論文 / 著書情報
Article / Book Information

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（博士課程）
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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 Doctor of　（　理学　）
学生氏名： Student's Name	梁　宇	指導教員（主）： Academic Advisor(main)	一瀬　宏
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要旨（英文 800 語程度）
Thesis Summary (approx.800 English Words)

Tetrahydrobiopterin (BH4) is an essential co-factor for aromatic amino acid hydroxylases and nitric oxide synthase. The de novo synthesis of BH4 is accomplished by a three-step reaction catalyzed by GTP cyclohydrolase I (GCH), 6-pyruvoyl-tetrahydropterin synthase, and sepiapterin reductase (SPR). GCH catalyzes the first as well as the rate limiting step in BH4 *de novo* synthesis. The expression of GCH can be stimulated by various stimuli, including nerve growth factor (NGF), cAMP, cytokines, and endotoxins, so that the synthesis of BH4 could be regulated for its appropriate physiological function. SPR is essential for both the *de novo* as well as the salvage pathway in BH4 synthesis. It belongs to the short-chain dehydrogenases/reductases (SDR) superfamily, but uniquely featured with its ability to reduce both carbonyl group on the side chain of 6-pyruvoyl-tetrahydropterin (PPH4). Although there are several studies describing the crystal structure of SPR with sepiapterin, the catalytic mechanism in BH4 *de novo* synthesis remains not fully understood.

In the first part of thesis, transcriptional regulation of GCH by lipopolysaccharides (LPS) is described. The transcriptional regulation of GCH by LPS was investigated with luciferase assay by transfecting a reporter vector inserted with human GCH promoter region into mouse macrophage RAW264.7 cells. Unlike that induced by nerve growth factor or cAMP, the GCH promoter could not respond to LPS stimulation. Later experiments showed that an enhancer region located in intron 1 acted as the response element. By using molecular biological strategies like electrophoretic mobility shift assay, chromatin immunoprecipitation and RNA interference assay, the trans-activators bound to this enhancer region were studied. It showed that C/EBP-β and Ets factors were bound to the enhancer region, which was responsible for LPS induced GCH transcription. This study showed for the first time the molecular mechanism of LPS induced GCH transcription, by specifying the cis-element as well as the trans-activators.

In the second part, the catalytic mechanism of SPR is discussed. First, an electrochemical HPLC detection system using an ion-exchange column for tetrahydro-form biopterins were described. The SPR catalyzed reaction was monitored using this system. Several mutant SPR proteins with Y170V, S157D single mutation in the catalytic pocket were expressed and the kinetics as well as biochemical features are examined. The SPR-Y170V, but not the SPR-S157D mutant could reduce the carbonyl group on 1' position of the side chain of PPH4, to produce 6-(1'-hydroxy-2'-oxopropyl)-PH4, indicating that the reduction of 1' carbonyl group can be accomplished by Ser157. On the other hand, when 6-(1'-oxo-2'-hydroxypropyl)-PH4, a tetrahydro-form of sepiapterin, was used as the substrate, neither mutant showed reductase activity. These data indicated that the binding property of PPH4 and 6-(1'-oxo-2'-hydroxypropyl)-PH4 to SPR differ slightly, so that the Ser157 residue could only catalyze the reduction of 1'-carbonyl group of PPH4. The wild type SPR also possesses isomerase activity, converting 6-(1'-oxo-2'-hydroxypropyl)-PH4 to 6-(1'-hydroxy-2'-oxopropyl)-PH4, but SPR-Y170V and SPR-S157D both failed in the isomerization reaction, suggesting that the isomerase activity, as well as the Y170 residue, is critical for the complete reduction of PPH4 to BH4. However, the role of Y170 residue still requires further examination, since the mutation of Ser157 to Asp might destroyed the binding between substrate and enzyme due to the negative charge of Asp. This part of study provided biochemical evidence of the SPR reaction for the first time, to show that the binding features of PPH4 to SPR differs from that of sepiapterin, which could not be uncovered by analyzing the crystal structure. In addition, the involvement of Try170 in the isomerase activity as well as the complete reduction of PPH4 to BH4 was confirmed.

備考 ： 論文要旨は、和文 2000 字と英文 300 語を 1 部ずつ提出するか、もしくは英文 800 語を 1 部提出してください。
Note : Thesis Summary should be submitted in either a copy of 2000 Japanese Characters and 300 Words (English) or 1copy of 800 Words (English).