MtrA 1 apoprotein and $[^{14}N]$cobalamine [11]. A: MtrA1 (His^{35}Lys) apoprotein isolated from *E. coli* cells grown on $(^{14}NH_4)_2SO_4$-containing minimal medium. B: MtrA1 (His^{35}Lys) apoprotein isolated from *E. coli* cells grown on $(^{15}NH_4)_2SO_4$-containing minimal medium. C: MtrA1 (His^{35}Lys) apoprotein isolated from *E. coli* cells grown on $(^{15}NH_4)_2SO_4$+$[^{14}N]$histidine-containing minimal medium. The instrument settings were as follows: microwave frequency, 9430 MHz; microwave power, 12.7 mW; modulation frequency, 100 kHz; modulation amplitude, 0.8 mT; temperature, 77 K.

Acknowledgements: This work was supported by the Max-Planck-Gesellschaft, by the Deutsche Forschungsgemeinschaft and by the Fonds der Chemischen Industrie.

References

[1] Deppenmeier, U., Müller, V. and Gottschalk, G. (1996) Arch. Microbiol. 165, 149–163.
[2] Harms, U., Weiss, D.S., Gärtner, P., Linder, D. and Thauer, R.K. (1995) Eur. J. Biochem. 228, 640–648.
[3] Harms, U. and Thauer, R.K. (1996) Eur. J. Biochem. 241, 149–154.
[4] Marsh, E.N.G. and Holloway, D.E. (1992) FEBS Lett. 310, 167–170.
[5] Drennan, C.L., Huang, S., Drummond, J.T., Matthews, R.G. and Ludwig, M.L. (1994) Science 266, 1669–1674.
[6] Ludwig, M.L. and Matthews, R.G. (1997) Annu. Rev. Biochem. 66, 269–313.
[7] Lienard, T. and Gottschalk, G. (1998) FEBS Lett. 425, 204–208.
[8] Sauer, K., Harms, U. and Thauer, R.K. (1997) Eur. J. Biochem. 243, 670–677.
[9] Burke, S.A., Sam, L.L. and Krzycki, J.A. (1998) J. Bacteriol. 180, 3432–3440.
[10] Paul, L. and Krzycki, J.A. (1996) J. Bacteriol. 178, 6599–6607.
[11] Harms, U. and Thauer, R.K. (1997) Eur. J. Biochem. 250, 783–788.
[12] Stupperich, E., Eisinger, H.J. and Albracht, S.P.J. (1990) Eur. J. Biochem. 193, 105–109.
[13] Zelder, O., Beatrix, B., Kroll, F. and Buckel, W. (1995) FEBS Lett. 369, 252–254.
[14] Padmakumar, R., Taoka, S., Padmakumar, R. and Banerjee, R. (1995) J. Am. Chem. Soc. 117, 7033–7034.
[15] Sauer, K. and Thauer, R.K. (1998) Eur. J. Biochem. 253, 698–705.
[16] Harms, U. and Thauer, R.K. (1998) in: Vitamin B_{12} and B_{12}-Proteins (Kräutler, B., Arigoni, D. and Golding, B.T., Eds.), pp. 157–165, Wiley-VCH, Weinheim.