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Potential role of serotonin as a biological reductant associated with copper transportation

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ABSTRACT

Serotonin (5-HT) is a neurotransmitter that is derived from tryptophan. Owing to a hydroxyl group attached to the indole nucleus, 5-HT exhibits a considerably higher redox activity than tryptophan. To gain insight into the biological relevance of the redox activity of 5-HT, the effect of Cu(I)-binding ligands on the 5-HT-mediated copper reduction was investigated. The d-d transition band of Cu(II) complexed with glycine [Cu(II)-Gly_2] was not affected by addition of 5-HT alone but was diminished when a thioether-containing compound coexists with 5-HT. Concomitant with disappearance of the d-d transition band of Cu(II)-Gly_2, the π-π* transition band of 5-hydroxyindole of 5-HT exhibits a red-shift which is consistently explained by oxidation of 5-HT and subsequent formation of a dimeric species. The redox reactions between 5-HT and copper are also accelerated by a peptide composed of a methionine (Met)-rich region in the extracellular domain of an integral membrane protein, copper transporter 1 (Ctr1). Since Ctr1 transports copper across the plasma membrane with specificity for Cu(I), reduction of extracellular Cu(II) to Cu(I) is required for copper uptake by Ctr1. Metalloreductases that can donate Cu(I) for Ctr1 have been identified in yeast but not yet been found in mammals. The results of this study indicate that the Met-rich region in the N-terminal extracellular domain of Ctr1 promotes the 5-HT-mediated Cu(II) reduction in order to acquire Cu(I) via a non-enzymatic process.

1. Introduction

Serotonin (5-HT) is a neurotransmitter widely distributed in the central nervous system (CNS) and contributes to the regulation of a variety of psychological or neural functions including mood, aggression, cognition and memory [1,2]. In spite of its significance in various aspects of biological activity, 5-HT is also known to be highly susceptible to oxidation by reactive oxygen species or metal ions [3,4]. For example, a previous study has shown that 5-HT is oxidized to a quinoid compound, such as quinone imine, in the presence of copper ion [5]. The highly reactive quinone imine may be an intermediate in the oxidation pathway of 5-HT and dimerizes into 5,5′-dihydroxy-4,4′-bitryptamine (DHBT), which is comprised of two 5-HT molecules linked via a C—C bond at the C4 carbon atoms of the indole nucleus [4].

Several previous studies have indicated that oxidation of 5-HT leads to formation of compounds which are assumed to be unfavorable for neuronal survival. For example, an interaction between Cu(II) and 5-HT has a potential risk of toxicity to PC12 cells [4]. DHBT is one of the candidate products that may be involved in neurotoxicity, since central administration of DHBT to mice resulted in death [6]. Besides the possibility that the products of 5-HT oxidation have toxic effects on cells, an interaction between Cu(II) and 5-HT is likely to cause deficiency of functional 5-HT [4].

The oxidation of 5-HT is accelerated in the presence of Cu(II), indicating that the oxidation reaction can be coupled to the reduction of Cu(II) to Cu(I). Indeed, previous studies have shown evidence for production of Cu(I) from a mixture of 5-HT and Cu(II) by using a Cu(I)-specific chelator, bathocuproinedisulfonic acid (BC) [4,5]. The 5-HT mediated copper reduction can also be problematic since it will cause oxidative damage to cells.

Copper is an essential trace element that serves as a protein cofactor in fundamental redox reactions involving cellular respiration, free radical defense, neurotransmitter function, and iron metabolism [7–9]. Although the ability of copper to cycle between its oxidized Cu(II) and reduced Cu(I) forms is critical for the physiological functions, an out-of-control reaction may lead to production of highly reactive oxygen species [10,11]. If 5-HT reduces Cu(II) in the extracellular space, the transiently formed Cu(I) can be readily oxidized back to Cu(II) which is preferred by an oxidizing extracellular environment. Thus, it is reasonable to speculate that the recycle between Cu(II) and Cu(I) promoted by 5-HT has a cytotoxic potential. For example, a mixture of 5-HT and Cu(II) was found to generate hydroxyl radicals that cause

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oxidative cleavage of DNA strands [5].

Although estimation of copper levels in the brain is not simple owing to its uneven distribution, copper concentration in the extracellular space of brain tissue is considered to be higher than that in the cerebrospinal fluid. For example, copper concentration in the synaptic cleft was estimated to reach 100–300 μM during neuronal depolarization [12–14]. Thus, it is generally assumed that 5-HT has a significant chance to coexist with copper in the extracellular space of the brain. As described above, not only the reduction of Cu(II) but also the oxidation of 5-HT, either of which can be caused by the interaction between 5-HT and Cu(II), are potentially harmful for cells or organisms. A reasonable explanation why copper-susceptible 5-HT is used as the major neurotransmitter in the CNS has not yet been given. One possibility is that there is some defense system against the redox reactions involving 5-HT and Cu(II). Another possibility is that either the reduction of Cu(II) by 5-HT or oxidation of 5-HT by Cu(II) has some physiological role which is worth pursuing in spite of risks of potential harms.

Copper is largely present as Cu(II) in the oxidizing extracellular environment, whereas Cu(I) is employed in the intracellular copper trafficking by copper chaperones [15]. Therefore, the reduction of Cu(II) to Cu(I) in the extracellular space is required for cellular homeostasis on certain restricted occasions, such as copper entry via copper transporter 1 (Ctr1) with specificity for Cu(I). Human Ctr1 is an integral membrane protein that has an overall structure consisting of an extracellular N-terminus, three membrane-spanning helices and an intracellular C-terminus [16]. The N-terminal domain of Ctr1 contains methionine (Met)-rich sequences, which have been recognized as binding sites for Cu(I). The Met residues are considered to be involved in trapping soft Cu(I) ions by using the soft thioether ligands and guiding the Cu(I) to the transmembrane pathway [17–19].

A search for the source of Cu(I) for Ctr1 has long been an important topic in copper homeostasis research. In budding yeast *Saccharomyces cerevisiae*, cell surface Fe(III)/Cu(II) reductases, Fre1 and Fre2, are known to reduce extracellular Cu(II) to Cu(I) prior to the transport across membranes by Ctr1 [20,21]. Based on our recent study, Ctr4 of fission yeast *Schizosaccharomyces pombe* has a cysteine and tryptophan-rich region which has a Cu(II) reductase activity and could act as a putative Cu(I) donor to the Met-rich motifs in the N-terminal extracellular domain [22]. In mammalian cells, several plasma membrane associated proteins such as prion protein and amyloid β-protein precursor have been shown to have copper reducing activity [23,24]. Although several lines of evidence suggest that a source of Cu(I) is required also for the copper transport activity of mammalian Ctr1 [25], participation of these membrane associated proteins in copper transportation is still unclear.

In the present study, we have examined a possibility that 5-HT-mediated Cu(II) reduction has relevance to copper uptake by Ctr1. The Cu(II)-reducing activity of 5-HT is dramatically enhanced in the presence of either a thioether-containing compound or a synthetic peptide composed of the Met-rich region in the N-terminal extracellular domain of Ctr1. The obtained results suggest that 5-HT is capable of reducing Cu(II) in the extracellular space under a specific condition when a Cu(I)-acceptor, such as Ctr1, coexists. The 5-HT-mediated Cu(II) reduction requires assistance of Ctr1, which may confer target selectivity on 5-HT as a Cu(I) donor.

2. Materials and methods

2.1. Materials

5-Hydroxytryptamine hydrochloride and bathocuproinedisulfonic acid (BC) were purchased from Wako Pure Chemical Corporation (Osaka, Japan). Copper(I) chloride, L-ascorbic acid, glycine (Gly), L-tryptophan (L-Trp) and 5-hydroxy-L-tryptophan (5-HW) were supplied by Nacalai Tesque Co. (Kyoto, Japan). Dithioether-containing compounds, 3,6-dithia-1,8-octanediol (3,6-DT) and 3,7-dithia-1,9-nonanediol (3,7-DT), are from Sigma–Aldrich (St. Louis, MO, USA). A pentapeptide corresponding to Met-rich M1 region of Ctr1, MGMSYMD (M1), and its mutant peptide AGASYAD [M1(M/A)] were synthesized by GenScript Corporation (Piscataway, NJ, USA) at ≥95% purity and certified by mass spectrometry and HPLC. Both M1 and M1(M/A) peptides have an acetylated N-terminal amino group.

2.2. Cu(II) reduction assay using bathocuproine

The Cu(II) reduction by 5-HT was assayed with a Cu(I)-specific chelator, BC. BC and Cu(I) form a 1:2 metal-ligand complex that exhibits a visible absorption band at 483 nm [26]. The concentration of Cu(I) was calculated by using the molar extinction coefficient of the Cu(I)-BC_2 complex, $\varepsilon_{483} = 12{,}250\ M^{-1}\ cm^{-1}$ [27]. A pH 7.0 phosphate buffer containing 5-HT, BC and NaCl was mixed with Cu(II) directly in a 5 cm-path length quartz optical cell and then a time-dependent increase in absorbance at 483 nm was monitored on Hitachi U-1800 spectrophotometer over a period of 600 s at 22 °C. The reaction mixture contains 0.4 μM 5-HT, 16 μM BC, 4 μM copper, 5 mM phosphate and 150 mM NaCl. Cu(II)-Gly 1:2 complex [Cu(II)-Gly_2] was employed as a source of Cu(II) to minimize the formation of insoluble metal-hydroxy and metal-oxy polymers at neutral pH [12,28]. The Cu(II) reduction assay was also performed on L-Trp and 5-HW.

2.3. Cu(II) reduction assay using visible absorption of Cu(II) complex

The effect of thioether-containing compounds on the 5-HT-mediated reduction of Cu(II) to Cu(I) was investigated by monitoring intensity decrease of the d-d transition band of Cu(II)-Gly_2 in visible absorption spectra. The 3d orbital of Cu(I) is filled with 10 electrons and thus the d-d transition is not expected to occur. A pH 7.0 phosphate buffer containing 5-HT, dithioether (3,6-DT or 3,7-DT), and NaCl was mixed with Cu(II)-Gly_2 and then visible absorption spectrum was measured on Hitachi U-2910 spectrophotometer using a 5 cm-path length quartz optical cell. Each sample contains 0.2 mM 5-HT, 0.1 mM Cu(II)-Gly_2, 1 mM phosphate, 150 mM NaCl and 0–1.0 mM dithioether. The concentration of 5-HT was determined from the UV absorption intensity at 308 nm ($\varepsilon_{308} = 3010\ M^{-1}\ cm^{-1}$).

2.4. Cu(II)-mediated oxidation of 5-HT

The effect of thioether-containing compounds on the Cu(II)-catalyzed oxidation of 5-HT was investigated using UV absorption of 5-HT. The solution condition for the UV absorption measurement was the same as that for the visible absorption (Section 2.3), except that a 1 cm-path length cell was employed instead of a 5-cm cell. The Cu(II)-mediated oxidation of 5-HT was also investigated in the presence of M1 peptide and its mutant. Peptide concentrations were calculated using $\varepsilon_{275} = 1410\ M^{-1}\ cm^{-1}$.

2.5. Electrochemical measurements

The voltammetry experiments were performed using a BAS ALS 1200A electrochemical analyzer. A three-electrode system including the working electrode, a platinum wire counter electrode and an Ag/AgCl reference electrode (RE-1B, BAS) was employed. A basal plane pyrolytic graphite electrode (3.0 mm diameter, BAS) was used as the working electrode. All potentials were reported with respect to Ag/AgCl. The electrolyte solution was a 10 mM phosphate buffer (pH 7.0) containing 100 mM Na_2SO_4. For the purpose of investigating the effect of thioether-containing compounds on the oxidation potential of Cu(I), an electrolyte solution containing 1 mM Cu(II)-Gly_2 and the desired concentration of 3,6-DT, 3,7-DT or M1 peptide was prepared. Cyclic voltammetry was performed in the range between 0.95 and −0.4 V. The data were recorded with linear potential scans at 100 mV s^{-1}, first in the negative direction from 0.95 V and then in the reverse direction. For

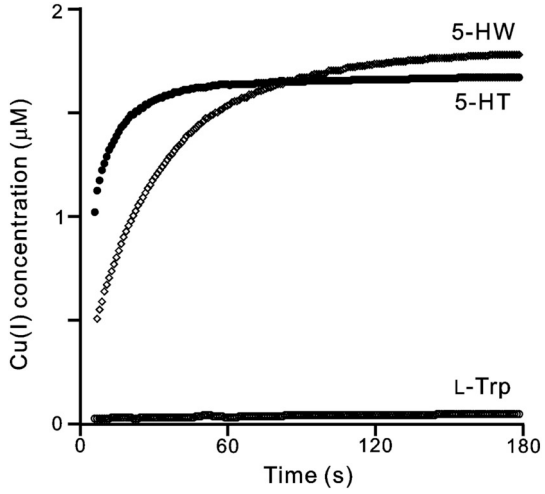

Fig. 1. Time courses of Cu(I) production in the presence of 5-HT, 5-HW and L-Trp, monitored as the increase of visible absorption by the Cu(I)-BC_2 complex. The reaction mixture was prepared containing 0.4 μM 5-HT (5-HW or L-Trp), 16 μM BC, 4 μM Cu(II)-Gly_2, 5 mM phosphate (pH 7.0) and 150 mM NaCl. The absorbance readings at 483 nm were recorded for 180 s at 1-s intervals. Each data point represents the average of three experiments.

the purpose of measuring the oxidation potential of 5-HT, the potential was scanned in the positive direction first.

3. Results

3.1. Cu(II) reduction by 5-HT

Cu(I) production by 5-HT-mediated Cu(II) reduction has been examined using an assay based on a Cu(I)-specific chelator, BC. The amount of Cu(I) formed in a reaction mixture was estimated based on visible light absorption by a characteristic orange-colored Cu(I)-BC_2 complex [27]. A concentration–time curve in Fig. 1 shows that a steep increase in concentration of Cu(I) occurs immediately after spiking Cu (II) into a phosphate buffered solution containing 5-HT and BC. The Cu (I) concentration quickly reached a plateau in spite of a low concentration of 5-HT (0.4 μM) compared to that of copper (4 μM) in the experimental condition of this assay. In order to elucidate the structural requirements for the Cu(II)-reducing ability of 5-HT, the Cu(I) production was also examined in the presence of the biological precursors of 5-HT. Although 5-HW has a comparable copper reducing activity as 5-HT, L-Trp reduces Cu(II) only to a very small extent. These results indicate that the presence of a hydroxy group on the indole nucleus is critical for the Cu(II) reduction by 5-HT.

The results obtained from the BC-based assay provide evidence that 5-HT has an ability to reduce Cu(II) to Cu(I). It is important to note that BC is not only a Cu(I) indicator but also a Cu(I)-specific chelator. Such a chelator, which sequesters Cu(I) from the reaction system, is likely to play a promoting role in the 5-HT-mediated Cu(II) reduction. Therefore, an alternative reduction assay was employed to examine the reducing ability of 5-HT in the absence of a Cu(I) chelator. Fig. 2 shows visible absorption spectra of Cu(II)-Gly_2 in the d-d transition region before and after addition of a biological reducing agent, ascorbic acid or 5-HT, at various concentrations. The d-d transition band of Cu(II)-Gly_2 appears as a weak broad band at 690 nm. This band was significantly diminished on addition of ascorbic acid (Fig. 2A). The disappearance of the d-d transition band is ascribed to reduction of Cu(II) to Cu(I) with fully occupied 3d orbitals. On the other hand, the d-d transition band remains unchanged at least in a short time interval of about 10 min (Fig. 2B), although spectral changes that are ascribed to formation of oxidative products of 5-HT were observed by acquiring the data over an extended period of time (Fig. S1). This indicates that 5-HT alone does

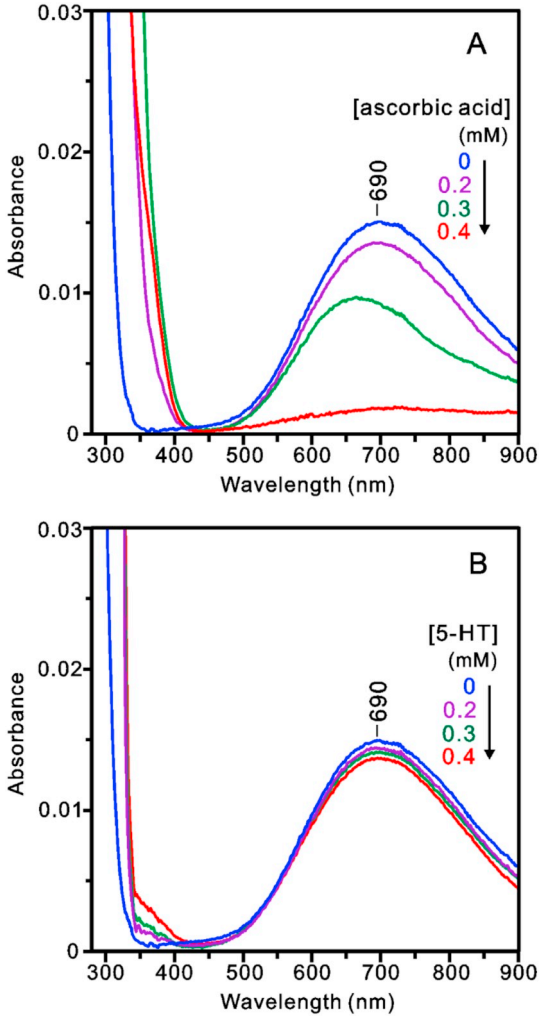

Fig. 2. Visible absorption spectra of Cu(II)-Gly_2 (0.1 mM) in the presence of various concentrations (0, 0.2, 0.3 and 0.4 mM) of reductants, ascorbic acid (A) and 5-HT (B). The solution was prepared with 5 mM phosphate buffer (pH 7.0) containing 150 mM NaCl. The spectrum was measured 5 min after the sample preparation. A broad band at 690 nm is ascribed to the d-d transition of Cu(II)-Gly_2.

not have a significant ability to reduce Cu(II). A chelator that stabilizes Cu(I) may be required for the 5-HT-mediated copper reduction.

3.2. Effect of thioether compounds on redox reactions between 5-HT and Cu (II)

Next, we investigated whether a thioether-containing compound is able to assist the 5-HT-mediated copper reduction. 3,6-DT with two thioether sulfur atoms was used as a potential Cu(I) chelator which mimics the Met-rich Cu(I) binding site of Ctr1. A high solubility in aqueous media is another reason why this compound was chosen. Fig. 3 shows UV–visible absorption spectra of a mixture of 5-HT and Cu(II)-Gly_2 in the absence and presence of 3,6-DT at various concentrations. To observe the d-d transition band of Cu(II)-Gly_2 with a small extinction coefficient (ca. 30 M^{-1} cm^{-1} at λ_{max}), a cell of 5-cm path length was used. The d-d transition band of Cu(II)-Gly_2 is observed at 690 nm as a broad band in the spectrum obtained in the absence of 3,6-DT. As was shown in Fig. 2B, the d-d transition band is diminished to only a small degree on addition of 5-HT in the absence of the Cu(I) chelator. However, the d-d transition band is reduced in intensity on addition of 3,6-DT in a concentration dependent manner (Fig. 3B). The 3,6-DT-induced intensity decrease of the d-d transition of Cu(II) did not occur in the

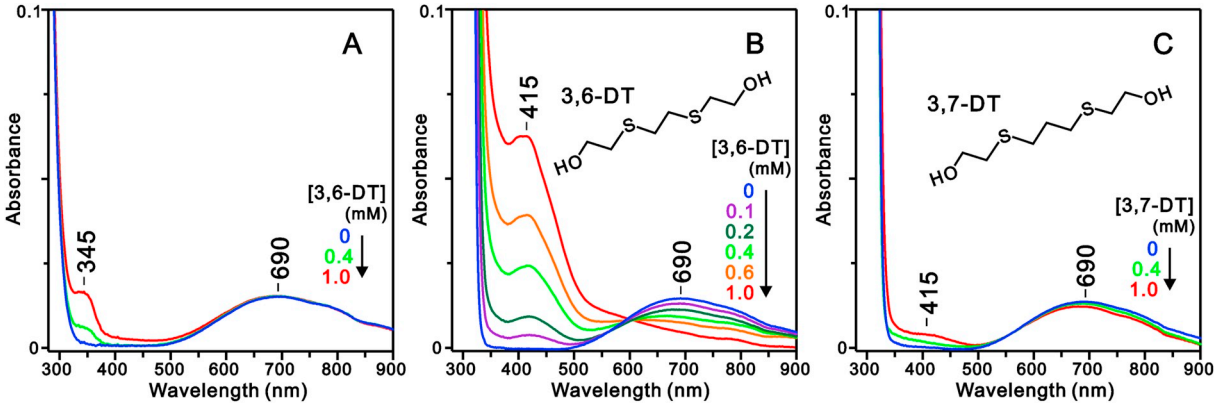

Fig. 3. Visible absorption spectra of Cu(II)-Gly_2 (0.1 mM) at various concentrations of thioether compound: at 0, 0.4 and 1.0 mM 3,6-DT in the absence of 5-HT (A); at 0, 0.1, 0.2, 0.4, 0.6 and 1.0 mM 3,6-DT in the presence of 0.2 mM 5-HT (B); and at 0, 0.4 and 1.0 mM 3,7-DT in the presence of 0.2 mM 5-HT (C). The molecular structures of 3,6-DT and 3,7-DT are shown as insets in B and C, respectively.

absence of 5-HT (Fig. 3A). Accordingly, the concentration-dependent change in the d-d transition intensity seen in Fig. 3B is attributable to a promoting effect of 3,6-DT on the 5-HT-mediated copper reduction. A visible absorption band at 415 nm with a broad shoulder around 600 nm, which increases in absorbance on the addition of 3,6-DT, may be attributable to compounds generated by oxidation of 5-HT. A weak 345-nm band in Fig. 3A is most probably due to the charge transfer transition in a Cu(II)-3,6-DT complex.

The copper reduction by 5-HT was also examined in the presence of another open-chain dithioether compound, 3,7-DT. In sharp contrast to the significant promoting effect of 3,6-DT, an intensity decrease of the d-d transition band on the addition of 3,7-DT is negligibly small (Fig. 3C). The dithioether compounds examined here have different number of methylene groups intervening between two thioether sulfur atoms. 3,6-DT, which is capable of forming a five-membered chelate ring, may have a greater stabilizing effect on Cu(I) [29].

To further understand the role of 3,6-DT in the redox reaction between 5-HT and copper, oxidation of 5-HT was investigated by measuring UV absorption of 5-HT. UV absorption spectrum of 5-HT in the presence of Cu(II)-Gly_2 is shown in Fig. 4A. The π-π* transition of 5-hydroxyindole group of 5-HT is observed as a band peaked at 274 nm with a 296-nm shoulder. The short-wavelength side of this band is partly overlapped by a weaker band around 230 nm due to Cu(II)-Gly_2. The spectrum of a solution consisting of a mixture of 5-HT and Cu(II)-Gly_2 can be quite well reproduced by the sum of the spectra of 5-HT alone and Cu(II)-Gly_2 alone (Fig. S2). Although copper is known to have a catalytic effect on the oxidation of 5-HT, the reaction may be suppressed by Gly that was added to stabilize Cu(II) under our experimental conditions [4]. A significant spectral change, which is characterized by a red-shift of the 296-nm shoulder peak, is induced by addition of 3,6-DT to 5-HT in the presence of Cu(II)-Gly_2 (Fig. 4A). This spectral change was completed immediately after the addition of 3,6-DT, and there was little further change over a 120-min period (Fig. 4A). Since 3,6-DT does not exhibit UV absorption in the wavelength region shown in Fig. 4, the red-shift is ascribed to a structural modification of the 5-hydroxyindole moiety, most likely due to oxidation of 5-HT. It has been confirmed that 3,6-DT does not induce a red-shift of the absorption band of 5-HT in the absence of Cu(II)-Gly_2 (not shown).

The oxidative pathways of 5-HT have been studied by previous investigators [4,5,30]. A quinone imine is considered to be a product at the initial step of the 5-HT oxidation, but highly reactive quinone imine may be soon converted to more stable species. A main product of copper-catalyzed oxidation of 5-HT has been identified by NMR and mass spectrometry as DHBT, which is comprised of two 5-HT molecules linked via a C−C bond at the C4 carbon atoms of the indole [4]. The UV absorption spectrum of DHBT, which has been obtained in an early work, shows two peaks around 300 nm in the region of L_a and L_b electronic transitions of indole ring [30]. Although there appear to be no drastic difference between the spectra of monomeric and dimeric 5-HT, the lower wavelength peak of DHBT is red-shifted by about 10 nm compared to that of 5-HT. The difference in absorbance at the two peaks becomes smaller on the dimer formation. A spectrum very similar to that of DHBT can be obtained by subtracting the spectrum of 5-HT before addition of 3,6-DT from that after addition of 3,6-DT using an appropriate subtraction factor to cancel the contribution from unoxidized 5-HT (Fig. S3). This indicates that the spectrum obtained after addition of 3,6-DT in Fig. 4A is contributed by both DHBT and unoxidized 5-HT. DHBT is considered to be the main product of the redox reaction between 5-HT and Cu(II) also in the case of reaction in which 3,6-DT acts as a promoter.

Fig. 4B shows 3,6-DT concentration dependence of UV absorption spectrum of 5-HT at constant concentrations of 5-HT and Cu(II)-Gly_2. The spectra of 3,6-DT-containing samples were measured within 5 min after addition of 3,6-DT. The 3,6-DT-induced red-shift of the 296-nm shoulder band, which is attributable to the dimer formation of 5-HT, occurs in a concentration-dependent manner. This spectral change is more clearly seen in the difference spectra (Fig. 4C). A positive peak appears at 315 nm in the difference spectra, which were obtained by subtraction of the spectrum of 5-HT before addition of 3,6-DT from those after addition of 3,6-DT, and increases in intensity with increasing 3,6-DT concentration. The spectra shown in Fig. 4B were measured under the same experimental conditions as those in Fig. 3B, except for the difference in the cell path length. Comparison of the 3,6-DT induced spectral changes in UV (Fig. 4B) and visible (Fig. 3B) regions demonstrates that the red-shift of the π-π* transition of 5-HT occurs in concert with the disappearance of the d-d transition band of Cu(II). Based on these results, it can be concluded that the 3,6-DT-promoted oxidation of 5-HT is coupled to the reduction of Cu(II) to Cu(I).

Addition of 3,7-DT to a mixture of 5-HT and Cu(II)-Gly_2 induces a much smaller spectral change of 5-HT (Fig. 4D) than that of 3,6-DT (Fig. 4C), indicating that 3,7-DT is less effective in promoting the redox reactions between 5-HT and Cu(II) compared to 3,6-DT. This result is consistent with that obtained from visible absorption spectra in the d-d transition region (Figs. 3B and C).

Next we examined the promoting effect of a pentapeptide corresponding to M1 region of Ctr1 (MGMSYMD) on the redox reactions between 5-HT and copper. This peptide has an amino acid sequence of the typical Met-rich motif MXMXXM (X is any other amino acid), which is assumed to act as the Cu(I) binding site in the extracellular N-terminal domain of Ctr family of proteins [31,32]. Following the above method for the dithioether compounds, UV absorption spectrum of 5-HT was measured after addition of M1 peptide in the presence of Cu(II)-Gly_2. Fig. 5A shows difference spectra between the spectra of 5-HT after and before addition of M1. Since M1 contains a tyrosine (Tyr) residue,

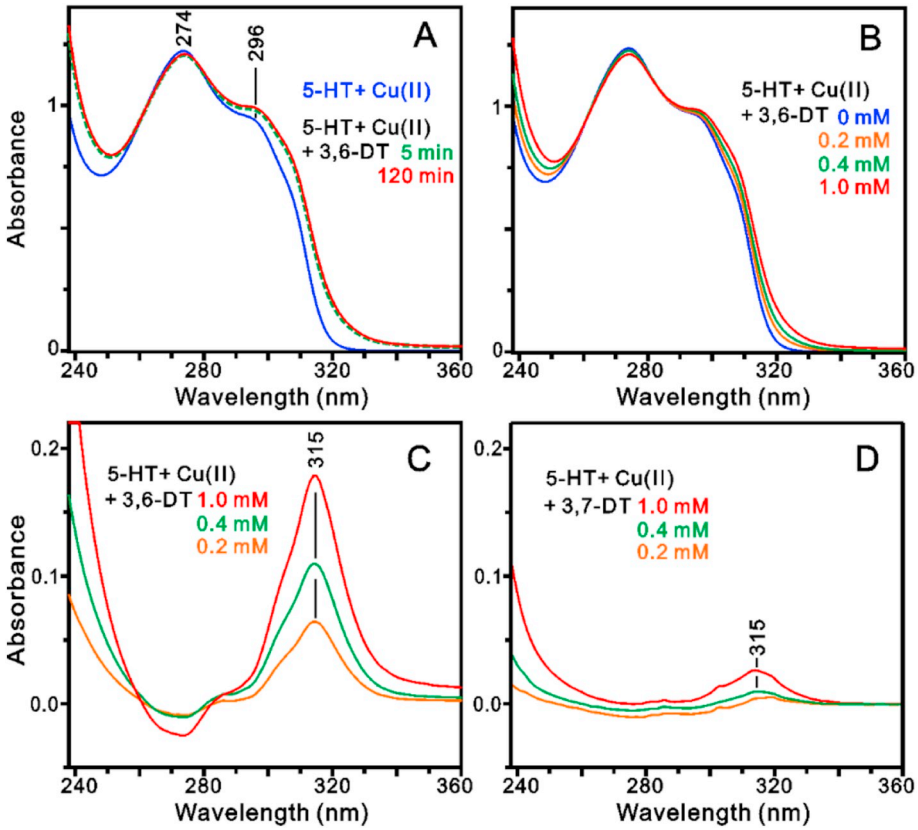

Fig. 4. UV absorption spectra of 0.2 mM 5-HT in the presence of 0.1 mM Cu(II)-Gly_2 and various concentrations of thioether compound. (A) Spectra of 5-HT before addition (blue) and 5 min (green, dashed) and 120 min (red) after addition of 3,6-DT. (B) Spectra of 5-HT at 0 (blue), 0.2 (orange), 0.4 (green) and 1.0 mM (red) 3,6-DT. (C) Difference spectra of 5-HT between after addition and before addition of 3,6-DT shown in panel B [0.2 mM – 0 mM (orange), 0.4 mM – 0 mM (green) and 1.0 mM – 0 mM (red)]. (D) Difference spectra of 5-HT between after addition and before addition of 3,7-DT [0.2 mM – 0 mM (orange), 0.4 mM – 0 mM (green) and 1.0 mM – 0 mM (red)]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the π-π* transition band of the phenol side chain of Tyr appears as a strong positive band at 274 nm in the difference spectra. A weaker additional peak at 315 nm is attributable to the spectral difference between DHBT and 5-HT, corresponding to the positive peak in Fig. 4C and D. The 315-nm peak increases in intensity with increasing M1 concentration, as in the case of 3,6-DT. In contrast, this peak is missing in the difference spectrum of a mutant peptide of M1, in which all Met residues are replaced by alanine [M1(M/A), Fig. 5B]. These results demonstrate that M1 peptide is able to accelerate the redox reactions between 5-HT and copper, and moreover, that the Met residues play a key role in promoting the reactions.

3.3. Effect of thioether compounds on oxidation potential of Cu(I)

Based on the results of previous sections, a peptide consisting of the Met-rich motif of Ctr1, as well as its model compound, has an ability to promote the redox reactions between 5-HT and copper. To gain insight into the underlying mechanism of the promotion of the redox reactions, electrochemical properties of the copper complexes were investigated by means of cyclic voltammetry (Fig. 6). The voltammetric experiments were performed at a neutral pH as in the case of the spectroscopic measurements in the present study. The uncomplexed copper ion is prone to form insoluble oxide/hydroxide species in neutral solution. In order to avoid formation of copper precipitates, which could complicate the interpretation of the voltammogram, copper was added as Cu(II)-Gly_2 [12,28]. The background voltammogram of electrolyte buffer (10 mM Na_2HPO_4/NaH_2PO_4, 100 mM Na_2SO_4) is shown as dotted lines in Fig. 6A. The voltammogram of 1 mM of Cu(II)-Gly_2 before addition of the dithioether compounds exhibits a reduction peak and an oxidation peak at −0.28 V and 0.15 V vs. Ag/AgCl reference, respectively (Fig. 6A). The −0.28 V peak can be attributed to the reduction of Cu(II) that is stabilized by the coordination of Gly ligands. The peak current decreases on addition of 3,6-DT, and concomitantly, a new reduction peak appears at around 0.05 V. The oxidation peak shifts to higher potentials with increased concentration of 3,6-DT, 0.39 and 0.56 V at 2 and 4-fold molar excess over copper ion, respectively (Fig. 6A). This

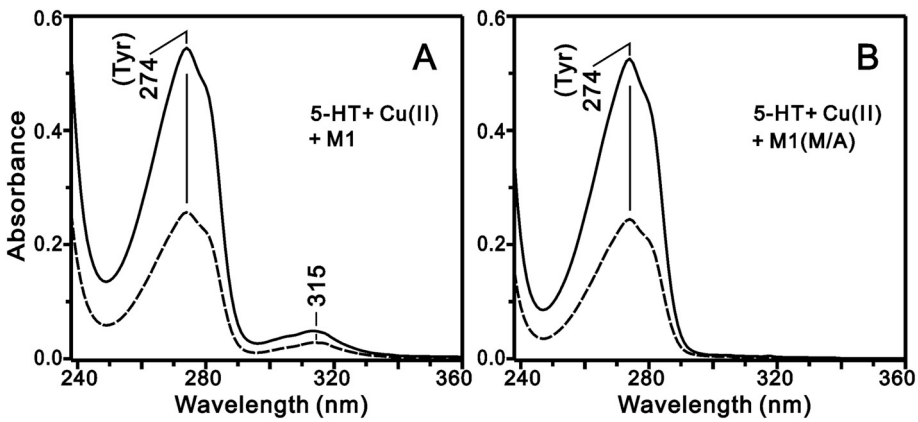

Fig. 5. UV absorption difference spectra of 5-HT between after addition and before addition of M1 peptide, wild type M1 (A) and mutant peptide M1(M/A) (B). Difference spectra 0.2 mM – 0 mM (dashed trace) and 0.4 mM – 0 mM (solid trace) are shown. The solution was prepared containing 0.2 mM 5-HT, 0.1 mM Cu(II)-Gly_2, 1 mM phosphate (pH 7.0), 150 mM NaCl and the desired concentration of peptide. A band at 274 nm is due to Tyr residue of the peptides.

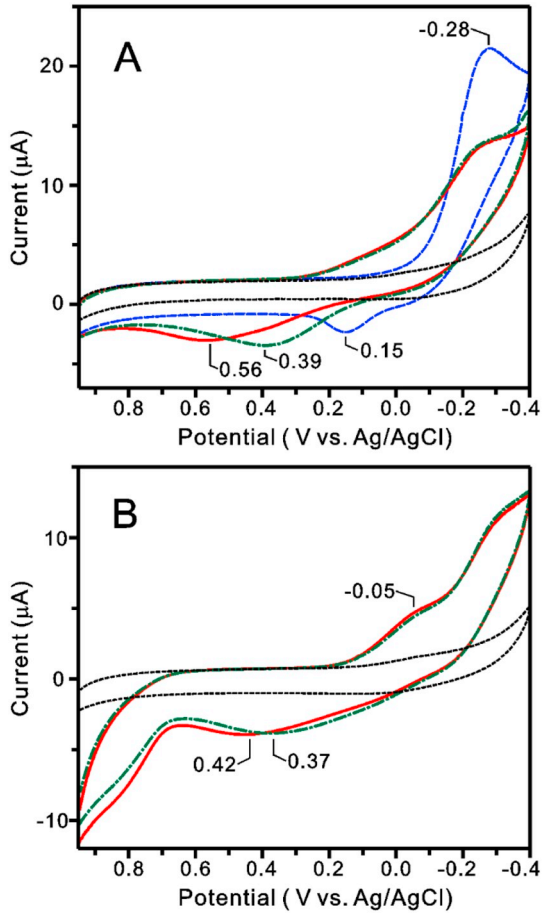

Fig. 6. Cyclic voltammograms of 1 mM Cu(II)-Gly_2 in the absence (blue dashed trace) and presence of 2 mM (green dash-dot-dash trace) and 4 mM (red solid trace) of 3,6-DT (A) and M1 peptide (B). All solutions were prepared with 10 mM phosphate buffer (pH 7.0) containing 100 mM Na_2SO_4. The background cyclic voltammogram is also shown (black dotted trace). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

demonstrates that 3,6-DT stabilizes the Cu(I) oxidation state. On the other hand, 3,7-DT, that is less effective in promoting the redox reactions between 5-HT and Cu(II) (Figs. 3 and 4), was not able to induce significant shift of the oxidation potential of Cu(I) at a 2-fold molar excess over copper ion (Fig. S4). The oxidation peak undergoes a small shift to 0.22 V even in the presence of a 4-fold excess of 3,7-DT.

Since a significant shift of the peak potential on increasing the 3,6-DT concentration from 2 to 4 mM occurs for the oxidation peak but not for the reduction peak, the peak-to-peak difference between oxidation and reduction potentials becomes larger especially at a higher 3,6-DT concentration. The solution contains Gly, phosphate and sulfate, in addition to copper and 3,6-DT. There is a considerable difference in preferred ligand species and/or coordination geometries between complexes of copper ions in the different oxidation states, Cu(II) and Cu(I) [19,33]. Cu(I) is expected to be mainly coordinated by thioether sulfur atoms, but contribution of thioethers to a Cu(II) complex may be secondary to Gly or phosphate. This is the reason why the stabilizing effect of thioethers on the Cu(I) oxidation state is estimated by using the oxidation potential but not the reduction–oxidation potential.

The effect of the Met-rich motif of Ctr1 on the oxidation potential of Cu(I) was examined by using M1 peptide (Fig. 6B). Similarly to 3,6-DT, the addition of M1 to Cu(II)-Gly_2 raises the oxidation potential to 0.37 and 0.42 V at 2 and 4-fold molar excess of M1 over copper ion, respectively. In sharp contrast, the mutant peptide M1(M/A), in which all Met residues of M1 are replaced by alanine, induces a downward shift of the oxidation potential to around 0 V (Fig. S4). These results demonstrate that the thioether side chain of Met residues play a critical role in stabilizing the Cu(I) oxidation state by M1 peptide.

We have also performed cyclic voltammetry of 5-HT itself in the same electrolyte buffer that was used for the copper/thioethers and copper/peptide systems mentioned above. When the scan is initiated in the negative direction, the cyclic voltammogram of 5-HT exhibits one irreversible oxidation peak at 0.38 V vs. Ag/AgCl (Fig. S5). 5-HT is incapable of reducing Cu(II) to Cu(I), when the redox potential of copper is lower than that of 5-HT. Although the reduction potentials of copper that is coordinated by thioethers are unclear, significant effects of 3,6-DT and M1 peptide on raising the oxidation potential of copper is consistent with that the thioether-containing compounds have a promoting effect on the redox reactions between 5-HT and Cu(II).

In addition to the oxidation peak around 0.4 V, the voltammogram of M1 exhibits another peak at around 0.8 V (Fig. 6B). The 0.4 V peak appears when both M1 peptide and Cu(II)-Gly_2 are present, while the 0.8 V peak is observed for M1 peptide even in the absence of copper (Fig. S4). In previous studies, a similar peak was also found in a voltammogram of a Tyr-containing peptide or Tyr alone, and has been attributed to the oxidation of the phenol side chain of Tyr [34,35].

4. Discussion

In the present study, copper reducing activity of 5-HT has been examined by using three different approaches based on the appearance of absorption band of the Cu(I)-BC_2 complex, disappearance of the d-d absorption band of Cu(II), and change in UV absorption due to the 5-HT oxidation. No significant progress of the redox reactions could be detected when the solution contains only 5-HT and Cu(II)-Gly_2. However, both the 5-HT-mediated reduction of Cu(II) and the Cu(II)-mediated oxidation of 5-HT are drastically accelerated by Cu(I) chelators such as BC and a dithioether compound. These results demonstrate that 5-HT is a Cu(II) reducing agent that requires a Cu(I)-binding ligand for its activation. If the 5-HT-mediated copper reduction has some physiological significance, the extracellular N-terminal domain of Ctr1 may be a potential candidate for such a Cu(I)-binding ligand.

Cu(I) has a higher polarizability than Cu(II) and thus preferentially complexes with soft sulfur-containing ligands, such as the cysteine (Cys) thiolate and the Met thioether. Proteins that are involved in copper sensing or trafficking in the reducing intracellular compartments mostly utilize Cys thiolates as ligands for Cu(I). In contrast, Met-rich Cu(I) binding sites are found in a more oxidizing environment including the periplasm of prokaryotic organisms and the extracellular space of eukaryotic cells [19,33]. The Met thioether group has a lower affinity for Cu(I) than Cys thiolate mainly due to lack of the electrostatic character of the Met-Cu(I) interaction [33,36]. However, Met is redox stable compared with Cys that has a propensity to form disulfide bond under an oxidizing condition. Presumably, for this reason, Met thioether is preferably used as the ligand for Cu(I) in the extracellular space [19].

Ctr family copper transporters are ubiquitously expressed in eukaryotes from yeast to mammals [31,37,38]. The N-terminal extracellular domain of human Ctr1 contains multiple closely spaced Met-rich motifs, MXXM and MXM (where X is any amino acid), which are proposed to provide binding sites for Cu(I) and to guide it to the membrane permeation pathway [31,32]. The Cu(I)-specific transportation by Ctr1 seems to be reasonable because Cu(I) is employed for copper trafficking in the intracellular compartment [15]. In the cytoplasm of mammalian cells, cysteine containing tripeptide glutathione exists at concentrations in the millimolar range [39]. The reduced thiol form of glutathione is predominant over the oxidized dimer and maintains a strong reducing environment in the cytoplasm. Glutathione is also important in stabilizing Cu(I) by the thiolate ligands. After the uptake from the extracellular space, the Cu(I) is considered to be initially bound by glutathione and then delivered to copper chaperones

[40]. In contrast, glutathione is present in the extracellular medium at much lower concentration (micromolar order) mainly as the oxidized form which is not available for the Cu(I) ligation [41]. Owing to a very different environment between the extracellular and intracellular spaces for the copper ion, the reduction of the extracellular Cu(II) to Cu (I) may be required for the transmembrane trafficking of copper.

In fission yeast *Schizosaccharomyces pombe*, copper uptake is carried out by a heteroprotein complex comprised of Ctr4 and Ctr5 [42,43]. Both of the copper transporters have a quasi-palindromic sequence rich in cysteine and aromatic amino acids termed "Cys/Trp motif", $CX_4YWNWYX_4C$ (where X is any amino acid), in the N-terminal extracellular domain in addition to several Met-rich motifs [37]. We have shown in previous studies that a peptide comprising Cys/Trp motif is capable of reducing Cu(II) to Cu(I) upon disulfide bond formation by the two cysteine residues [22,44]. The resulting Cu(I) is stabilized by the unoxidized peptide through both the cysteine thiolate coordination and the cation-π interaction with tryptophan side chains. These results suggest a possibility that the N-terminal extracellular domain of Ctr4 and/or Ctr5 acts as not only a Cu(I) acceptor but also a Cu(II) reductase. On the other hand, copper is transported into cells by Ctr1 and Ctr3 in budding yeast *Saccharomyces cerevisiae* [,[17,45]]. Ctr3 has a Cys/Trp motif in the N-terminal extracellular domain. Yeast Ctr1 lacks Cys/Trp motif but has multiple repeats of Met-rich motifs containing a total of 30 Met residues. Since thioether of Met is redox-inactive, metalloreductases that convert Cu(II) to Cu(I) is essential for the copper uptake by Ctr1. Plasma membrane metalloreductases, Fre1 and Fre2, are known to be responsible for the Ctr1-mediated copper uptake in *Saccharomyces cerevisiae* [46,47]. Although human Ctr1 also has thioether-only binding sites for Cu(I) in the N-terminal extracellular domain, membrane associated metalloreductases corresponding to Fre1 and Fre2 of yeast have not yet been found in human and other mammals.

If one supposes that 5-HT is involved in the Cu(I) donation to Ctr1, 5-HT may have advantages over plasma membrane metalloreductases from the viewpoint of efficient Cu(I) transport in the extracellular space. Cu(I) is poorly soluble in aqueous medium at neutral to basic pH unless the ion is stabilized by ligand binding. In the case of yeast, membrane associated proteins, Fre1 and Fre2, produce Cu(I) and then the Met-rich region of Ctr1 may capture the Cu(I). Even if Fre1 and Fre2 reduce Cu(II) independently of Ctr1, the resulting Cu(I) is expected to remain soluble in the extracellular medium, since yeast acidify their environment to pH 4–5 [48]. Cu(I) may be unstable in neutral and oxidizing extracellular space in the mammalian CNS. However, Cu(I) is expected to be efficiently delivered to Ctr1 if the reduction of Cu(II) to Cu(I) is triggered by Ctr1 itself, like the 5-HT-mediated copper reduction.

Non-enzymatic biological reductants that are able to reduce Cu(II) to Cu(I), including ascorbic acid, could be potential candidates for the Cu(I) donor involved in the copper-uptake machinery [49]. However, we speculate that the use of 5-HT as an extracellular copper reductant has a merit from the viewpoints of prevention of copper-induced toxicity. Reduction of Cu(II) to Cu(I) in the oxidizing extracellular space is potentially risky, because Cu(I) can react with H_2O_2 to generate highly reactive hydroxyl radical via Fenton reaction. Thus, basically all biological reductants have a potential to promote oxidative stress [41]. For example, ascorbic acid is known to have various antioxidant properties such as scavenging of reactive oxygen species. However, ascorbic acid also behaves as a strong prooxidant under conditions which allow it to reduce Cu(II) or Fe(III) in the extracellular medium [41]. Although 5-HT could be another extracellular reducing agent, the reducing ability is distinct from that of ascorbic acid which reduces Cu(II) even in the absence of a Cu(I) acceptor (Fig. 2). The copper reduction, which is activated only in the presence of a Cu(I) acceptor, is expected to minimize toxic effects of copper. In the case that Met-rich motif of Ctr1 acts as the Cu(I) acceptor, the Cu(I) will be sequestrated from the extracellular medium and then stabilized by intracellular glutathione or copper chaperones. Based on these considerations, 5-HT is supposed to be an ideal reductant for promotion of the copper uptake via Ctr1.

Ctr1 is ubiquitously distributed in mammalian brain [50,51]. The importance of Ctr1 as a copper transporter in brain has been demonstrated by a previous study using Ctr1 gene knock-out mice, which showed about 50% reduction in total copper level in brain of mice heterozygous for *Ctr1* (*Ctr1*$^{+/-}$) [52]. Therefore, if extracellular reducing agents instead of plasma membrane metalloreductases provide Cu (I) for Ctr1, the reducing agents must be widely distributed in brain. Cell bodies of 5-HT-containing neurons located in the brain stem has branches which ramify extensively and project throughout the CNS. A single cell possesses a large number of varicosities in the order of 10^5 [53,54]. The majority of these varicosities make no synaptic contacts and may release 5-HT directly into the extrasynaptic space [55]. These previous findings suggest that 5-HT is widely distributed in the brain and plays some regulatory role in the CNS, in addition to acting as a neurotransmitter [56].

Since copper is essential for a variety of biological processes, Ctr1 protein is widely expressed not only in brain but also outside the CNS such as liver canaliculi, kidney cortex tubules, and small intestinal enterocytes [51]. Unlike other neurotransmitters, the vast majority of 5-HT is also found outside the CNS [2,57]. Notably, intestinal enterochromaffin cells synthesize > 90% of 5-HT and release it in the small intestinal mucosa, where dietary copper is acquired [57]. Although further research is needed to establish the full relationship between 5-HT and Ctr1, the results of the present study suggest a possibility that the copper reducing ability of 5-HT is a crucial part of the cellular copper uptake machinery.

5. Conclusions

Integral membrane protein Ctr1 transports copper across the plasma membrane to the intracellular delivery pathway with specificity for Cu (I). The Met-rich motifs in the N-terminal extracellular domain of human Ctr1 provide binding sites for Cu(I), but do not have copper reductase activity. Membrane associated metalloreductases that provide Cu(I) for Ctr1 have not yet been found in human and other mammals. We have shown in the present study that the reduction-oxidation reactions between 5-HT and Cu(II) are promoted by thioether-containing compounds, such as a peptide composed of a Met-rich region of Ctr1. Since 5-HT is not able to reduce Cu(II) to Cu(I) in the absence of thioethers, 5-HT is considered to be a copper reductant that is activated only in the presence of Ctr1. Although reduction of Cu(II) to Cu(I) in the oxidizing extracellular space is potentially risky, the use of 5-HT as a copper reductant is expected to minimize toxic effects of copper.

Abbreviations

5-HT	5-hydroxytryptamine (serotonin)
CNS	central nervous system
Ctr1	copper transporter 1
DHBT	5,5′-dihydroxy-4,4′-bitryptamine
BC	bathocuproinedisulfonic acid
3,6-DT	3,6-dithia-1,8-octanediol
3,7-DT	3,7-dithia-1,9-nonanediol
Fre1	cell surface Fe(III)/Cu(II) reductase
Fre2	cell surface Fe(III)/Cu(II) reductase
M1	a pentapeptide corresponding to M1 region of Ctr1 (MGMSYMD)
M1(M/A)	a mutant peptide of M1 (AGASYAD)

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Appendix A. Supplementary data

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References

[1] W.A. Weiger, Biol. Rev. Camb. Philos. Soc. 72 (1997) 61–95, https://doi.org/10.1111/j.1469-185X.1997.tb00010.x.
[2] M. Berger, J.A. Gray, B.L. Roth, Annu. Rev. Med. 60 (2009) 355–366, https://doi.org/10.1146/annurev.med.60.042307.110802.
[3] M.Z. Wrona, G. Dryhurst, Chem. Res. Toxicol. 11 (1998) 639–650, https://doi.org/10.1021/tx970185w.
[4] C.E. Jones, C.K. Underwood, E.J. Coulson, P.J. Taylor, J. Neurochem. 102 (2007) 1035–1043, https://doi.org/10.1111/j.1471-4159.2007.04602.x.
[5] N. Hadi, A. Malik, S. Azam, N.U. Khan, J. Iqbal, Toxicol. in Vitro 16 (2002) 669–674, https://doi.org/10.1016/S0887-2333(02)00083-8.
[6] M.Z. Wrona, R.N. Goyal, D.J. Turk, C.L. Blank, G. Dryhurst, J. Neurochem. 59 (1992) 1392–1398, https://doi.org/10.1111/j.1471-4159.1992.tb08452.x.
[7] I. Bremner, J.H. Beanie, Annu. Rev. Nutr. l0 (1990) 63–83, https://doi.org/10.1146/annurev.nu.10.070190.000431.
[8] R.I. Cousins, Physiol. Rev. 65 (1985) 238–309, https://doi.org/10.1152/physrev.1985.65.2.238.
[9] M.M. Peña, J. Lee, D.J. Thiele, J. Nutr. 129 (1999) 1251–1260, https://doi.org/10.1093/jn/129.7.1251.
[10] B. Halliwell, J.M. Gutteridge, Biochem. J. 219 (1984) 1–4, https://doi.org/10.1042/bj2190001.
[11] B. Halliwell, J.M. Gutteridge, Meth. Enzymol. 186 (1990) 1–85, https://doi.org/10.1016/0076-6879(90)86093-B.
[12] D.R. Brown, K. Qin, J.W. Herms, A. Madlung, J. Manson, R. Strome, P.E. Fraser, T. Kruck, A. von Bohlen, W. Schulz-Schaeffer, A. Giese, D. Westaway, H. Kretzschmar, Nature 390 (1997) 684–687, https://doi.org/10.1038/37783.
[13] J. Kardos, I. Kovacs, F. Hajos, M. Kalman, M. Simonyi, Neurosci. Lett. 103 (1989) 139–144, https://doi.org/10.1016/0304-3940(89)90565-X.
[14] D.E. Hartter, A. Barnea, Synapse 2 (1988) 412–415, https://doi.org/10.1002/syn.890020408.
[15] A.C. Rosenzweig, T.V. O'Halloran, Curr. Opin. Chem. Biol. 4 (2000) 140–147, https://doi.org/10.1016/S1367-5931(99)00066-6.
[16] J.F. Eisses, J.H. Kaplan, J. Biol. Chem. 277 (2002) 29162–29171, https://doi.org/10.1074/jbc.M203652200.
[17] A. Dancis, D.S. Yuan, D. Haile, C. Askwith, D. Eide, C. Moehle, J. Kaplan, R.D. Klausner, Cell 76 (1994) 393–402, https://doi.org/10.1016/0092-8674(94)90345-X.
[18] J.T. Rubino, P. Riggs-Gelasco, K.J. Franz, J. Biol. Inorg. Chem. 15 (2010) 1033–1049, https://doi.org/10.1007/s00775-010-0663-9.
[19] A.V. Davis, T.V. O'Halloran, Nat. Chem. Biol. 4 (2008) 148–151, https://doi.org/10.1038/nchembio0308-148.
[20] L.J. Martins, L.T. Jensen, J.R. Simons, G.L. Keller, D.R. Winge, J. Biol. Chem. 273 (1998) 23716–23721, https://doi.org/10.1074/jbc.273.37.23716.
[21] E.M. Rees, D.J. Thiele, J. Biol. Chem. 282 (2007) 21629–21638, https://doi.org/10.1074/jbc.M703397200.
[22] M. Okada, T. Miura, J. Inorg. Biochem. 159 (2016) 45–49, https://doi.org/10.1016/j.jinorgbio.2016.02.004.
[23] T. Miura, S. Sasaki, A. Toyama, H. Takeuchi, Biochemistry 44 (2005) 8712–8720, https://doi.org/10.1021/bi0501784.
[24] M. Suazo, C. Hodar, C. Morgan, W. Cerpa, V. Cambiazo, N.C. Inestrosa, M. Gonzalez, Biochem. Biophys. Res. Commun. 382 (2009) 740–744, https://doi.org/10.1016/j.bbrc.2009.03.096.
[25] M.M.O. Peña, S. Puig, D.J. Thiele, J. Biol. Chem. 275 (2002) 33244–33251, https://doi.org/10.1074/jbc.M005392200.
[26] G.F. Smith, D.H. Wilkins, Anal. Chem. 25 (1953) 510–511, https://doi.org/10.1021/ac60075a037.
[27] X. Huang, M.P. Cuajungco, C.S. Atwood, M.A. Hartshorn, J.D. Tyndall, G.R. Hanson, K.C. Stokes, M. Leopold, G. Multhaup, L.E. Goldstein, R.C. Scarpa, A.J. Saunders, J. Lim, R.D. Moir, C. Glabe, E.F. Bowden, C.L. Masters, D.P. Fairlie, R.E. Tanzi, A.I. Bush, J. Biol. Chem. 274 (1999) 37111–37116, https://doi.org/10.1074/jbc.274.52.37111.
[28] H. Dobbie, W.O. Kermack, H. Lees, Biochem. J. 59 (1955) 240–246, https://doi.org/10.1042/bj0590240.
[29] R.D. Hancock, J. Chem. Educ. 69 (1992) 615–621, https://doi.org/10.1021/ed069p615.
[30] N. Eriksen, G.M. Martin, E.P. Benditt, J. Biol. Chem. 235 (1960) 1662–1667, https://doi.org/10.1007/978-3-7091-9113-2_35.
[31] A. Dancis, D. Haile, D.S. Yuan, R.D. Klausner, J. Biol. Chem. 269 (1994) 25660–25667.
[32] J. Jiang, I.A. Nadas, M.A. Kim, K.J. Franz, Inorg. Chem. 44 (2005) 9787–9794, https://doi.org/10.1021/ic051180m.
[33] J.T. Rubino, K.J. Franz, J. Inorg. Biochem. 107 (2012) 129–143, https://doi.org/10.1016/j.jinorgbio.2011.11.024.
[34] M.-D. Vestergaard, K. Kerman, M. Saito, N. Nagatani, Y. Takamura, E. Tamiya, J. Am. Chem. Soc. 127 (2005) 11892–11893, https://doi.org/10.1021/ja052522q.
[35] D. Jiang, L. Men, J. Wang, Y. Zhang, S. Chickenyen, Y. Wang, F. Zhou, Biochemistry 46 (2007) 9270–9282, https://doi.org/10.1021/bi700508n.
[36] J.T. Rubino, M.P. Chenkin, M. Keller, P. Riggs-Gelasco, K.J. Franz, Metallomics 3 (2011) 61–73, https://doi.org/10.1039/c0mt00044b.
[37] H. Zhou, D.J. Thiele, J. Biol. Chem. 276 (2001) 20529–20535, https://doi.org/10.1074/jbc.M102004200.
[38] B. Zhou, J. Gitschier, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 7481–7486, https://doi.org/10.1073/pnas.94.14.7481.
[39] J.H. Kaplan, E.B. Maryon, Biophys. J. 110 (2016) 7–13, https://doi.org/10.1016/j.bpj.2015.11.025.
[40] E.B. Maryon, S.A. Molloy, J.H. Kaplan, Am. J. Physiol. Cell Physiol. 304 (2013) C768–C779, https://doi.org/10.1152/ajpcell.00417.2012.
[41] B. Halliwell, J.M. Gutteridge, Arch. Biochem. Biophys. 280 (1990) 1–8, https://doi.org/10.1016/0003-9861(90)90510-6.
[42] J. Beaudoin, D.J. Thiele, S. Labbé, S. Puig, Microbiology 157 (2011) 1021–1031, https://doi.org/10.1099/mic.0.046854-0.
[43] R. Ioannoni, J. Beaudoin, A. Mercier, S. Labbé, Copper-dependent trafficking of the Ctr4-Ctr5 copper transporting complex, PLoS ONE 5 (2010) e11964, , https://doi.org/10.1371/journal.pone.0011964.
[44] M. Okada, T. Miura, T. Nakabayashi, J. Inorg. Biochem. 169 (2017) 97–105, https://doi.org/10.1016/j.jinorgbio.2017.01.009.
[45] S.A.B. Knight, S. Labbé, L.F. Kwon, D.J. Kosman, D.J. Thiele, Genes Dev. 10 (1996) 1917–1929, https://doi.org/10.1101/gad.10.15.1917.
[46] R. Hassett, D.J. Kosman, J. Biol. Chem. 270 (1995) 128–134, https://doi.org/10.1074/jbc.270.1.128.
[47] E. Georgatsou, L.A. Mavrogiannis, G.S. Fragiadakis, D. Alexandraki, J. Biol. Chem. 272 (1997) 13786–13792, https://doi.org/10.1074/jbc.272.21.13786.
[48] M.E. van der Rest, A.H. Kamminga, A. Nakano, Y. Anraku, B. Poolman, W.N. Konings, Microbiol. Rev. 59 (1995) 304–322.
[49] K.L. Haas, A.B. Putterman, D.R. White, D.J. Thiele, K.J. Franz, J. Am. Chem. Soc. 133 (2011) 4427–4437, https://doi.org/10.1021/ja108890c.
[50] K.M. Davies, D.J. Hare, V. Cottam, N. Chen, L. Hilgers, G. Halliday, J.F. Mercer, K.L. Double, Metallomics 5 (2013) 43–51, https://doi.org/10.1039/C2MT20151H.
[51] Y.M. Kuo, A.A. Gybina, J.W. Pyatskowit, J. Gitschier, J.R. Prohaska, J. Nutr. 136 (2006) 21–26, https://doi.org/10.1093/jn/136.1.21.
[52] J. Lee, J.R. Prohaska, D.J. Thiele, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 6842–6847, https://doi.org/10.1073/pnas.111058698.
[53] M.A. Audet, L. Descarries, G. Doucet, J. Chem. Neuroanat. 2 (1989) 29–44, https://doi.org/10.1002/cne.902740302.
[54] E.S. Vizi, J.P. Kiss, B. Lendvai, Neurochem. Int. 45 (2004) 443–451, https://doi.org/10.1016/j.neuint.2003.11.016.
[55] P. Séguéla, K.C. Watkins, L. Descarries, J. Comp. Neurol. 289 (1989) 129–142, https://doi.org/10.1002/cne.902890111.
[56] W.J. McEntee, T.H. Crook, Psychopharmacology 103 (1991) 143–149, https://doi.org/10.1007/BF02244194.
[57] M.D. Gershon, J. Tack, Gastroenterology 132 (2007) 397–414, https://doi.org/10.1053/j.gastro.2006.11.002.