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Identification and expression of a new splicing variant of FAD-sulfhydryl oxidase in adult rat brain

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Abstract

Flavoproteins of the quiescin/sulfhydryl oxidase (QSOX) family catalyze oxidation of peptide and protein thiols to disulfides with the reduction of oxygen to hydrogen peroxide. We report here the molecular cloning of a new putative sulfhydryl oxidase cDNA, *rQSOX-L* (GenBank Accession no AY623665), from adult rat brain and its expression studied by RT-PCR, Northern and Western blots in rat tissues. DNA-sequencing demonstrated the existence of two cDNAs in rat cortex, corresponding to a long transcript (*rQSOX-L*) and a short transcript (*rQSOX-S*) which differed by 851 nucleotides due to alternative splicing. The new transcript, *rQSOX-L* (3356 nucleotides), was specifically expressed in brain, hypophysis, heart, testis and seminal vesicle. The distribution of this variant is not homogeneous in the different tissues studied and suggests a complex gene regulation. The full-length *rQSOX-L* cDNA has an open reading frame of 2250-bp encoding a protein of 750 amino acids that contains a signal peptide sequence, a protein-disulfide-isomerase-type thioredoxin and ERV1-ALR domains and a long form specific C-terminal extension. The rQSOX-L protein is highly homologous to members of the sulfhydryl oxidase/Quiescin family and contains particularly two potential sites for *N*-glycosylation. This protein isoform was specifically detected in rat brain tissues in opposition to the low molecular form that was ubiquitous. Matrix-assisted laser desorption/ionization time of flight mass spectrometry analysis of the immunoprecipitate tryptic fragments allowed the identification of rQSOX-L protein.

Keywords: rQSOX-L sulfhydryl oxidase; Rat adult brain; Alternative splicing; Genomic organization; Expression pattern; MALDI-TOF MS

1. Introduction

Alternative mRNA polyadenylation, splicing, and mRNA editing are post-transcriptional events that introduce diversity in mammalian mRNAs, thereby regulating the number of structurally and functionally different proteins encoded by a single gene. The flavin-dependent sulfhydryl oxidases represent a newly discovered family of proteins quiescin/sulfhydryl oxidase (QSOX) with a wide range of cellular localizations and putative roles [1–8]. The avian and mammalian proteins can catalyze the direct oxidation of protein cysteine residues to disulfides with the reduction of oxygen to hydrogen peroxide [9–12] without mediation of other proteins or small molecules [2,11]. QSOX enzymes contain an N-terminal redox active thioredoxin domain [8,13] and an ERV1/ALR domain [14–20] closer to the C-terminus. The general importance of QSOX in cellular development is indicated by the finding that, during evolution, the enzyme of the yeast ERV1 prototype was fused to thioredoxin/disulfide isomerase domains and generated a new catalyst for disulfide formation with dramatically enhanced enzymatic activity [8,21]. QSOX like other redox-active proteins may have diverse functions in the regulation of cell growth and differentiation [6,18,19,22]. Immunohistochemical and biochemical studies indicated the important role of these proteins in spermatogenesis [23,24]. It was reported that the guinea pig sulfhydryl oxidase *CpQSOX* was a potentially estrogen-regulated gene and could be implicated in the negative cell cycle control [6]. The CpQSOX protein appeared to be specific of

Abbreviations: ALR, augmenter of liver regeneration; CBB, Coomassie brilliant blue; *ERV1*, gene essential for respiration and vegetative growth; MALDI-TOF MS, matrix-assisted laser-desorption/ionization-time-of-flight mass spectrometry; PDI, protein disulfide isomerase; QSOX, quiescin sulfhydryl oxidase; *rQSOX-L*, rat *QSOX* long transcript; *rQSOX-S*, rat *QSOX* short transcript

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epithelial cells in uterus and its expression level varied during the estrus cycle [6]. Human QSOX, rat seminal vesicle FAD-linked enzymes, QSOX from mouse epidermis and CpQSOX proteins are clearly distinct from the Erv1p/ALR sulfhydryl oxidase. The QSOX-related enzymes are monomeric proteins larger than 60 kDa that are excreted from cells [4,5]. In contrast, Erv1p/ALR are small proteins of approximately 21 kDa that form dimers localized in the mitochondrial intermembrane space and represent the first components of this compartment with a role in the biogenesis of cytosolic Fe/S proteins [16]. It was reported that in adult rat central nervous system, QSOX protein is specifically expressed by neurons throughout the rostrocaudal extent of the brain as well as in the spinal cord, and this study also demonstrated that this enzyme is associated with the Golgi apparatus [25]. Recently, a neuroblastoma-derived sulfhydryl oxidase gene (*SOXN*), which comprises 12 exons and maps to 9q34.3, was described [26]. This gene encodes a putative protein of 698 amino acids predominantly located in the plasma and in the nuclear membrane and regulates sensitization to interferon γ-induced cell death in human neuroblastoma cells. The characterization of the different QSOX isoforms in rat adult brain is important for further investigations of their physiological functions in the nervous system, especially their role in initiating and promoting neuronal oxidative damage, because brain is highly vulnerable to oxidative injury due to its high oxygen tension and elevated levels of polyunsaturated fatty acids. Oxidative stress in the brain has been increasingly associated with the development of various neurological diseases. Recent data have shown that oxidative injury was involved in the pathogenesis of neurodegenerative diseases such as Parkinson's disease, Huntington's disease and Alzheimer's disease [27] and have further reinforced the need for understanding the antioxidant potential of brain, particularly with respect to the ability to recover from oxidative stress. It has been reported that at least two transcripts of *rQSOX* were detected in rat brain, suggesting a tissue-specific splicing of its mRNA. The shorter transcript, likely corresponding to the mRNA identified from rat seminal vesicles, was highly expressed in diencephalon and telencephalon but nothing was known about the long transcript [7].

In this paper, we report the cloning, the characterization and the tissue distribution of a new QSOX transcript in the rat brain: *rQSOX-L* which encodes an 82.5 kDa protein highly expressed in the adult rat brain tissues.

2. Materials and methods

2.1. Animals

Sprague–Dawley male rats (3 months old, IFFA Credo, France) weighing 250–300 g housed under natural daylight conditions with food and water provided

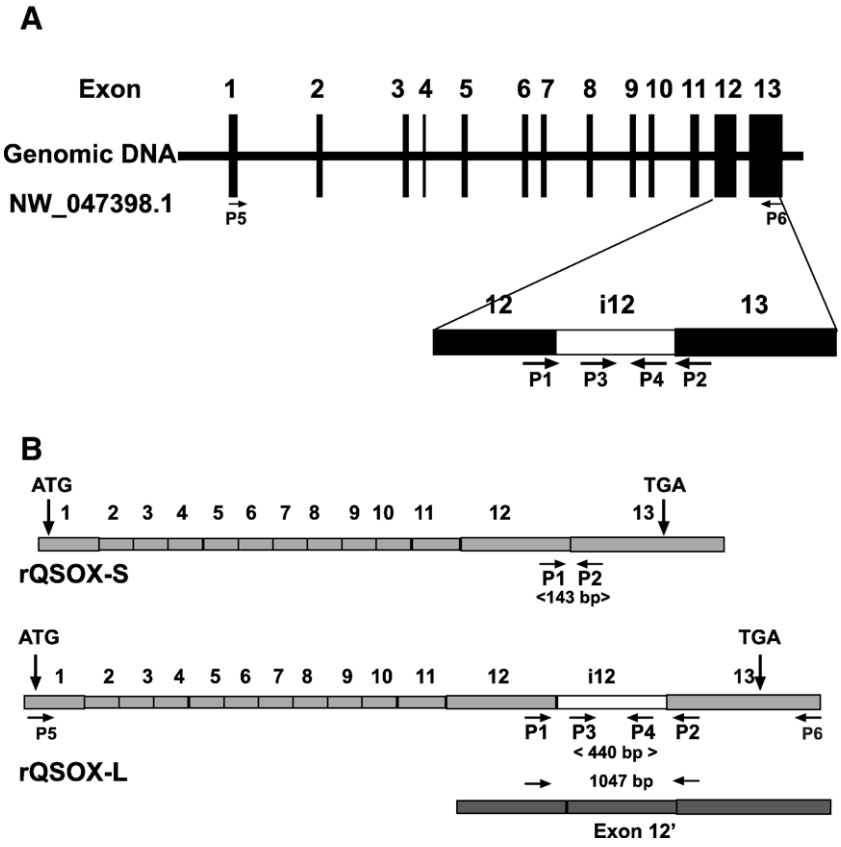

Fig. 1. Genome structure and alternative splicing of rat *QSOX* were predicted by alignment of cDNAs with genomic DNA. (A) Structure speculated by comparison of *rQSOX*-S with rat *QSOX* gene: vertical and horizontal lines indicate exons and introns, respectively; intron 12 is noted i12; P1, P2, P3, P4, P5 and P6 show the positions of primers for RT-PCR. (B) Organization of the two *rQSOX* cDNAs: the start codon ATG and stop codon TGA are marked. The same exon is show by the same number in the two cDNAs. The black shading of a box indicates a non-common sequence.