Volume 50(1-2):83-87, 2006
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Protein-protein interaction of cytochrome b_{561} in chromaffin vesicle membranes studied by two-dimensional blue-native/sodium dodecyl sulfate gel electrophoresis and co-immunoprecipitation analysis

Fusako Takeuchi[1], Yohei Yamamoto[2], Yuka Nishimura[2], Sam-Yong Park[1], Motonari Tsubaki[2,3]*

[1]Division of Supramolecular Biology, International Graduate School of Arts and Sciences, Yokohama City University, Yokohama, Japan, [2]Department of Molecular Science and Material Engineering, Graduate School of Science and Technology, Kobe University, Kobe, Japan; [3]Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, Kawaguchi, Japan

ABSTRACT We analyzed a protein-protein interaction in solubilized chromaffin vesicles using two-dimensional electrophoresis (1st, Blue-Native PAGE; 2nd, Tricine-SDS-PAGE). Cytochrome b_{561} band, which was verified by immunoblotting, was observed in the two-dimensional gel with an apparent molecular weight of ~100~400kDa. On the other hand, purified cytochrome b_{561} showed a monomeric band (28 kDa) in Blue-Native PAGE. These results indicated that cytochrome b_{561} interacts with other proteins in the chromaffin vesicles to form a large protein complex(es). To clarify the nature of the interaction, we performed co-immunoprecipitation experiments, where the solubilized membrane proteins were treated with immunopurified anti- b_{561} IgG antibodies followed by sedimentation with protein-A-Sepharose. We found that there were no other proteins co-sedimented with cytochrome b_{561}. Since the immunopurified anti- b_{561} IgG antibodies bound specifically to the C-terminal hydrophilic portion of cytochrome b_{561} protein, we concluded that such binding of the IgG antibodies to the C-terminal portion might cause an inhibition of protein-protein interaction with other proteins in the solubilized state.
Acta Biol Szeged 50(1-2):83-87 (2006)

KEY WORDS

Cytochrome b_{561} protein-protein interaction
Blue-Native PAGE
two-dimensional gel electrophoresis
chromaffin vesicles
co-immunoprecipitation

In the matrix and membranes of chromaffin vesicles, there are many proteins, peptides and enzymes responsible for various physiological functions (Winkler et al. 1986). In the vesicular matrix, three major secretory proteins (chromogranin A and secretogranins I and II) together with peptides derived from them, and smaller amounts of neuropeptides, and several different endo- and exo-proteinase for the production of these peptides exist (Apps 1997). In the membranes, those responsible for catecholamine biosynthesis (dopamine β-hydroxylase and cytochrome b_{561}), active transport of vesicle components (V-type ATPase, carriers for monoamines, nucleotides, and small ions) and exocytosis (synaptotagmin, synaptobrevin and other proteins) are known to exist. To perform these specific biological roles, these proteins and other proteins with unknown functions must have many kinds of interactions each other and, in sometimes, form stable or transient molecular complexes. Such formations of the protein complexes are usually crucial to manifest most of their physiological functions.

Among these proteins residing in chromaffin vesicles, cytochrome b_{561} is very unique to have a role for transporting electrons from cytosolic ascorbate (AsA) to intravesicular monodehydroascorbate (MDA) radical to regenerate AsA (Njus and Kelley 1993). This electron transfer is essential for the production of neurotransmitters by intravesicular copper-containing monooxygenases, dopamine β-hydroxylase (DBH) and peptidylglycine α-amidating monooxygenase (PAM) (Apps 1997; Winkler and Fischer-Colbrie 1998). It is assumed that cytochrome b_{561} exists in the membrane as a monomer and plays a role as an electron conduit from cytosolic AsA to intravesicular MDA radical. However, there have been some reports describing a small intravesicular protein, which may interact with cytochrome b_{561} and participate in some role for the electron transfer or its modulation (Grigoryan et al. 1981; Markossian et al. 1986). Further, a direct interaction between cytochrome b_{561} and the membrane-bound form of DBH was inferred (Ahn and Klinman 1987; Wimalasena and Wimalasena 1995). In the present study, we examined such possibilities of a direct protein-protein interaction of cytochrome b_{561} with other proteins in chromaffin vesicles using a various biochemical techniques, including two-dimensional Blue-Native PAGE, co-immunoprecipitation, and immunoblotting. Blue-Native electrophoresis was originally developed to study mitochondrial membrane protein complexes (Schägger and von Jagow 1991; Schägger et al. 1994; Schägger and Pfeiffer 2000) and has since then been used widely (van Lis et al. 2003; Culvenor et al. 2004; Claeys et al. 2005). Us-

*Corresponding author. E-mail: mtsubaki@kobe-u.ac.jp

ing this technique, membrane proteins and their complexes were solubilized with a nonionic detergent and native protein-protein complexes were resolved in a first-dimensional PAGE in the presence of Coomassie Brilliant Blue (CBB) G250 dye according to their apparent molecular mass. In a second dimension, an SDS-PAGE was employed, where SDS denatured the complex and separates them into their respective subunits according to their molecular mass during electrophoresis. A preliminary study on chromaffin vesicle membrane proteins with this technique was reported previously by Apps (Apps 1997), in which a single protein-staining band corresponding to cytochrome b_{561} was observed together with DBH (monomer and tetramer) and V-type ATPase (115-,39- and 16-kDa subunits of the V_o segment). However, since then, there has been no detailed study concerning such protein-protein interactions in chromaffin vesicle membranes. In the present study, we found that cytochrome b_{561} is likely to have protein-protein interactions with other proteins and that the C-terminal hydrophilic tail may have some roles for such interactions.

Materials and Methods

Purification of anti-cytochrome b_{561} IgG

Antisera against purified bovine cytochrome b_{561} (Tsubaki et al. 1997) were raised in a Japanese white rabbit (Takara-Bio, Japan) and were partially purified by ammonium sulfate fractionation (0~0.2M). The partially purified IgG fraction was further purified at 4°C using HiTrap-NHS- b_{561} affinity column, in which purified bovine cytochrome b_{561} was covalently attached to HiTrap-NHS activated HP resin (1.0 mL volume pre-packed in a column; Amersham Biosciences, USA) according to the manufacturers recommendation. The adsorbed anti-cytochrome b_{561} IgG was washed with 1M NaCl, 1% Triton X-100, 20 mM Tris-HCl (pH 7.5) at 4°C and, then, eluted with 0.1 M Gly-HCl pH 2.5. The eluted IgG fraction was neutralized immediately by mixing with 1 M Tris and was further mixed with 1.0 mg/ml of bovine serum albumin to increase the stability.

Sample preparation for blue-native electrophoresis

Chromaffin vesicles were obtained from bovine adrenal medullae as previously described (Tsubaki et al. 1997). The purified vesicles were saved in –80°C until use. To solubilize chromaffin membrane proteins and their complexes, the membrane pellet was vigorously pipetted in extraction buffer (final volume of 100 µl with 4.0 mg protein/ ml) containing 750 mM aminocaproic acid, 50 mM BisTris/HCl (pH 7.0) at 4°C. Fifteen µl of 10% (w/v) dodecyl maltoside were then added to the suspension (final 0.34% (w/v)). After incubation on ice for 20 min with vortex mixing in every 5 minutes, insoluble membrane materials were removed by centrifugation at 10,500 rpm for 20 min. To the supernatant (155 µl), 2.5 µl of 5% (w/v) Coomassie Brilliant Blue G250 diluted in 500 mM aminocaproic acid was added. Samples were then briefly centrifuged at 10,500 rpm to eliminate any precipitate and kept on ice until loading on Blue-Native (BN) gel.

Blue-Native PAGE and immuno blot analyses

Blue-Native (BN)-PAGE for soluble and membrane proteins was carried out according to a published protocol (Schägger and von Jagow 1991; Schägger et al. 1994) using 5-18% gradient gel. The native high-molecular-weight markers (66~669 kDa) used for calibration were as follows; thyroglobulin (669 kDa), ferritin (440 kDa), catalase (250 kDa), and bovine serum albumin (66 kDa) (from Sigma, USA). Additionally, purified cytochrome *c* oxidase (from bovine heart mitochondria, 220 kDa (dimer)) and dopamine β-hydroxylase (from bovine adrenal medulla (350 kDa (tetramer)) were used. One complete lane was excised from the fist dimensional, and the gel was resolved by a denaturing Tris-SDS-PAGE in the second dimension, after an appropriate pre-treatment. After the electrophoresis, proteins in the gels were transferred to a nitrocellulose membrane (0.45 µm, BioRad, USA) by semidry blotting by using a discontinuous transfer system in 25 mM Tris, 192 mM glycine, 20% (v/v) methanol with 150 mA for 1.5 h. Blocking and immunodecorations were performed in Tween-20/phosphate-buffered saline containing 1% (w/v) BSA using anti-bovine cytochrome b_{561}-IgG(rabbit) (500-fold dilution) as primary antibodies and anti-rabbit IgG-IgG(goat)-HRP conjugate (500-fold dilution) as secondary antibodies. Immunodetection was done by addition of 0.2% 4-chloro-1-napthol in 50 mM Tris-HCl (pH7.4) followed by 30% hydrogen peroxide (final 0.006%).

Co-immunoprecipitation analyses

Bovine chromaffin vesicle membranes were solubilized with 1.0% (w/v) dodecyl maltoside in the same buffer used for Blue-Native PAGE. After the centrifugation (10,500 rpm, 10 min) to remove insoluble materials, the solution was incubated with purified anti-cytochrome b_{561} IgG (rabbit) using a rotary incubator (30min) followed by precipitation with protein-A-Sepharose beads (centrifugation at 2,000 rpm; 2 min). The precipitate was washed with 0.1% (w/v) Triton X-100 in 0.15 M NaCl and 50 mM Tris-HCl (pH 7.4) to remove non-specifically bound proteins. Then, the precipitate was boiled to release proteins from the bound complex. Then, the released proteins were analyzed by Tricine-SDS-PAGE (Schägger and von Jagow 1987) in the absence of disulfide-bond reducing reagents (dithiothreitol or β-mercaptoethanol). As control experiments, non-specifically interacting proteins, either in the solubilized chromaffin vesicles or in the purified IgG fraction, to protein-A-Sepharose beads were analyzed in the same condition.

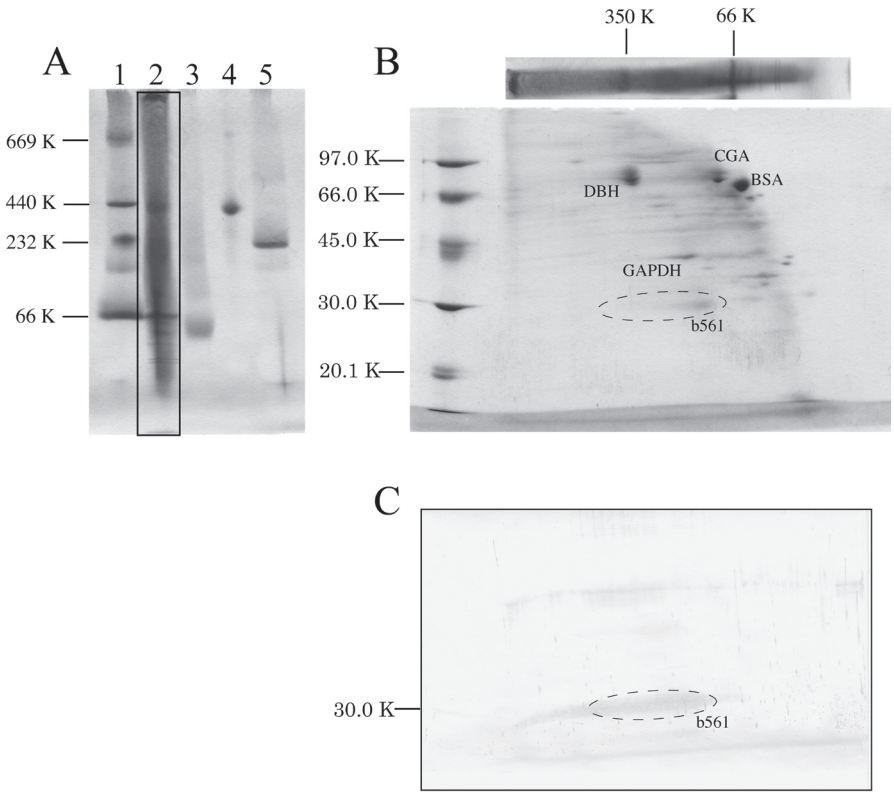

Figure 1. One-dimensional and two-dimensional resolution of bovine chromaffin vesicle membrane proteins using Blue-Native PAGE. (Panel A) Bovine adrenal chromaffine vesicle membranes were solubilized, and resolved by Blue-Native PAGE in first native dimension (lane 2). Purified bovine cytochrome b_{561} (12.9 μg, lane 3), bovine dopamine β-hydroxylase (lane 4), bovine cytochrome *c* oxidase (lane 5), and a mixture of protein markers (lane 1: thyroglobulin, 669 kDa; ferritin, 440 kDa; catalase, 232 kDa; bovine serum albumin, 66 kDa) were also electrophoresed under the same condition. Gel concentration, 5~18% linear gradient; 0.085% CBB G-250; 0.34% dodecyl maltoside. (Panel B) One complete lane (lane 2) was excised from the fist dimensional gel (indicated by a rectangle) was resolved by a denaturing Tris-SDS-PAGE in the second dimension, after an appropriate pre-treatment. In the far-left of the gel, a mixture of low-molecular weight markers (97.0 kDa, 66.0 kDa, 45.0 kDa, 30.0 kDa, 20.1 kDa, 14.4 kDa; LMW Marker Kit, Amersham Biosciences, USA) was applied. The gels were stained with Coomassie Brilliant Blue R-250. Cytochrome b_{561} protein spot was circled for easiness of the identification. Other identified proteins (DBH, BSA, CGA, and GAPDH) were also indicated. (Panel C) The resolved proteins in the gel after the second dimensional SDS-PAGE were transferred to a nitrocellulose membrane and cytochrome b_{561} protein was visualized using immunochemical technique as described in the text and was circled for easiness of the identification.

NH_2-terminal amino acid sequencing of protein spots in 2D gel

NH_2-terminal amino acid sequence of each protein spot in 2D gel was analyzed as follows. Proteins in the 2D gel were electro-blotted onto a PVDF membrane (Sequi-Blot, 0.2 μm; BioRad, USA). The Ponceau S-stained protein band on the PVDF membrane was cut using a clean razor and was directly analyzed with an ABI protein sequencer (Model 492; Applied Biosystems, USA) up to 10 cycles.

Results and Discussion

Blue-Native PAGE analyses of chromaffin vesicle membrane proteins and cytochrome b_{561}

The solubilized chromaffin vesicle membranes were analyzed by Blue-Native PAGE with 5~18% linear gradient polyacrylamide gel (Fig. 1A, lane 2). It showed several proteins or protein complexes with apparent molecular weights at ~30 kDa, 66 kDa and 350 kDa. These protein bands were overlapped with several broad bands centered 50~500 kDa. To analyze these overlapping protein bands in the first dimension, the rectangular section in Fig. 1A was excised and was resolved further in a denatured condition by Tricine-SDS-PAGE. A representative 2D gel stained with CBB R-250 after the electrophoresis is shown in Fig. 1B. The 350 kDa and 66 kDa band in the first Blue-Native PAGE were found as a tetrameric form of DBH (87.5 kDa) and a monomeric form of BSA (66 kDa). In addition, protein bands for chromogranin-A precursor (CGA) (50 kDa) and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) (36 kDa) were identified. The identification of these 4 protein bands was based on the NH_2-terminal amino acid sequences up to 10 cycles on the

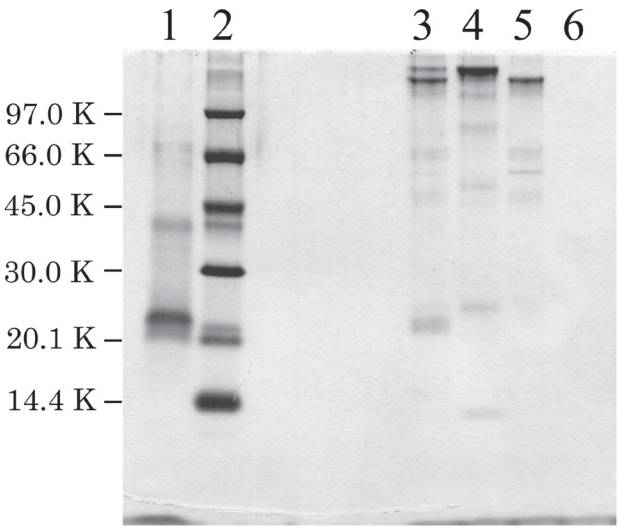

Figure 2. Immunoprecipitation of bovine cytochrome b_{561} using purified anti- b_{561} IgG and its interaction with other proteins analyzed by SDS-PAGE. Bovine chromaffin vesicle membranes were solubilized with 1.0% (w/v) dodecyl maltoside and the solution was incubated with purified anti-cytochrome b_{561} followed by precipitation with protein-A-Sepharose beads. Then the precipitate was boiled and released proteins were analyzed (lane 3), showing a band of cytochrome b_{561} and other non-specifically bound proteins with a higher molecular weight. As controls, non-specifically bound proteins in chromaffin vesicles (lane 4) and non-specifically bound proteins in purified IgG fraction (lane 5) to protein-A-Sepharose beads are shown. There was no release of proteins from protein-A-Sepahrose beads after boiling (lane 6).

transferred proteins to PVDF membranes from the gel and MALDI-TOF analyses for in-gel-digested tryptic peptides. The CGA showed an apparent molecular weight of ~100 kDa whereas GAPDH indicated a mobility of about 250 kDa, both in the Blue-Native gel. These results suggested that the formation of a stable molecular complex with other proteins for CGA (Yoo et al. 2005) and a dimer of tetrameric complex for GAPDH in native conditions.

Cytochrome b_{561} in the solubilized chromaffin vesicle membranes did not form a sharp protein spot in the 2D gel (Fig. 1B). Instead, it formed a very broad and spread protein band ranging from 100 kDa to 400 kDa in the first dimension and 28kDa in second dimension. This broad band as an authentic cytochrome b_{561} protein was confirmed by immunodetection using anti-cytochrome b_{561} IgG(rabbit) and anti-rabbit IgG-IgG(goat)-HRP conjugate (Fig. 1C). However, when the purified bovine cytochrome b_{561} was analyzed in the native form under the same buffer and detergent condition, it showed a clear protein spot (~30kDa) in the first Blue-Native PAGE gel (Fig. 1A, lane 3). These results suggested that cytochrome b_{561} is likely to form a large protein complex with other membrane (or soluble) proteins in the solubilized chromaffin vesicle membranes. Formation of a homo-oligomeric forms of cytochrome b_{561} might be not so significant, since the formation of such complexes were not so obvious for the purified cytochrome b_{561} in the native form (Fig. 1A, lane 3). To clarify these interacting proteins with cytochrome b_{561} in chromaffin vesicles, we conducted co-immunoprecipitation analyses.

Specificity of anti-b_{561}-IgG antibodies

Before the immunoprecipitation analyses, we investigated a binding specificity of the immunopurified anti- b_{561}-IgG antibodies to the purified bovine cytochrome b_{561} by Western blotting. When a partially digested mixture (with endogenous proteases) of cytochrome b_{561} was analyzed, the immunopurified anti- b_{561} IgG antibodies recognized the full-length of cytochrome b_{561} protein. However, the immunopurified anti-b_{561} IgG antibodies did not recognize the partially digested form in which only the C-terminal portion was specifically cleaved off (not shown). Therefore, we concluded that the immunopurified anti- b_{561} IgG antibodies recognized specifically the C-terminal hydrophobic peptide. Being consistent with this conclusion, when a purified fused protein DHFR-Bb561C, in which a C-terminal part (219-252) of bovine cytochrome b_{561} was fused to the C-terminus of DHFR and was expressed in *E. coli*, was analyzed similarly, the immunopurified anti- b_{561} IgG antibodies recognized this protein very clearly (not shown).

Co-immunoprecipitation analyses

Immunopurified anti-b_{561}-IgG antibodies were mixed with the solubilized chromaffin vesicle membranes to form putative (anti-b_{561}-IgG)-(b_{561})-(interacting protein) ternary complexes in the solution. Such putative protein complexes were sedimented by mixing with protein-A-Sepharose beads, washed, and followed by boiling to release the individual protein molecules from the complex. The released proteins were then analyzed with Tricine-SDS-PAGE (Schägger and von Jagow 1987), but in the absence of disulfide-bond reducing reagents (dithiothreitol or mercaptoethanol). A protein band corresponding to cytochrome b_{561} (28 kDa) and other faint protein bands with a higher molecular weight were observed (Fig. 2, lane 3). As control experiments, non-specifically bound proteins in chromaffin vesicles (Fig. 2, lane 4) and non-specifically bound proteins in purified IgG fraction (Fig. 2, lane 5) to protein-A-Sepharose beads were also analyzed. There was no release of proteins from protein-A-Sepharose beads after boiling (Fig. 2, lane 6). Since all the protein bands with a higher molecular weight appeared in lane 3 were identified as non-specifically bound proteins to protein-A-Sepharose beads (lanes 4 and 5), we reached a conclusion that there was no protein having a direct interaction with cytochrome b_{561} in the solubilized vesicle membranes, at least in the present experimental condition.

Protein-protein interacting domain of cytochrome b_{561}

To explain the present discrepant results, we thought a possibility that binding of purified anti- b_{561} IgG antibodies to the C-terminal part of cytochrome b_{561} protein may inhibit the interaction with other proteins. Since cytochrome b_{561} does not contain a cleavable signal peptide, it is predicted that both N-terminus and C-terminus would be localized to the cytoplasmic side of the secretory vesicle membrane (Perin et al. 1988). According to our membrane-spanning model of cytochrome b_{561} (Okuyama et al. 1998), most of the hydrophilic sequences of cytochrome b_{561} are on the cytoplasmic face of the vesicles, as has been suggested from biochemical experiments (Abbs and Phillips 1980). Indeed, we have shown that some parts of these cytosolic hydrophilic segments containing negatively-charged amino acid residues are responsible for the interaction with ascorbate (Tsubaki et al. 2000; Takeuchi et al. 2001) and the N-terminal Met residue is acetylated (Nakamura et al. 2003). The remaining C-terminal hydrophilic peptide (34 amino acid residue-long) is the longest part in cytochrome b_{561} exposed to the cytosolic surface but its physiological roles are still not well-understood. The most likely story is that this segment contains multiple routing signals (such as a targeting signal for the transit from Golgi to chromaffin vesicles and/or a retrieving signal from the plasma membrane to *trans* Golgi network), as has been identified for peptidiylglycine α-hydroxylating monooxygenase (Milgram et al. 1996). Experiments are currently in progress to identify proteins that may interact with this segment.

References

Abbs MT, Phillips JH (1980) Organisation of the proteins of the chromaffin granule membrane. Biochim Biophys Acta 595:200-221.

Ahn NG, Klinman JP (1987) Activation of dopamine β-monooxygenase by external and internal electron donors in resealed chromaffin granule ghosts. J Biol Chem 262:1485-1492.

Apps DK (1997) Membrane and soluble proteins of adrenal chromaffin granules. Seminar. Cell Develop Biol 8:121-131.

Claeys D, Geering K, Meyer BJ (2005) Two-dimensional Blue Native/sodium dodecyl sulfate gel electrophoresis for analysis of multimeric proteins in platelets. Electrophoresis 26:1189-1199.

Culvenor JG, Ilaya NT, Ryan MT, Canterford L, Hoke DE, Williamson NA, McLean CA, Masters CL, Evin G (2004) Characterization of presenilin complexes from mouse and human brain using Blue Native gel electrophoresis reveals high expression in embryonic and minimal change in complex mobility with pathogenic presenilin mutations. Eur J Biochem 271:375-385.

Grigoryan NA, Nalbandyan RM, Buniatian HC (1981) An extremely acidic copper-containing protein from chromaffin granules. Biochem Biophys Res Commun 100:921-928.

Markossian KA, Paitian NA, Mikaelyan MV, Nalbandyan RM (1986) On the physiological role of neurocuprein: Aponeurocuprein is an inhibitor of dopamine β-monooxygenase. Biochem Biophys Res Commun 138:1-8.

Milgram SL, Mains RE, Eipper BA (1996) Identification of routing determinants in the cytosolic domain of a secretory gramule-associated integral membrane protein. J Biol Chem 271:17526-17535.

Nakamura M, Takeuchi F, Tsubaki M (2003) Cytochrome b_{561} is not fatty acylated but acetylated at the amino terminus in the chromaffin vesicle membranes: An approach for identification of the posttranslational modification of transmembrane proteins. Protoplasma 221:41-46.

Njus D, Kelley PM (1993) The secretory-vesicle ascorbate-regenerating system: a chain of concerted H^+/e^--transfer reactions. Biochim Biophys Acta 1144:235-248.

Okuyama E, Yamamoto R, Ichikawa Y, Tsubaki M (1998) Structural basis for the electron transfer across the chromaffin vesicle membranes catalyzed by cytochrome b_{561}: Analyses of cDNA nucleotide sequences and visible absorption spectra. Biochim Biophys Acta 1383:269-278.

Perin MS, Fried VA, Slaughter CA, Südhof TC (1988) The structure of cytochrome b561, a secretory vesicle-specific electron transport protein. EMBO J 7:2697-2703.

Schägger H, Cramer WA, von Jagow G (1994) Analysis of molecular masses and oligomeric states of protein complexes by blue native electrophoresis and isolation of membrane protein complexes by two-dimensional native electrophoresis. Anal Biochem 217:220-230.

Schägger H, Pfeiffer K (2000) Supercomplexes in the respiratory chains of yeast and mammalian mitochondria. EMBO J 19:1777-1783.

Schägger H, von Jagow G (1987) Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Anal Biochem 166:368-379.

Schägger H, von Jagow G (1991) Blue native electrophoresis for isolation of membrane protein complexes in enzymatically active form. Anal Biochem 199:223-231.

Takeuchi F, Kobayashi K, Tagawa S, Tsubaki M (2001) Ascorbate inhibits the carbethoxylation of two histidyl and one tyrosyl residues indispensable for the transmembrane electron transfer reaction of cytochrome b_{561}. Biochemistry 40:4067-4076.

Tsubaki M, Kobayashi K, Ichise T, Takeuchi F, Tagawa S (2000) Diethylpyrocarbonate-modification abolishes fast electron accepting ability of cytochrome b_{561} from ascorbate but does not influence on electron donation to monodehydroascorbate radical: Distinct roles of two heme centers for electron transfer across the chromaffin vesicle membranes. Biochemistry 39:3276-3284.

Tsubaki M, Nakayama M, Okuyama E, Ichikawa Y, Hori H (1997) Existence of two heme B centers in cytochrome b_{561} from bovine adrenal chromaffin vesicles as revealed by a new purification procedure and EPR spectroscopy. J Biol Chem 272:23206-23210.

van Lis R, Atteia A, Mendoza-Hernández G, González-Halphen D (2003) Identification of novel mitochondiral protein components of *Chlamydomonas reinhardtii*. Apoteomic approach. Plant Physiol 132:318-330.

Wimalasena K, Wimalasena DS (1995) The reduction of membrane-bound dopamine β-monooxygenase in resealed chromaffin granule ghosts. Is intragranular ascorbic acid a mediator for extragranular reducing equivalents? J Biol Chem 270:27516-27524.

Winkler H, Apps DK, Fischer-Colbrie R (1986) The molecular function of adrenal chromaffin granules: Established facts and unresolved topics. Neuroscience 18:261-290.

Winkler H, Fischer-Colbrie R (1998) Regulation of the biosynthesis of large dense-core vesicles in chromaffin cells and neurons. Cell Mol Neurobiol 18:193-209.

Yoo SH, You SH, Huh YH (2005) Presence of syntaxin 1A in secretory granules of chromaffin cells and interaction with chromogranins A and B. FEBS Lett 579:222-228.