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Cytochrome b561 protein family: Expanding roles and versatile transmembrane electron transfer abilities as predicted by a new classification system and protein sequence motif analyses

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Abstract

Cytochrome b561 family was characterized by the presence of "b561 core domain" that forms a transmembrane four helix bundle containing four totally conserved His residues, which might coordinate two heme *b* groups. We conducted BLAST and PSI-BLAST searches to obtain insights on structure and functions of this protein family. Analyses with CLUSTAL W on b561 sequences from various organisms showed that the members could be classified into 7 subfamilies based on characteristic motifs; groups A (animals/neuroendocrine), B (plants), C (insects), D (fungi), E (animals/TSF), F (plants+DoH), and G (SDR2). In group A, both motif 1, {FN(X)HP$(X)_2$M$(X)_2$G$(X)_5$G(X)ALLVYR}, and motif 2, {YSLHSW(X)G}, were identified. These two motifs were also conserved in group B. There was no significant features characteristic to groups C and D. A modified version of motif 1, {LFSWHP$(X)_2$M$(X)_3$F$(X)_3$M(X)EAIL(X)SP$(X)_2$SS}, was found in group E with a high degree of conservation. Both motif 3, {DP(X)WFY(L)H$(X)_3$Q}, and motif 4, {K(X)R(X)YWN(X)YHH$(X)_2$G(R/Y)} ,were found in group F at different regions from those of motifs 1 and 2. The "DoH" domain common to the NH_2-terminal region of dopamine β-hydroxylase was found to form fusion proteins with the b561 core domains in groups F and G. Based on these results, we proposed a hypothesis regarding structures and functions of the 7 subfamilies of cytochrome b561.

Keywords: Protein structural motif; Cytochrome b561; Transmembrane electron transfer; Membrane protein; Ascorbate

1. Introduction

It is becoming evident that most of eukaryotic cells have similar membranous proteins belonging to a unique protein family named "cytochrome b561". Originally, cytochrome b561 was found to reside specifically in neuroendocrine vesicle membranes [3–5] and to have a function for supplying electron equivalents across the membranes [6–8] to the intravesicular copper-containing monooxygenase, such as dopamine β-hydroxylase (DBH) and peptidylglycine α-amidating monooxygenase (PHM) [9–11]. To perform this specific physiological role, this very hydrophobic protein with a putative six-transmembrane α-helices structure contains two heme *b* prosthetic groups on both sides of the membranes [12–14]. The extravesicular heme group has a role for electron acceptance from cytosolic ascorbate (AsA) and the other heme on the intravesicular side is likely to donate electrons to intravesicular monodehydroascorbate (MDA) radical after conducting the transmembrane electron transfer reaction [15–17]. Re-generated AsA can donate electrons to the copper-containing monooxygenase inside the vesicles, leading to the biosynthesis and activation of various neurotransmitters [9,18].

Abbreviations: DBH, dopamine β-hydroxylase; PHM, peptidylglycine α-amidating monooxygenase; AsA, ascorbate; MDA, monnodehydroascorbate; TSF, tumor suppression factor; Dcytb, duodenal cytochrome b561; SDR2, stromal cell-derived receptor 2

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Upon finding of duodenal cytochrome b561 (Dcytb) as a ferric reductase for the transport of ferrous iron into intestinal duodenal mucosa cells [19], we and other investigators had begun to realize that there were a significant number of new members belonging to the cytochrome b561 family. Indeed, it had been proposed that there were *b*-type cytochromes reducible with AsA in the plasma membranes from various plants [20–22] but with unknown physiological functions. Further, Ponting proposed that there were additional members of the cytochrome b561 family in animals, including stromal cell-derived receptor 2 (SDR2), tumor suppression factor[1] (TSF), etc, on the basis of PSI-BLAST analyses conducted on various data bases [23]. It must be noted that the "cytochrome b561 family" (IPR004877, IPR006593; InterPro) is distinctly different from the "bacterial cytochrome b561 protein family" (IPR011577), although the latter bacterial family members are also integral membrane proteins having electron transport activities by using two non-covalently bound heme prosthetic groups [24]. The "bacterial b561 domain" has a completely different architecture in the coordination of two hemes and has been found in a number of nickel-dependent hydrogenase subunits (IPR000516, IRP006471) [25].

In our previous short review [26], we proposed that the cytochrome b561 family members in animals and plants could be categorized into four major subfamilies based on the analysis for the core domain sequences of cytochromes b561. Properties and physiological functions of this protein family are still not clear at this stage. In the present study, we have extended our study to obtain further insights on the structure and functions of the cytochrome b561 protein family.

2. Methods

2.1. BLAST, PSI-BLAST, and SMART searches on cytochrome b561 family members

The deduced amino acid sequences of several members of the cytochrome b561 family, including cytochromes b561 from planaria (AB049567) [27], pig (D88157), sheep (D88158) [14], *Arabidopsis thaliana* (AB049627 and AB049628)(Asada et al, unpublished), and *Zea mays* (AB182641)(Nakanishi et al, unpublished), all of which were determined by our research group, were each used as a query for BLAST searches [28]. The BLAST searches were performed using the DDBJ server (http://www.ddbj.nig.ac.jp/Welcome-e.html) at National Institute of Genetics (Mishima, Shizuoka, Japan) employing firstly entire b561 sequences as queries. The retrieved nucleotide sequences were then translated into amino acid sequences. As supportive analyses, we also conducted PSI-BLAST searches [28] using the same queries employed for the BLAST searches. The retrieved b561-like amino acid sequences were each examined by a multiple sequence alignment analysis using CLUSTAL W (v.1.83) [29] of the DDBJ server for comparison with several cytochrome b561 sequences, such as bovine cytochrome b561 [30]. Conservation of two pairs of His residues at the appropriate positions {i.e., the first and the third His residues being on the putative cytosolic side and the second and the fourth His residues being on the boundary region between the external (or intravesicular) hydrophilic loops and the transmembrane α-helices} was checked after the hydrophobicity plot analysis using Kyte–Doolittle-type parameters [31] with a window size of 19 amino acid residue length. Proteins without such two pairs of His residues were discarded as a non-member of the cytochrome b561family, even if there might be some distinct similarities in the amino acid sequences to an authentic cytochrome b561 (see later).

As an independent approach, we conducted several web-based searches. Those included InterPro (release 8.1, http://www.ebi.ac.uk/interpro/) [32] and its subdivisions, SMART (SMART 4.0, http://smart.embl-heidelberg.de/) [33,34], Pfam (release 16, http://www.sanger.ac.uk/Software/Pfam/) [35] and PROSITE (release 18.0, http://www.expasy.org/prosite/) [36]. This kind of multiple approaches was very powerful and important for the confirmation of the domain structure and identification of membrane spanning α-helices of the retrieved sequences.

2.2. Sequence analysis of cytochrome b561-domain

After collecting a number of sequences with authentic "b561" domain structure, we conducted a multiple sequence alignment for all these sequences using CLUSTAL W of the DDBJ server. The aligned sequences were evaluated by a visual inspection and the alignment was further corrected manually, if necessary. After such manual adjustments were completed, we defined the "core domain" of cytochrome b561 protein family. Initially, the core domain consisted of central four α-helices among the six α-helices of the protein. The extent of the conservation of the first and the last putative α-helices were very low and these portions were considered as non-essential parts for the function of transmembrane electron transfer [23,37]. Indeed, some members of the family lacked the first α-helix, being consistent with the notion that central four α-helices are essential part of cytochrome b561 family [23]. However, in a later stage, we extended the core region towards COOH-terminus further including most part of the sixth α-helix segment to increase the accuracy of our analysis since this last α-helix segment was always retained in cytochrome b561 family members. However, the very end of the COOH-terminus was not included in the analysis since the sequences of this end portion showed a significant diversity and may have an unidentified routing signal(s) characteristic to each member of the family.

After the definition of the core domain, the restricted b561-domain sequences were again multiply aligned using the CLUSTAL W of the DDBJ server. Based on the bootstrap values (bootstrap count, 1000) obtained from the CLUSTAL W analysis, a phylogenetic tree was constructed for the b561 domain sequences in a radial (an unrooted tree) form or in a phylogram form using TreeView (v.1.66) [38].

2.3. Sequence analysis of DoH domain

It has been known that several members of cytochrome b561 family contain a homologous domain residing in DBH ("DoH"-domain, or "DOMON" domain) [23,39], although its exact function(s) is still not known. Usual cytochrome b561 family members contained about 230~250 amino acid residues in length. Therefore, sequences longer than 300 amino acid residues were examined with CLUSTAL W using the DoH-domains of SDR2 (human) or DBH (human) as a query sequence whether the DoH domain being present or not. After the identification of the DoH-domain in each b561 sequence followed by confirmation with other Web-based databases (InterPro, SMART, Pfam, and PROSITE), the core domain of DoH-sequence was defined using CLUSTAL W of the DDBJ server. Based on the bootstrap values obtained from the CLUSTAL W analysis, a phylogenetic tree was constructed for the DoH core domain in a radial (an unrooted tree) form or in a phylogram form using TreeView (v.1.66) [38]. Sequences of the DoH domains from DBH, CG6 protein, and Air12 proteins [23,39,40] were also analyzed for comparison.

3. Results and discussions

The BLAST search using cytochrome b561 sequences as queries showed many b561-like sequences from various organisms. The results were assessed by PSI-BLAST and SMART analyses. We restricted our search within several re-

[1] Tumor suppression factor (TSF) (101F6 protein) is one of the products from putative tumor suppressor genes identified in a 120-kb critical tumor homozygous deletion region (found in lung and breast cancer) of human chromosome 3p21.3 [1,2].