Inhibitory Act of Selenoprotein P on Cu^{+}/Cu^{2+} -induced tau aggregation and neurotoxicity

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Estimation of Cu^{+} Binding Constants of tau-R2

Bicinchoninic acid is a colorimetric Cu^{+} chelator, which can form a stable purple complex with Cu^{+} in a 2:1 ratio as $Cu^{+}(BCA)_2$ that exhibits absorption maxima at 562 nm (ε= 7900 $M^{-1}\cdot cm^{-1}$) and 358 nm (ε= 42 900 $M^{-1}\cdot cm^{-1}$).[1] Therefore, the transfer of Cu^{+} from BCA to tau-R2 was evaluated by monitoring the changes in UV-Vis absorbance of $Cu^{+}(BCA)_2$ at 562 nm. At the wavelength between 450 nm and 650 nm, neither apo- nor copper-bound proteins gave any absorption, while UV absorption from BCA occurs at $\lambda \leq 370$ nm.

Experiment of competition reaction between tau-R2 and BCA was carried out under anaerobic conditions as described previously.[2] Briefly, Cu^{+} solutions in the form of $[Cu(MeCN)_4]PF_6$ were mixed with BCA in a molar ratio of 1:3 in Tris/HCl buffer (50 mM, pH 7.4) as a stock solution. Protein solutions

at various concentrations were then mixed with the stock solution to give rise to final concentrations of Cu^+ of 20 μM and BCA of 60 μM. Ascorbic acid was kept at 1 mM to maintain the oxidation state of Cu^+ in the reaction mixture. The results were corrected for dilution and base-line absorbance at 800 nm.

The absorbance data obtained in tau-R2 titrated into $Cu^+(BCA)_2$ (Fig. 1) were used to calculate the binding constants for Cu^+-tau-R2 complex, using the following equation,

$$\frac{1}{Y} = \frac{1}{n} + \frac{1}{nK_a} \cdot X \qquad \text{(Eq.1)}$$

where the fractional saturation Y =(concentration of Cu^+ bound to P)/(total concentration of all forms of P), $X = \frac{[BCA]^2}{[Cu^+(BCA)_2]}$, K_a is the intrinsic binding constant, n is the stoichiometry value. The detailed information for the calculation of K_a and n value could be found in Table S1. The slope of the plot of 1/Y versus X (Fig. S1) gives $\log K_a = 1.9\times10^{-5}$ (correlation coefficient r = 0.94, n= 0.34, Fig. 1, Fig. S1 and Table S1). Using the known value for the association constant of $Cu(BCA)_2$ of $\log \beta_2$ = 17.30, binding constants of $K_{tau\text{-}R2}$=12.58 was calculated for Cu^+ binding to tau-R2.

Reference

1. Xiao, Z.; Donnelly, P. S.; Zimmermann, M.; Wedd, A. G. Transfer of copper between bis(thiosemicarbazone) ligands and intracellular copper-binding proteins. insights into mechanisms of copper uptake and hypoxia selectivity. *Inorg. Chem.* **2008,** 47, 4338-47.

2. Yatsunyk, L. A. and Rosenzweig, A. C. Cu(I) binding and transfer by the N terminus of the Wilson disease protein. *J Biol Chem,* **2007**, 282, 8622-8631.

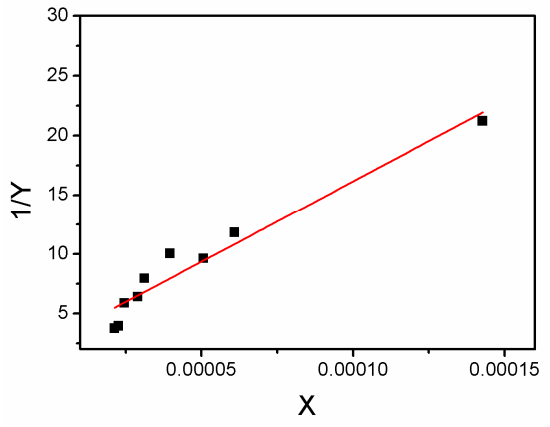

Figure S1 Plot of 1/Y versus of X to give the intrinsic binding constants and binding number of tau-R2 for Cu^{+}. $X = \frac{[BCA]^2}{[Cu^{+}(BCA)_2]}$ $Y = \frac{[nCu^{+} - P]}{[P]_T}$

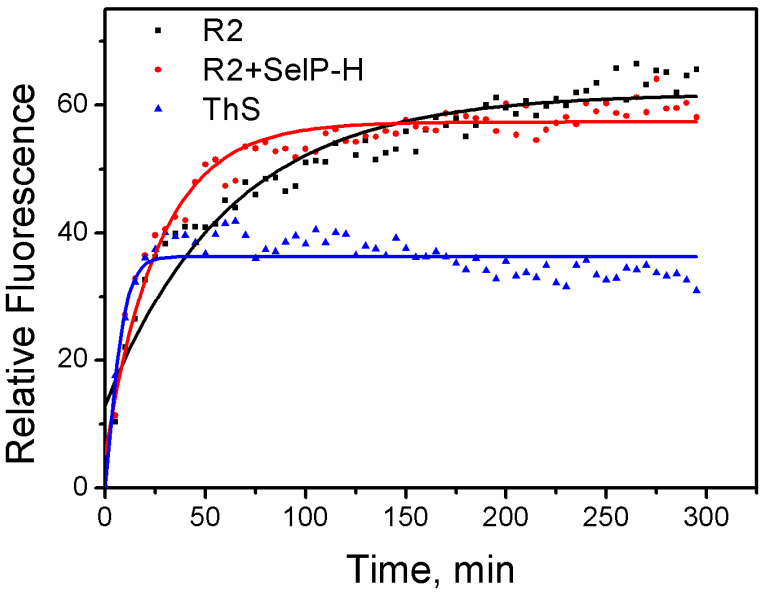

Figure S2. Fibrillation of tau-R2 in the absence of copper ions. Tau-R2 (15 μM) was incubated with heparin (3.8 μM) in the presence or absence of SelP-H (10 μM). All experiments were carried out in 50 mM Tris-HCl, 100 mM NaCl, containing 20 μM ThS at pH 7.4, 37 ºC. λ_{ex}=440 nm, λ_{em}=480 nm. Solid lines represent the fits of the data to Boltzmann equation.

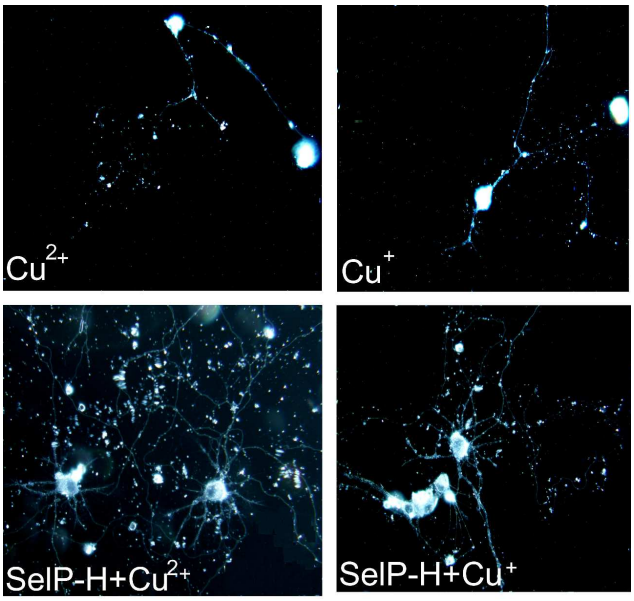

Figure S3. Typical dark field microscopy (DFM) images of primary neurons upon 12 h treatment with copper ions (3.5 μM) in the presence or absence of SelP-H (5 μM). Cu^{+} was prepared by reducing $CuSO_4$ with 1 mM H_2Asc.

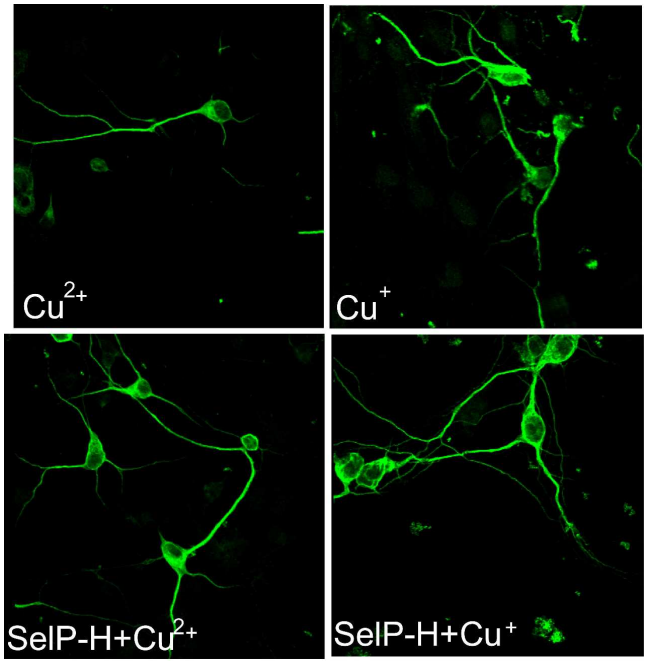

Figure S4. Typical confocal images showing the level and distribution of MAP-2 in primary neurons upon 12 h treatment with copper ions (3.5 μM) in the presence or absence of SelP-H (5 μM). Cu^{+} was prepared by reducing $CuSO_4$ with 1 mM H_2Asc.

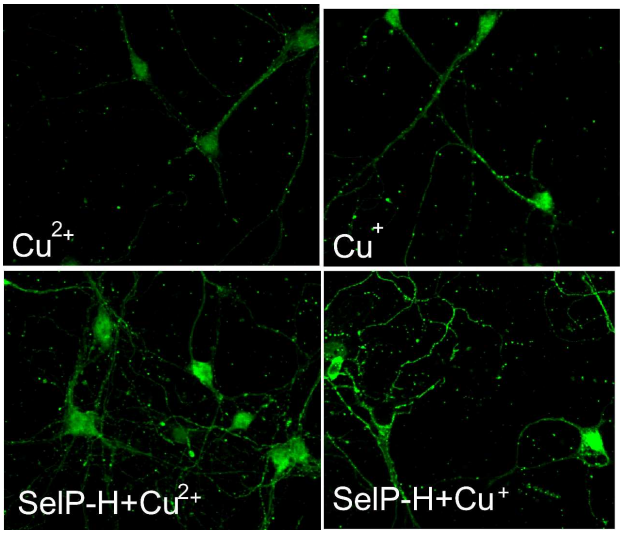

Figure S5. Typical confocal images showing the level and distribution of synaptophysin in primary neurons upon 12 h treatment with copper ions (3.5 μM) in the presence or absence of SelP-H (5 μM). Cu^{+} was prepared by reducing $CuSO_4$ with 1 mM H_2Asc.

Table S1 Determination of intrinsic binding constants and binding number of tau-R2 for Cu^{+} by competition with BCA for Cu^{+}[a, b]

$[P]_T$, μM	0	1	2	5	10	15	30	40	50	60	200
Absorbance	0.2144	0.2116	0.2090	0.2053	0.1977	0.1942	0.1824	0.1698	0.1600	0.1512	0.0738
$[Cu^{+}$-2BCA], μM	20	19.7332	19.4926	19.1494	18.4387	18.1104	17.0118	15.8403	14.9245	14.1056	6.8793
$[BCA]_f$, μM	20	20.5335	21.0147	21.7012	23.1226	23.7792	25.9765	28.3193	30.1511	31.7889	46.2414
$[Cu]_f \times 10^{-11}$, M	0.025	0.0234	0.0221	0.0203	0.0172	0.0160	0.0126	0.0099	0.0083	0.0070	0.0016
$[nCu^{+}$-P], μM	0	0.2667	0.5074	0.8506	1.5613	1.8896	2.9882	4.1597	5.0755	5.8944	13.1207
X	0.00002	2.14E-05	2.27E-05	2.46E-05	2.9E-05	3.12E-05	3.97E-05	5.06E-05	6.09E-05	7.16E-05	0.000311
1/Y	n.d.	3.7490	3.9419	5.8783	6.4050	7.9383	10.0393	9.6161	9.8512	10.1791	15.2431
n						0.34					
K_a						1.9E-5					
r						0.9432					

n.d.: not determined

[a] Experiments were performed in Tris-HCl (50 mM, pH 7.4) and H_2Asc (1 mM) under anaerobic conditions.

[b] Total Cu^{+} and BCA concentration in all equilibrium solutions are 20 and 60 μM, respectively.

$$X = \frac{[BCA]^2}{[Cu^{+}(BCA)_2]}$$

$$Y = \frac{[nCu^{+} - P]}{[P]_T}$$