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Short Communication

Cloning and Sequencing of cDNA Encoding Human Sepiapterin Reductase¹⁾

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Sepiapterin reductase (7,8-dihydrobiopterin: NADP⁺ oxidoreductase, EC 1.1.1.153; SPR) catalyzes the NADPH-dependent reduction of various carbonyl substances including derivatives of pteridines, and belongs to a group of enzymes called aldo-keto reductases. Many reports supports that SPR plays an important role for the biosynthesis of BH₄. It is uncertain, however, whether two carbonyl groups on the side chain of 6-pyruvoyl tetrahydropterin, an intermediate involved in BH₄ formation, are reduced, *in vivo*, by the sequential action of SPR alone or by the two enzymatic reactions with SPR and aldose reductase (EC 1.1.1.21). Molecular probes are required to study the metabolism of BH₄ in a new aspect.

Citron *et al.* reported a partial nucleotide sequence of rat SPR, which lacks the 5' sequence corresponding to the first 3 amino acids in the N-terminus of the protein (1). We determined 96% of the amino acid sequence of rat SPR including the N-terminal structure by protein analysis (2). The complete amino acid sequence of the mature form of rat SPR was provided by these two reports. In this paper (3), we report the cloning of a cDNA for SPR from human liver and the complete amino acid sequence of human SPR deduced from the nucleotide sequence of the cDNA.

We also discuss the amino acid sequence homology among human SPR, human carbonyl reductase, and some dehydrogenases.

First we amplified the cDNA for rat SPR by the polymerase chain reaction. The sequence of synthetic primer A was 5'-GGGAATTCCATGGAGGG (ATGC)GGCAGGCTAGG-3' which was designed based on the N-terminal amino acid sequence of rat SPR previously described (2) and the nucleotide sequence reported by Citron *et al.* (1) with an additional *EcoRI* site. The sequence of synthetic primer B is 5'-GAGGATCCTTAAATGTCATAGAAGTCCACG TG-3' which is corresponding to the nucleotide sequence of 757-781 (1) including an additional *BamHI* site. Total RNA (10 µg) from rat brain was reverse transcribed using primer B. The single stranded cDNA generated was amplified by the PCR with primers A and B, and cloned into a Bluescript KS (Stratagene) vector.

The rat cDNA was labeled and then used for screening through plaque hybridization of 3×10^5 recombinants from a human liver cDNA library. Seven strongly hybridized clones were isolated, subcloned into the *EcoRI* site of a Bluescript vector and sequenced.

By analogy with the rat sequence, it was suggested that clone hSPR 8–25 contained an entire coding region of human SPR. Its sequence in both orientations was determined using synthetic primers (Fig. 1). It contained an open reading frame encoding 261 amino acid residues following the first ATG, 22 bp of 5' untranslated and 25 bp of 3' untranslated regions.

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Enzymes: aldose reductase (EC 1.1.1.21), carbonyl reductase (EC 1.1.1.184), estradiol 17 β -dehydrogenase (EC 1.1.1.62), 3 β -hydroxy-5-ene steroid dehydrogenase (EC 1.1.1.145), sepiapterin reductase (EC 1.1.1.153).

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

      70      80      90      100     110     120
TGGAGCTGGCCCGCTCCTGGCTCGCTGCTGCGCCGGCTCCGTGCTTGTCTTAGC
P 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
TTTTCACAGGAGGACGCGCCAGCTGGAGGCCGAGCTGGGCGCCGAGCGGTCTGGC
I F N D E A L R Q L E A E L G A E R S G

      190     200     210     220     230     240
TTTTCGCTGGTGGGGTGCCCGCGACCTGGGCGCCGAGGCGGCTTGCAGCAGCTGCTC
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
TGGCGCTGGCGAGCTCCCGCGCCCAAGGGGTGCGAGCGACTGCTGCTATCAACAA
P 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
TGGGGCTCTCTTGGGGATGTGTCCAAAGGCTTCGTGGACCTGAGTGACTCACTCAAGTG
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
AACAACTACTGGGCACTGAACCTTGAACCTCCATGCTCTGCTGACTTCCAGCGTCTGAAG
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
GCCTTCCCGACAGTCTCGCTCAACAGACCGTGGTTAATCATCTCGTCCCTCTGTGCC
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
CTGCAACCTTTCAAAGGCTGGGCGCTGTACTGTGAGGAAAGGCTCGCTGATATGCTG
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
TTCCAGTCTTGGCGCTGGAGGAACCTAATGTGAGGGTCTGAAGTATGCCCCAGGTCCT
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
CTGGACACAGACATGACGAGTGGCCCGGAGACCTCCGTGGACCCAGACATGCGAAA
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
GGGCTGACGAGCTGAAGGCAAGGGAGCTGGTGGATTGCAAGGTGTCAGCCAGAGAAA
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
CTGTGAGCTTACTGGAAAAGGACGAGTTCAAGTCTGGAGCCACGTTGGACTTCTATGAC
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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Figure 1. The nucleotide sequence of cDNA encoding human sepiapterin reductase and the deduced amino acid sequence. The predicted amino acid sequence is shown below the nucleotide sequence by one-letter code.

A poly(A)⁺ tail was not found in the clone, because the cDNA library used was made using random hexamers for priming cDNA synthesis, not but oligo(dT). In the previous paper, we determined the N-terminal sequence of the mature form of rat SPR, N-acetyl-Met-Glu-Gly-Gly- (2). The amino acid sequence deduced from the nucleotide sequence of hSPR 8–25 began with Met-Glu-Gly-Gly-, suggesting that the ATG codon acts as the translation initiation signal. Based on the predicted amino acid sequence, the molecular mass was calculated to be 28 047 daltons for the protein. The predicted amino acid sequence

of human SPR showed a 74% identity with the rat enzyme (1, 2).

It is known that SPR has a dehydrogenase activity, a reverse reaction against reductase (4), and shows the activity of carbonyl reductases (5). Thus we compared the amino acid sequence of human SPR with human carbonyl reductase (EC 1.1.1.184), human estradiol 17 β -dehydrogenase (EC 1.1.1.62), and human 3 β -hydroxy-5-ene steroid dehydrogenase (EC 1.1.1.145). They exhibit a striking homology with SPR especially in their N-terminal regions. Gaps were introduced to obtain a maximal alignment of their sequences. With this alignment, the sequence of residues 1–57 in human SPR show 37% identity (19 out of 52 positions compared) with that of residues 1–45 in human estradiol 17 β -dehydrogenase, and 63% similarity when conservative substitutions of amino acids are also taken into account. Similarly 25% identity (13 out of 52 positions) and 62% similarity with that of residues 1–51 in human 3 β -hydroxy-5-ene steroid dehydrogenase, 33% identity (19 out of 58 positions) and 71% of similarity with that of residues 1–58 in human carbonyl reductase are observed.

A putative pterin binding site and a NADPH binding consensus sequence on SPR are also located on the 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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