S-17-5 6R-Tetrahydrobiopterin Biosynthesis: Structures of Rat and Human Sepiapterin Reductase

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I. INTRODUCTION

(6R)-2-Amino-6-(L-erythro-1,2-dihydroxypropyl)-5,6,7,8-tetrahydro-4(3H)-pteridinone (tetrahydrobiopterin, BPH4) is an essential cofactor for aromatic amino acid monooxygenases, phenylalanine, tyrosine, and tryptophan hydroxylases [1,2]. Tyrosine and tryptophan hydroxylases are the rate-limiting enzymes in the biosynthesis of monoamine neurotransmitters, i.e., catecholamines (dopamine, noradrenaline, and adrenaline) and serotonin, and their activities are regulated by several factors. Animals synthesize BPH4 in vivo from GPT through several enzymatic reactions. The concentration of BPH4 within the monoaminergic neurons and the adrenal medullary cells is thought to be one of the regulatory factors.

II. BPH4 BIOSYNTHESIS AND SEPIAPTERIN REDUCTASE (SPR)

Sepiapterin reductase (7,8-dihydrobiopterin: NADP⁺ oxidoreductase, EC 1.1.1.153, SPR) [3] catalyzes the final step of BPH4 biosynthesis, and is thought to play an important role for the biosynthesis [4].

SPR shows a high specificity against a few carbonyl derivatives of pteridines. However, the enzyme can catalyze the NADPH-dependent reduction of various carbonyl substances other than pteridines, and is considered to be one of the 'aldo-keto reductases', which have a broad specificity for endogenous and exogenous carbonyl compounds. It is important to clarify the complete structure of the enzyme molecule to analyze the complicated reaction mechanism of the enzyme. The enzyme purified from rat erythrocytes consists of two identical subunits each with an Mr of about 28,000. We have determined primary structures of rat SPR [5] and human SPR [6] by protein analysis and by cDNA cloning. We have found homology among human SPR, human carbonyl reductase, and human dehydrogenases.

III. STRUCTURE OF RAT SPR

Citron et al. [7] recently reported a partial nucleotide sequence of rat SPR. We determined 96% of the amino acid sequence of rat SPR including the N-terminal structure by protein analysis [5]. The complete amino acid sequence of the mature form of rat SPR was provided by these two reports [5,7].

We purified rat SPR from erythrocytes by the method of Sueoka and Kato [3], i.e., ammonium sulfate, hydroxyapatite, Matrix Red A, and isoelectric focusing. The purified SPR was reduced with dithiothreitol and carboxymethylated with monoiodoacetamide. The

S-carboxyamidomethylated-protein was enzymatically and chemically cleaved. For enzymatic cleavage, *Achromobacter* protease I, TPCK-trypsin and α -chymotrypsin were used. Chemical cleavage was carried out by cyanogen bromide and BNPS-skatole.

Peptides were separated by reversed-phase HPLC. Sequence analysis was performed with an Applied Biosystems Model 470A Protein Sequencer on line connected to a 120A PTH analyzer.

The sequencer analysis could not identify the N-terminal sequence of the enzyme. Digestion of a N-blocked peptide was carried out with acyl amino acid releasing enzyme, and the analysis of the peptide with a sequencer yielded the sequence of N-acetyl-Met-Glu-Gly-Gly-Arg-.

We have sequenced 96.2% of the entire sequence of the mature form of the enzyme. Three important informations emerged from the present results. (1) Our results clearly demonstrated that the N-terminus of the mature form of the enzyme is the methionine-1 and processed by N-acetylation. (2) Citron et al. [7] recently reported a partial nucleotide sequence of a cDNA encoding rat SPR, which lacks the 5' sequence corresponding to the first 3-amino acids in the N-terminus of the protein. The amino acid sequence of rat SPR presented by us [7] completely agrees with the sequence derived from the rat cDNA by Citron et al. [5]. Comparison of the results with the predicted amino acid sequence provided the complete amino acid sequence of the mature form of the enzyme. (3) This also indicates that no proteolytic processing event occurs at the C-terminus of the enzyme, and also no covalent processing event other than N-acetylation, such as glycosylation or methylation, appears to occur in the entire molecule. The Mr of the subunit was calculated to be 28169.06 from this complete amino acid sequence of 262 residues including N-terminal acetyl group. Therefore, the Mr of the native enzyme should be 56338.12

IV. STRUCTURE OF HUMAN SPR

We [6] have cloned a full-length cDNA for human SPR from liver, and have determined the complete amino acid sequence of human SPR deduced from the nucleotide sequence of the cDNA. A clone (hSPR8-25) contained an entire coding region of human SPR. The nucleotide sequence of cDNA encoding human SPR and the deduced amino acid sequence are shown in Fig.1. The clone encoded a protein of 261 amino acids with a calculated Mr of 28047.

The predicted amino acid sequence of human SPR was compared to that of the rat enzyme [5,7]. The putative pterin binding site (Ala-Gly-Leu-Leu-Ser) suggested by Citron et al. [7] in rat SPR is shown on a solid line. As the amino acid sequence in human SPR corresponding to this sequence was Ala28-Ser-Leu-Leu-Ser32, the consensus sequence for a pterin-binding peptide should be Ala-Xxx-Leu-Leu-Ser. A NADPH binding consensus sequence, TL-L-G-G---G---L---L---L-L-D, spanning 28 amino acids from residues 13-40 was well conserved between human and rat SPR.

SPR has a dehydrogenase activity, a reverse reaction against reductase, and also carbonyl reductase activity. Therefore, we compared the amino acid sequence of human SPR with those of human estradiol 17 β -dehydrogenase (EDH) [8], 3 β -hydroxy-5-ene steroid dehydrogenase (HSD) [9] and carbonyl reductase (CBR) [10]. They exhibit a striking homology with SPR, especially in their N-terminal region. The pattern of homology suggests that these molecules contain a similar core structure in the N-terminal portion containing the determinants for the enzymatic activity.

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                                -20      -10      -1
                                GCCGCCGGCGGAGAACAGGAGC

1       10       20       30       40       50       60
ATGGAGGGCGGGCTGGGGCGTGCTGTGTGCTTGCTGACCGGGGCTCCCGCGGCTTCGGC
M E G G L G R A V C L L T G A S R G F G

70       80       90       100      110      120
CGGACGCTGGCCCCGCTCCTGGCCTCGCTGTGCTGCGCCGGCTCCGTGCTTGTCTTAGC
R T L A P L L A S L L S P G S V L V L S

130      140      150      160      170      180
GCCCCAACGACGAGGCACTGCGCCAGCTGGAGGCCGAGCTGGGCGCCGAGCGGTCTGGC
A R N D E A L R Q L E A E L G A E R S G

190      200      210      220      230      240
CTGCGCGTGGTGGGGTGGCCCGGACCTGGGCGCCGAGGCCGCTTGCAGCAGCTGTCTC
L R V V R V P A D L G A E A G L Q Q L L

250      260      270      280      290      300
GGCGCCCTGCGCGAGCTCCCCGGGCCAAGGGGCTGCAGCGACTGTGCTTATCAACAAC
G A L R E L P R P K G L Q R L L L I N N

310      320      330      340      350      360
GGGGGCTCTCTTGGGGATGTGTCCAAAGGCTTCGTGGACCTGAGTGACTCCACTCAAGTG
A G S L G D V S K G F V D L S D S T Q V

370      380      390      400      410      420
AACAACTACTGGGCACTGAACCTGACCTCCATGCTCTGCCTGACTTCAGCGTCTGAAG
N N Y W A L N L T S M L C L T S S V L K

430      440      450      460      470      480
GCCTTCCCGGACAGTCTCTGGCCTCAACAGAACCGTGGTTAACATCTCGTCCCTCTGTGCC
A F P D S P G L N R T V V N I S S L C A

490      500      510      520      530      540
CTGCAACCTTTCAAAGGCTGGGCGCTGTACTGTGCAGGAAAGGCTGCTCGTGATATGCTG
L Q P F K G W A L Y C A G K A A R D M L

550      560      570      580      590      600
TTCCAGGTCTTGGCGCTGGAGGAACCTAATGTGAGGGTGTGTAAGTATGCCCCAGGTCTCT
F Q V L A L E E P N V R V L N Y A P G P

610      620      630      640      650      660
CTGGACACAGACATGCAGCAGTTGGCCCGGAGACCTCCGTGGACCCAGACATGCGAAAA
L D T D M Q Q L A R E T S V D P D M R K

670      680      690      700      710      720
GGGCTGCAGGAGCTGAAGGCAAAGGGGAAGCTGGTGGATTGCAAGGTGTGAGCCAGAAA
G L Q E L K A K G K L V D C K V S A Q K

730      740      750      760      770      780
CTGCTGAGCTTACTGGAAAAGGACGAGTTCAAGTCTGGAGCCACGTGGACTTCTATGAC
L L S L L E K D E F K S G A H V D F Y D

790      800      810
AAATAAGCCCATGTTTTTGGCTTCCTGAACC
K *

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Fig.1. The nucleotide sequence encoding human sepiapterin reductase (SPR) and the deduced amino acid sequence. The consensus sequence for a pterin-binding site is shown on a solid line, and that of a NADPH-binding site is shown on a dotted line.

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