論文 / 著書情報
Article / Book Information

題目(和文)	
Title(English)	Studies on the disturbed metabolism caused by genetic ablation of quinonoid dihydropteridine reductase
著者(和文)	徐峰
Author(English)	Feng Xu
出典(和文)	学位:博士（理学）, 学位授与機関:東京工業大学, 報告番号:甲第9724号, 授与年月日:2015年3月26日, 学位の種別:課程博士, 審査員:一瀬　宏,村上　聡,久堀　徹,梶原　将,林　宣宏
Citation(English)	Degree:, Conferring organization: Tokyo Institute of Technology, Report number:甲第9724号, Conferred date:2015/3/26, Degree Type:Course doctor, Examiner:,,,,
学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

専攻： Department of	分子生命科学　専攻	申請学位（専攻分野）： Academic Degree Requested	博士　（　理学　） Doctor of
学生氏名： Student's Name	Xu Feng	指導教員（主）： Academic Advisor(main)	一瀬　宏　教授
		指導教員（副）： Academic Advisor(sub)	

要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Tetrahydrobiopterin (BH4) is an essential cofactor for the hydroxylation of tyrosine and tryptophan, which is the rate-limiting step in dopamine and serotonin synthesis, respectively. Dopamine and serotonin are well known to be involved in brain function as neurotransmitters, and consequently, monoamine deficiency due to BH4 depletion causes a variety of neuropsychiatric disorders. Besides, BH4 is also required for phenylalanine hydroxylase (PAH) and all three isoforms of nitric oxide synthases. Therefore, it is of great significance to know how the level of biopterin is maintained and regulated in the cells.

BH4 is *de novo* synthesized from GTP via three enzymatic reactions, which are catalyzed by GTP cyclohydrolase I (GCH), 6-pyruvoyl-tetrahydropterin synthase and sepiapterin reductase. During the hydroxylation reactions, BH4 is oxidized and then dehydrated to *quinonoid* dihydrobiopterin (*q*BH2), which is reduced to BH4 by *quinonoid* dihydropteridine reductase (QDPR). *q*BH2 is unstable and easily converted to 7, 8-dihydrobiopterin (BH2), which can be a substrate for dihydrofolate reductase (DHFR).

The intracellular BH4 contents are regulated at many levels and much investigation has been focused on the regulation of *de novo* synthesis and the rate-limiting enzyme GCH. However, little is known for the roles of recycling pathway. Therefore, in this work, I studied a recently established *Qdpr*-deficient mouse model, which has a total ablation in the *Qdpr* gene, and explored the roles of QDPR and DHFR in the BH4 metabolism.

To elucidate whether the absence of QDPR enzyme alters the levels of BH4, I quantified the contents of BH4 and its oxidized forms BH2 and biopterin (BP) in the $Qdpr^{-/-}$ mice. Unexpectedly, in spite of the absence of recycling pathway, BH4 contents were not decreased in the brain or liver, and even increased in the kidney, lung and heart, whereas those of BH2 and BP were significantly increased in all the tissues examined. Additionally, the contents of neopterin, an intermediate metabolite of BH4 *de novo* synthesis were increased as well. However, expression level or *in vitro* activity of GCH in the transgenic mice was not elevated. These data suggest that BH4 is maintained via an alternative pathway other than QDPR-mediated recycling pathway in the $Qdpr^{-/-}$ mice.

In vitro studies have demonstrated that DHFR can catalyze the reduction of BH2 to produce BH4, but the *in vivo* role of DHFR is not fully understood. I therefore inhibited its activity with methotrexate (MTX), a specific inhibitor of DHFR. The results showed that MTX greatly reduced the contents of BH4 and increased those of BH2 and BP, which indicates that DHFR is deeply involved in

the BH4 regeneration in the *Qdpr*$^{-/-}$ mice than that in the wild-type mice.

Metabolism of biopterin and folate seems to be independent, but clinical cases and *in vivo* involvement of DHFR in the BH4 regeneration suggest a close connection between them. To investigate how folates are associated with biopterins, I quantified the folate profiles and revealed significant deficiency in the levels of folate derivatives. I performed metabolome analysis as well, and found wide alterations in the levels of metabolites which were associated with folate metabolism. These data corroborate universal disturbances in the folate-associated metabolism. Of note, increase in the oxidative stress biomarkers was observed in the *Qdpr*$^{-/-}$ mice, such as glutathione and homocysteine, most of which were also related to folate metabolism.

In order to explore why folate metabolism was disturbed in the *Qdpr*$^{-/-}$ mice, I proposed several hypotheses and examined them using *in vitro* kinetic assays and *in silico* molecular dynamics (MD) simulations. The involvement of DHFR in the BH4 regeneration may interfere with the reduction of dihydrofolate (DHF) in the *Qdpr*$^{-/-}$ mice, which is originally catalyzed by DHFR. In other words, BH2 becomes a potential inhibitor for the reduction of DHF. In order to examine the hypothesis, I first compared the binding affinity of BH2 and DHF for DHFR through MD simulations and the result showed that BH2 bound to DHFR in much weaker affinity compared with DHF. I further corroborated this with kinetic assays and inhibition studies yielded a K_i value of approx. 88 μM for DHFR, which was far higher than the estimated intracellular BH2 level (approx. 1 μM). These data suggest that increase in the BH2 contents may not be a plausible reason for the folate deficiency in the *Qdpr*$^{-/-}$ mice. Further studies on the *in vivo* catalytic activity of DHFR would be required to solve the question.

Additionally, I explored the reason for hyperphenylalaninemia in the *Qdpr*$^{-/-}$ mice, which have adequate amount of PAH and sufficient hepatic BH4 supply. Quantification of pteridines in each subcellular fraction suggested decreased BH4 levels in the cytosol of the *Qdpr*$^{-/-}$ mouse hepatocytes, where the hydroxylation of Phe principally occurs.

Collectively, I characterized a mouse model with a genetic defect in the *Qdpr* gene and revealed that genetic ablation of *Qdpr* caused wide alterations in the biopterin, folate and phenylalanine metabolism. It is the first report revealing the *in vivo* involvement of DHFR in the BH4 metabolism, and proving the genetic defect in the BH4 metabolic process as a possible cause of folate deficiency.