HAL
open science

Chaperones in maturation of molybdoenzymes: Why specific is better than general?

Olivier N Lemaire, Sophie Bouillet, Vincent Méjean, Chantal Iobbi-Nivol, Olivier Genest

To cite this version:

Olivier N Lemaire, Sophie Bouillet, Vincent Méjean, Chantal Iobbi-Nivol, Olivier Genest. Chaperones in maturation of molybdoenzymes: Why specific is better than general?. Bioengineered, 2016, 8 (2), pp.133-136. 10.1080/21655979.2016.1218579 . hal-01413222

HAL Id: hal-01413222

https://hal.science/hal-01413222v1

Submitted on 25 Apr 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Chaperones in maturation of molybdoenzymes: why specific is better than general?

Olivier N. Lemaire, Sophie Bouillet, Vincent Méjean, Chantal Iobbi-Nivol*, Olivier Genest*

*Co-corresponding

Aix Marseille Univ, CNRS, BIP, Marseille, France

*ogenest@imm.cnrs.fr; *iobbi@imm.cnrs.fr

KEYWORDS: Molybdoenzymes, specific chaperone, general chaperone, maturation, molybdenum cofactor, pyranopterin monophosphate

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

Our research is supported by the Centre National de la Recherche Scientifique (CNRS) and Aix-Marseille Université (AMU). O.N. L. and S. B. are supported by a MESR fellowship and AMU.

ABSTRACT

Molybdoenzymes play essential functions in living organisms and as a result in various geochemical cycles. It is thus crucial to understand how these complex proteins become highly efficient enzymes able to perform a wide range of catalytic activities. It has been established that specific chaperones are involved during their maturation process. Here, we raise the question of the involvement of general chaperones acting in concert with dedicated chaperones or not.

Molybdoenzymes have been studied for decades, and to our knowledge the first publication that proposed a common cofactor for nitrate reductase and xanthine dehydrogenase in *Aspergillus nidulans* was published in Nature in 1964.[1] In this article,

Pateman and coworkers proposed that the unknown common cofactor they named CNX was associated to molybdenum and was responsible for the catalytic activity of both enzymes. Since then, numerous items of information have been accumulated concerning molybdoenzymes. Indeed we can distinguish those belonging to LUCA from those more recently evolved, we can classify them into three families according to the modification of the basic form of the molybdenum cofactor and we now know the major steps of their biosynthesis.[2-5] In the three domains of life, molybdoenzymes are essential. Indeed, molybdoenzymes are involved in detoxification processes and in bacterial anaerobic respiration. In humans, molybdenum cofactor deficiency (MoCD) is a severe neonatal onset metabolic disorder with death in early infancy first described about forty years ago. Thirty years later, a new therapy strategy based on the infusion of the precursor form of the molybdenum cofactor, the cyclic pyranopterin monophosphate (cPMP) has been developed and it was shown that it can improve outcomes of one form of MoCD.[6,7] Among all living organisms, bacteria possess the highest number and diversity of molybdoenzymes.[4] Indeed, even if it is not exhaustive yet, the large panel of enzymatic activity they catalyze make them key players in fundamental geochemical cycles and efficient tools for bioremediation and depollution.[8-11]

In bacteria, among the three families of molybdoenzymes (the xanthine oxidase (XO) family, the sulfite oxidase (SO) family and the dimethyl sulfoxide (DMSO) reductase family) only members of the SO family can be matured without the support of auxiliary proteins while molybdoenzymes of the two others have been described to require specific accessory proteins dedicated to their biogenesis.[12,13] To study the maturation of molybdoenzymes of the DMSO reductase family, we have chosen to dissect the maturation process of TorA.[5] TorA is a trimethylamine oxide (TMAO) reductase and the terminal electron acceptor of the Tor respiratory system present in bacteria, especially in aquatic Proteobacteria. TorA turned out to be an exquisite model because it is a simple enzyme with respect of its composition, a soluble monomer containing the molybdenum cofactor (bis-molybdopterin guanine dinucleotide cofactor (bisMGD) form) as sole prosthetic group. The study of this basic model led to establish the various steps of molybdoenzyme maturation that were then also studied in other molybdoenzymes and that are extensively described in various recent reviews.[5, 12] TorA is a protein of about 90 kDa and its fold is organized into four domains with only the fourth C-terminal domain being made with a continuous amino acid stretch and two domains mainly involved in the binding of the cofactor.[14] Moreover, the cofactor is deeply buried at the extremity of a funnel-like deep depression in the core of the enzyme. The latter

point shared by the numerous enzymes of the DMSO reductase family crystallized so far was also observed in solved crystal structures of enzymes of the XO family members.[4, 5] The maturation process of enzymes of the XO family has also been well studied, with the xanthine dehydrogenase of *Rhodobacter capsulatus* as a model. Members of these family are multimeric complexes containing either the basic Mo-MPT or the MCD (molybdopterin cytosine dinucleotide) form of the molybdenum cofactor and also iron sulfur clusters and FAD.[13]

Altogether, these considerations imply the support of a chaperone activity, and families of dedicated chaperones have been discovered, including: TorD, XdhC, NapD and FdhD/FdsC families.[15] An interesting feature is that molybdoenzymes and their cognate chaperones, which are usually genetically related, seem to have coevolved. This latter point could be an explanation concerning the high level of specificity between the molybdoenzyme and the associated chaperone and it can also be the reason why, although the fold of the chaperones is conserved in a given family, their overall sequence homology is low. During the maturation process, the challenge is to insert the molybdenum cofactor into the core of the folded apoprotein. In general, dedicated chaperones are multitask proteins. They interact with several partners including their cognate molybdoenzymes and proteins involved in the maturation process of the molybdenum cofactor, they protect the apomolybdoenzyme from degradation by proteases, they act on the conformation of the molybdoenzyme to induce a competent fold for the insertion of the molybdenum cofactor and also probably to facilitate the folding of the enzyme after the insertion of the cofactor. In addition, each chaperone possesses specific tasks linked to the characteristic of each enzyme as, for example, the acquisition of other prosthetic groups, the subunit assembly, the final maturation or sulfuration of the cofactor and the targeting of the mature form of the enzyme to the TAT export machinery (all these findings have been largely described in several reviews, see refs 5, 12, 13, 15).

Most of the time, there is a synteny between genes encoding the molybdoenzyme and the chaperone, but sometimes the genes are located in different loci of the chromosome, therefore making the identification of the gene coding for the specific chaperone more difficult. Deletion of the gene encoding a potential specific chaperone is a convenient approach to decipher whether the stability/maturation of the molybdoenzyme is affected in such a context. However, in some cases no synteny and no obvious potential mate chaperone have been found. Moreover, by listing the operons encoding orthologous molybdoenzymes, it appears that depending on the bacteria, the gene encoding the

chaperone could be missing as exemplified by the absence of *arrD* encoding a TorD-like chaperone in the genomic arsenic locus of *Shewanella* sp. ANA-3 compared to that of other bacteria.[5, 16] This observation raises the question of why for some enzymes a dedicated chaperone is required and why some others can insert their cofactor without the support of accessory proteins. The fact that a minimal amount of TorA can be matured in the absence of the chaperone TorD indicates that the maturation process can be done even without specific chaperone, although this appears not to be the rule.[12, 17] One can thus wonder whether general molecular chaperones play a role in the maturation process of molybdoenzymes (figure 1). Furthermore, the absence of specific chaperones dedicated to the maturation and/or the stability of molybdoenzymes of the SO family could be counterbalanced by the involvement of general chaperones. So far, to our knowledge, this point has not been elucidated.

General chaperones are present in every organism and assist most of the proteins in the cell by preventing their aggregation and allowing their correct folding or activation.[18] Therefore, it is most likely that molybdoenzymes, like the other proteins, are at some point clients of general chaperones. Since the insertion of the molybdenum cofactor is performed in a folded apoenzyme, one can imagine that general chaperones may support the folding of these complex apoenzymes by acting in the early steps. In addition, general chaperones could help for the maturation of the molybdoenzymes in non-stress conditions. However, when a stress arises, general chaperones become probably less available for the maturation of molybdoenzymes since chaperones are titrated by the bulk of the other unfolded proteins in the cell. Since molybdoenzymes are often involved in essential processes like respiration [19], the cell cannot allow to lose these enzymes, and consequently it has evolved specific chaperones that are dedicated to their molybdoenzymes and are thus always available for their maturation.[17]

In addition, as explained above, efficient maturation of molybdoenzymes is a very complex process that requires skills from specific chaperones that go well beyond the basic roles general molecular chaperones can have. For example, the specific chaperone TorD interacts with the molybdenum cofactor and with proteins involved in molybdenum cofactor maturation, protects the molybdoenzyme from degradation and allows its maturation (figure 1).[20] It is thus improbable that a general chaperone protein that has not evolved with its mate molybdoenzyme could efficiently be able to fulfill these actions. Some specific chaperones have evolved from general chaperones. An example of such an evolution is given by the specific chaperone of a toxin-antitoxin system from *Mycobacterium tuberculosis* that

has evolved from the general chaperone SecB to specifically protect the antitoxin protein.[21, 22] However, it does not seem that the specific chaperones involved in molybdoenzyme maturation have evolved from general chaperone proteins.

Few studies so far have been undertaken to decipher the involvement of general molecular chaperones during molybdoenzyme biogenesis. Moreover, when they were done, it was on well-known enzymes pairing with a specific chaperone. For example, the general chaperone trigger factor was proposed to interact with the signal peptide of TorA.[23] In addition, DnaK was shown to stabilize overproduced GFP chimera containing the signal sequence of TorA.[24] However, in this study the authors found that when physiologically expressed, the maturation of TorA was not affected by the absence of the DnaK chaperone system. On the other hand, other studies have proposed that the specific chaperone itself interacts with general chaperones, as it has been shown for DmsD, a TorD homolog, that could interact with general chaperones including DnaK and GroEL.[25-27] It is still not clear if the role of the general chaperone is to help in the folding or stabilization of the specific chaperone, or if this interaction could allow the hand off of the molybdoenzyme between the general and the specific chaperones.

The question can be raised in another way. Indeed, to assess the role of general chaperone, molybdoenzymes without specific chaperone could be a better model and could, at first, allow to avoid competitive effect between the two types of chaperones. With production of molybdoenzymes increasingly becoming an important challenge with respect to their capacity of bioremediation and depollution, it will be crucial to elucidate if a combination of specific and general chaperones could improve the production and maturation of the molybdoenzymes.

Acknowledgments

We thank Kevin Copp for reviewing the manuscript.

References

1. Pateman JA, Cove DJ, Rever BM, Roberts DB. A common co-factor for nitrate reductase and xanthine dehydrogenase which also regulates the synthesis of nitrate reductase. Nature 1964; 201:58–60.

2. Schoepp-Cothenet B, van Lis R, Philippot P, Magalon A, Russell MJ, Nitschke W. The ineluctable requirement for the trans-iron elements molybdenum and/or tungsten in the origin of life. Sci Rep 2012; 2:263.

3. Mendel RR, Leimkühler S. The biosynthesis of the molybdenum cofactors. J Biol Inorg Chem JBIC Publ Soc Biol Inorg Chem 2015; 20:337–47.

4. Hille R, Hall J, Basu P. The mononuclear molybdenum enzymes. Chem Rev 2014; 114:3963–4038.

5. Leimkühler S, Iobbi-Nivol C. Bacterial molybdoenzymes: old enzymes for new purposes. FEMS Microbiol Rev 2016; 40:1–18.

6. Atwal PS, Scaglia F. Molybdenum cofactor deficiency. Mol Genet Metab 2016; 117:1–4.

7. Schwahn BC, Van Spronsen FJ, Belaidi AA, Bowhay S, Christodoulou J, Derks TG, Hennermann JB, Jameson E, König K, McGregor TL, et al. Efficacy and safety of cyclic pyranopterin monophosphate substitution in severe molybdenum cofactor deficiency type A: a prospective cohort study. Lancet Lond Engl 2015; 386:1955–63.

8. Grein F, Ramos AR, Venceslau SS, Pereira IAC. Unifying concepts in anaerobic respiration: insights from dissimilatory sulfur metabolism. Biochim Biophys Acta 2013; 1827:145–60.

9. Kraft B, Strous M, Tegetmeyer HE. Microbial nitrate respiration--genes, enzymes and environmental distribution. J Biotechnol 2011; 155:104–17.

10. Madsen EL. Microorganisms and their roles in fundamental biogeochemical cycles. Curr Opin Biotechnol 2011; 22:456–64.

11. Zhang Y, Rump S, Gladyshev VN. Comparative Genomics and Evolution of Molybdenum Utilization. Coord Chem Rev 2011; 255:1206–17.

12. Genest O, Méjean V, Iobbi-Nivol C. Multiple roles of TorD-like chaperones in the biogenesis of molybdoenzymes. FEMS Microbiol Lett 2009; 297:1–9.

13. Neumann M, Leimkühler S. The role of system-specific molecular chaperones in the maturation of molybdoenzymes in bacteria. Biochem Res Int 2011; 2011:850924.

14. Iobbi-Nivol C, Hazer R, Méjean V, Czjzek M. Trimethylamine N-oxide reductase. In: Handbook of Metalloproteins 2001. John Wiley and Sons Limited, Chichester, UK; 2001.

15. Iobbi-Nivol C, Leimkühler S. Molybdenum enzymes, their maturation and molybdenum cofactor biosynthesis in Escherichia coli. Biochim Biophys Acta 2013; 1827:1086–101.

16. Yamamura S, Amachi S. Microbiology of inorganic arsenic: From metabolism to bioremediation. J Biosci Bioeng 2014; 118:1–9.

17. Ilbert M, Méjean V, Iobbi-Nivol C. Functional and structural analysis of members of the TorD family, a large chaperone family dedicated to molybdoproteins. Microbiol Read Engl 2004; 150:935–43.

18. Kim YE, Hipp MS, Bracher A, Hayer-Hartl M, Hartl FU. Molecular chaperone functions in protein folding and proteostasis. Annu Rev Biochem 2013; 82:323–55.

19. Grimaldi S, Schoepp-Cothenet B, Ceccaldi P, Guigliarelli B, Magalon A. The prokaryotic Mo/W-bisPGD enzymes family: a catalytic workhorse in bioenergetic. Biochim Biophys Acta 2013; 1827:1048–85.

20. Redelberger D, Genest O, Arabet D, Méjean V, Ilbert M, Iobbi-Nivol C. Quality control of

a molybdoenzyme by the Lon protease. FEBS Lett 2013; 587:3935–42.

21. Bordes P, Cirinesi A-M, Ummels R, Sala A, Sakr S, Bitter W, Genevaux P. SecB-like chaperone controls a toxin-antitoxin stress-responsive system in Mycobacterium tuberculosis. Proc Natl Acad Sci U S A 2011; 108:8438–43.

22. Sala A, Calderon V, Bordes P, Genevaux P. TAC from Mycobacterium tuberculosis: a paradigm for stress-responsive toxin-antitoxin systems controlled by SecB-like chaperones. Cell Stress Chaperones 2013; 18:129–35.

23. Jong WSP, ten Hagen-Jongman CM, Genevaux P, Brunner J, Oudega B, Luirink J. Trigger factor interacts with the signal peptide of nascent Tat substrates but does not play a critical role in Tat-mediated export. Eur J Biochem FEBS 2004; 271:4779–87.

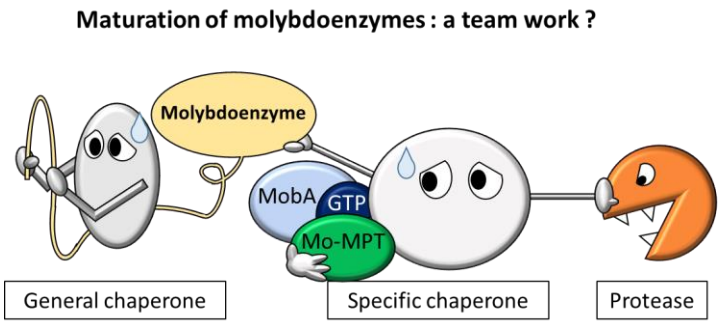
24. Pérez-Rodríguez R, Fisher AC, Perlmutter JD, Hicks MG, Chanal A, Santini C-L, Wu L-F, Palmer T, DeLisa MP. An essential role for the DnaK molecular chaperone in stabilizing over-expressed substrate proteins of the bacterial twin-arginine translocation pathway. J Mol Biol 2007; 367:715–30.

25. Li H, Chang L, Howell JM, Turner RJ. DmsD, a Tat system specific chaperone, interacts with other general chaperones and proteins involved in the molybdenum cofactor biosynthesis. Biochim Biophys Acta 2010; 1804:1301–9.

26. Chan CS, Song X, Qazi SJS, Setiaputra D, Yip CK, Chao T-C, Turner RJ. Unusual pairing between assistants: interaction of the twin-arginine system-specific chaperone DmsD with the chaperonin GroEL. Biochem Biophys Res Commun 2015; 456:841–6.

27. Chan CS, Bay DC, Leach TGH, Winstone TML, Kuzniatsova L, Tran VA, Turner RJ. “Come into the fold”: A comparative analysis of bacterial redox enzyme maturation protein members of the NarJ subfamily. Biochim Biophys Acta 2014; 1838:2971–84.

Figure 1

Maturation of molybdoenzymes: A product of team work?

MoMPT: molybdenum cofactor; MobA: bacterial enzyme involved in the final step of molybdenum cofactor biogenesis.