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Structure, gene expression, and evolution of primate copper chaperone for superoxide dismutase

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ABSTRACT

Copper chaperone for superoxide dismutase (CCS) is essential for transporting copper ion to Cu,Zn-superoxide dismutase (Cu,Zn-SOD). We cloned cDNAs for six primate species' CCSs. The total number of amino acid residues of primate CCSs is 274. Similarities between primates were over 96%. Important residues for the CCS function were well conserved. A phylogenetic tree of CCSs and Cu,Zn-SODs from various organisms showed that these two proteins were derived from a common ancestor, diverging very early on during eukaryote evolution. The high frequency of nonsynonymous substitutions was found in the lineage to Old World monkeys and apes. Expression of the CCS gene in various tissues of Japanese monkey was found to be high in the liver and adrenal gland, followed by the kidney and small intestine. Such expressional pattern was similar with that of Cu,Zn-SOD gene (Fukuhara et al., 2002).

1. Introduction

Reactive oxygen species (ROS) are produced as a consequence of aerobic respiration and substrate oxidation. Since ROS are highly reactive, the occurrence of high levels of ROS may cause metabolic malfunctions and damage to biological macromolecules, resulting in the generation of various diseases (Matés et al., 1999), although low levels of ROS are indispensable in many biochemical processes such as the defense system against micro-organisms (Michell, 1984). Several enzymes are involved in generating and scavenging ROS and it is appropriate to clarify tissue-specific ROS-generating and scavenging systems, considering the different tissue distribution of these enzymes (Fukuhara and Kageyama, 2003; Fukuhara et al., 2001).

Copper/zinc superoxide dismutase (Cu,Zn-SOD) is a major ROS-scavenging enzyme and functions as a dimeric protein in which each identical subunit contains one copper and one zinc ion (Fridovich, 1995). Copper is an essential trace element for eukaryotes and most prokaryotes (Bertini et al., 2010) and is also essential for Cu,Zn-SOD activity (Rigo et al., 1977). In recent studies, copper trafficking proteins have been identified in plant, yeast, rodent, and human cells (Askwith and Kaplan, 1998; Harrison et al., 1999). Intracellular copper trafficking proteins are soluble factors that transport copper ion to specific proteins. The fast copper-transfer pathways require metal-mediated protein–protein interactions and protein–protein specific recognitions (Banci et al., 2006). The thermodynamic data showed that copper is drawn to the enzymes that require it by passing from one copper protein site to another, exploiting gradients of increasing copper-binding affinity (Banci et al., 2010). The copper chaperone for SOD (CCS), which delivers copper ion to Cu,Zn-SOD in the cytoplasm, has been isolated from yeast, fly, rodent, and human cells (Casareno et al., 1998; Hiromura et al., 2000; Schmidt et al., 1999).

The CCS consists of three domains, i.e., an N-terminal domain (domain I), a middle domain that resembles the target enzyme Cu, Zn-SOD (domain II), and a C-terminal domain (domain III) (Lamb et al., 1999; Schmidt et al., 1999). CCS transports copper ion to Cu, Zn-SOD by direct interaction to the enzyme at the domain II region of the protein (Lamb et al., 2000), and there is high similarity between Cu,Zn-SOD and CCS domain II (Lamb et al., 1999). Although molecular evolutional analyses have not been done to date, it is anticipated that there are phylogenetic relationships between Cu,Zn-SODs and CCSs.

Abbreviations: SOD, superoxide dismutase; CCS, Copper chaperone for superoxide dismutase; Cu,Zn-SOD, copper/zinc-superoxide dismutase; ROS, Reactive oxygen species; RT-PCR, reverse transcription-polymerase chain reaction; Hs, Homo sapiens; *Pt*, *Pan troglodytes*; *Pp*, *Pongo pygmaeus*; *Hl*, *Hylobates lar*; *Mff*, *Macaca fuscata*; *Ca*, *Cebus paella*; *Cj*, *Callithrix jacchus*; *Rn*, *Rattus norvegicus*; *Mm*, *Mus musculus*; *Gg*, *Gallus gallus*; *Xl*, *Xenopus laevis*; *Dr*, *Danio rerio*; *Dm*, *Drosophila melanogaster*; *Sc*, *Saccharomyces cerevisiae*; *Ec*, *Escherichia coli*; NJ, neighbor-joining; MP, maximum parsimony; ML, maximum likelihood; SDS, sodium dodecyl sulfate; His, histidine; Asp, Aspartic acid; Gly, glicine; Val, valine; Ile, isoleucine; Tyr, tyrosine; Arg, arginine; Cys, cystein; Ser, serine; Glu, glutamic acid; mRNA, messenger ribo nucleic acid; kb, kilobase; FD, prefrontal lobe; FA, motor lobe; PC, somatosensory lobe; PG, parietal lobe; OC, occipital lobe; TA, superior temporal lobe; TE, inferior temporal lobe; Hi, hippocampus; Cl, cerebellum; He, heart; Lu, lung; St, stomach; SI, small intestine; Li, liver; Pa, pancreas; Sp, spleen; Ad, adrenal gland; Ki, kidney; Mu, muscle; Te, testis.

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In this study, we first cloned primate CCS cDNAs and determined their nucleotide sequences. Molecular phylogenetic analyses of CCSs and Cu,Zn-SODs from various organisms including primates showed that these two proteins diverged from a common ancestor very early on during eukaryote evolution. Expressional patterns of the CCS gene in monkey tissues were found to be similar to those of the Cu,Zn-SOD gene.

2. Materials and methods

2.1. Chemicals

Titan One Tube RT-PCR System and Expand High Fidelity^{PLUS} PCR System were purchased from Roche Molecular Biochemicals, Mannhein, Germany; QIAEX II was from QIAGEN K. K., Tokyo, Japan; pGEM-T Easy vector was from Promega Corp., Maison, WI; Thermo Sequenase cycle sequencing kit was from Amersham, Cleveland, OH. All other chemicals were of reagent grade.

2.2. Primate tissues

Fresh placenta from a chimpanzee (*Pan troglodytes*; 17-year-old female) was obtained just after delivery at the Primate Research Institute, Kyoto University. The stomach of orang-utan (*Pongo pygmaeus*; 10-year-old male) was removed from an animal which died of disease, and the livers of a Japanese monkey (*Macaca fuscata*; 6-year-old male), a capuchin (*Cebus apella*; newborn male), and a common marmoset (*Callithrix jacchus*; 1-year-old male) were obtained immediately after sacrifice by exsanguination *via* the bilateral carotid arteries under deep anesthesia using ketamine hydrochloride and sodium pentobarbital, in accordance with the guidelines of the Primate Research Institute, Kyoto University. Fresh stomach from a white-handed gibbon (*Hylobates lar*; adult male) was obtained from an animal that died of disease at the Japan Monkey Center, Inuyama. Portions of these tissues were subjected to RNA extraction.

2.3. PCR cloning of CCS cDNAs

cDNAs for primate CCSs were synthesized by RT-PCR and the succeeding nested PCR. The first-round RT-PCR was done using the Titan One Tube RT-PCR System with a pair of primers of 5′-GTC TCG GGG TGG TGA CTG and 5′-AGG ACA GCA ACA GAG CCA AG. Nested PCR was carried out using Expand High Fidelity^{PLUS} PCR System with a pair of primers of 5′-TGG TGA CTG GGT CCA GAA TG and 5′-CCA AGG TGA GGT CCT GCT CA. These primers were designed based upon the cDNA sequence of the human CCS (Culotta et al., 1997). Nested PCR products were resolved by agarose-gel electrophoresis and the band of the CCS cDNA was subjected to DNA extraction with QIAEX II. cDNAs were ligated to the pGEM-T Easy vector, and the vector was used for the transformation of *E. coli* JM109.

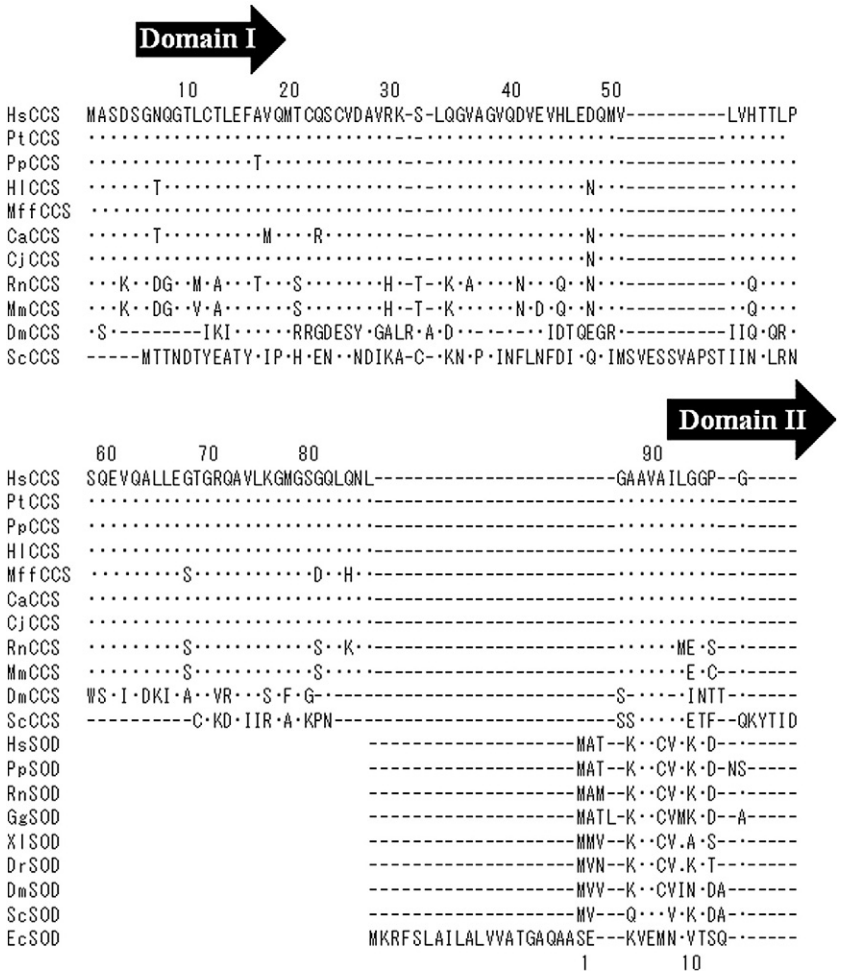

Fig. 1. Comparison of the amino acid sequences of the CCSs of chimpanzee (GenBank/EMBL/DDBJ accession number: AB120990), orang-utan (AB120991), gibbon (AB120992), Japanese monkey (AB120993), capuchin monkey (AB120994), and common marmoset (AB120995) with those of the human and rat, and Cu,Zn-SODs of the various organisms. Dots indicate that amino acids are identical to human CCS. Residue numbers in the upper and lower parts of the sequences are those of human CCS and Cu,Zn-SOD, respectively. Copper and zinc binding residues of Cu,Zn-SODs and residues at the corresponding positions in CCSs are in the filled boxes. Hs, human; Pt, chimpanzee Pp, orang-utan; Hl, gibbon; Mff, Japanese monkey; Ca, capuchin monkey; Cj, common marmoset; Rn, rat; Mm, mouse; Gg, chicken; Xl, clawed toad; Dr, zebra fish; Dm, fly; Sc, yeast; Ec, *Escherichia coli*.