Cisplatin Inhibits the Formation of a Reactive Intermediate during Copper-Catalyzed Oxidation of Amyloid β Peptide

Gulshan R. Walke[1], Srikanth Rapole[2] and Prasad P. Kulkarni*[1]

[1]Biometry and Nutrition Group, Agharkar Research Institute, Pune, India, [2]Proteomics Laboratory, National Centre for Cell Science, University of Pune Campus, Pune, India.

Supporting information

Experimental Section

Materials

All chemicals and reagents were of highest purity grade. The commercially available, synthesized Aβ1-16 peptide was obtained from BioResource Biotech Pvt. Ltd. India with >95% purity. Stock solution of peptide was prepared by dissolving the amorphous powder in deionized water. The peptide concentration was determined by UV-visible absorption of Tyrosine (ε_{280} = 1280 $M^{-1}cm^{-1}$), considered as a free tyrosine using JASCO V-630 spectrophotometer. Then the solution was diluted to get exact stock concentration of 2.5mM. $CuSO_4.5H_2O$, tris(hydroxymethyl)aminomethane (Tris) and glacial acetic acid were purchased from Sisco Research Laboratory Pvt. Ltd. India. The cis diammineplatinum(II)dichloride (cisplatin) was obtained from Sigma-Aldrich, India. Stock solution of cisplatin was prepared by dissolving known amount of compound in deionised water to give final concentration of 2.5mM (0.75mg/mL). The cisplatin stock was freshly prepared and protected from light. Ascorbic acid and 3,3',5,5'_Tetramethylbenzidine (TMB) were purchased from Sigma-Aldrich. The ammonium acetate was obtained from Qualigens, India. ZipTip C18 Pipette Tips, Millipore, India were used for sample preparation. The deionized water was used for all experiments, was obtained from Sartorius arium 611 water purification system with specific resistance of 18.2 MΩcm.

Methods

Copper-catalyzed Oxidation Reactions:

Copper-catalyzed oxidation of Aβ1-16 was performed in the presence of 500μM copper solution in 50mM Tris buffer (pH- 7.4). Briefly, 500μM Aβ1-16 was first incubated with 500μM aqueous copper solution for 12 hrs at 4°C. After incubation, the mixture was kept at room temperature for 30 minutes. Then 40 folds of ascorbic acid were added, followed by incubation for 30 minutes at 37°C with tubes open to atmospheric oxygen. In the case of oxidation of Aβ1-16 in the presence of cisplatin, the mixture of 500μM Aβ1-16:Cu(II) in 1:1 ratio was incubated at 4°C for 6 hrs. Further, 4 μL cisplatin stock was added to make final concentration of 500 μM and the resultant reaction mixture was incubated at 4°C for additional 6 hrs. After incubation, the mixture was kept at room temperature for 30 minutes. Then 40 folds of ascorbic acid were added, followed by incubation at 37°C for 30 minutes with tubes open to atmospheric oxygen. The reactions were stopped by the addition of 1% (v/v) glacial acetic acid. The samples were then immediately desalted using ziptip C18 before ms analysis.

Copper-catalyzed oxidation reactions of Aβ1-16 pre incubated with cisplatin:

500μM Aβ1-16 in presence of 500μM cisplatin incubated for 3 hr at 4°C in 100mM ammonium acetate buffer (pH-7.4) with subsequent addition of 500μM Cu(II) followed by 3 hr incubation at 4°C. After incubation, the reaction mixture was kept at room temperature for 30 minutes. Then 40 folds of L-ascorbic acid were added to the reaction mixture, followed by incubation for 30 minutes at 37°C with tubes open to atmospheric oxygen. The reaction was stopped by the addition of 1% (v/v) glacial acetic acid. The sample was then immediately used for ms analysis.

Mass Spectrometry:
Mass spectra were collected on a AB Sciex 4800 MALDI-TOF/TOF mass spectrometer (AB Sciex, Framingham, MA) linked to 4000 series explorer software (v.3.5.3). Samples were mixed with CHCA matrix in 1:1 ratio and spotted on a MALDI target plate. Spots were then allowed to dry and loaded on MALDI. Mass spectra were recorded within a mass range from 800 to 4000 Da, using a Nd:YAG 355 nm laser. The acceleration voltage used was 20 kV and extraction voltage was 18 kV. The instrument was calibrated using 6 peptide standard mix that was purchased from AB Sciex. MS spectra were obtained in reflector mode using 900 laser shots with 4000 laser intensity. Further MS/MS spectra were acquired with a total accumulation of 1500 laser shots and collision energy of 2 kV.

Electrochemical Measurements:
Voltammetric experiments were carried out on Model 600B Series Electrochemical analyzer, CH instruments, India. Screen printed electrodes (TE-100), used in the experiments, were purchased from CH instruments, India. The screen printed electrode (SPE) composed of a glassy carbon as working electrode (3 mm in diameter) and auxiliary electrode, and a Ag/AgCl reference electrode. Before performing experiment, SPE were subjected to oxygen plasma treatment (50mA current and 0.1mbar pressure for 5minutes) which increases the hydrophilicity of the electrode, removes the impurities, activates carbon surface, enhances electron transfer rate and electrochemical response.[1,2] The electrolyte solution was 50mM Tris buffer (pH-7.4) containing 0.1 M KCl. The experiments were done using 500μM Aβ1-16-Cu(II) complex in the absence and presence of 500μM cisplatin, at scan rate 25mV/s, at temperature 293K. The samples were prepared as per procedure mentioned in 'copper-catalyzed oxidation reactions' section.
References:

1. Wang, S. C.; Chang, K. S.; Yuan, C.J. *Electrochimica Acta*, **2009**, 4937.
2. Evans, J. F.; Kuwana, T. *Analytical Chemistry*, **1979**, 359.

Ascorbate Consumption Experiments:
The traces of kinetics of ascorbate consumption were recorded on BioTek SYNERGY-HT microplate reader, at 25°C, in 96 UV transparent well plate (Corning). The absorbance of ascorbate was monitored at λ=265nm as a function of the time in 100mM ammonium acetate buffer (pH-7.4). The Aβ1-16 was incubated in presence of Cu(II) for 6hr at 4°C in ammonium acetate buffer (10μL). Similarly, Aβ1-16 was incubated in presence of Cu(II) for 3 hr at 4°C in ammonium acetate buffer with subsequent addition of cisplatin followed by 3 hr incubation at 4°C (10μL) to form reaction mixture of Aβ1-16-Cu(II)-cisplatin. The kinetics of ascorbate (190μL) was measured for each 30 second of interval for 5 min. Then, the ascorbate consumption was started by addition of reaction mixtures Aβ1-16-Cu(II) and Aβ1-16-Cu(II)-cisplatin. The final concentration of Aβ1-16, Cu(II) and cisplatin were 25μM and ascorbate concentration was 250μM each in the final volume of 200μL.

Peroxidase Activity:
The peroxidase activity of Aβ1-16-Cu(II) was measured, in the absence and presence of cisplatin. 3,3',5,5'_Tetramethylbenzidine (TMB) was used as a substrate. 10mg of TMB was dissolved in 0.5mL of glacial acetic acid and diluted to 10mL 1M AcOH/NaOAc buffer, pH-4.5. The Aβ1-16 was incubated in presence of Cu(II) for 6hr at 4°C in AcOH/NaOAc buffer (10μL). Similarly, Aβ1-16 was incubated in presence of Cu(II) for 3 hr at 4°C in 1M AcOH/NaOAc buffer with subsequent addition of cisplatin followed by 3 hr incubation at 4°C (10μL) to form reaction mixture of Aβ1-16-Cu(II)-cisplatin. Then, 10μL preformed Aβ1-16-Cu(II) and Aβ1-16-Cu(II)-cisplatin were added in 185μL of TMB solution. 5μL of 40mM H_2O_2 solution was added in the above solution. The final concentration of Aβ1-16, Cu(II), cisplatin and H_2O_2 was 25μM, 25μM, 25μM and 1mM respectively. The total volume of the mixture was 200μL. The traces of kinetics were recorded, using absorbance at λ=652nm with 3min interval. The absorbance was measured using BioTek SYNERGY-HT microplate reader, at 25°C, in 96 well plate (TARSONS).

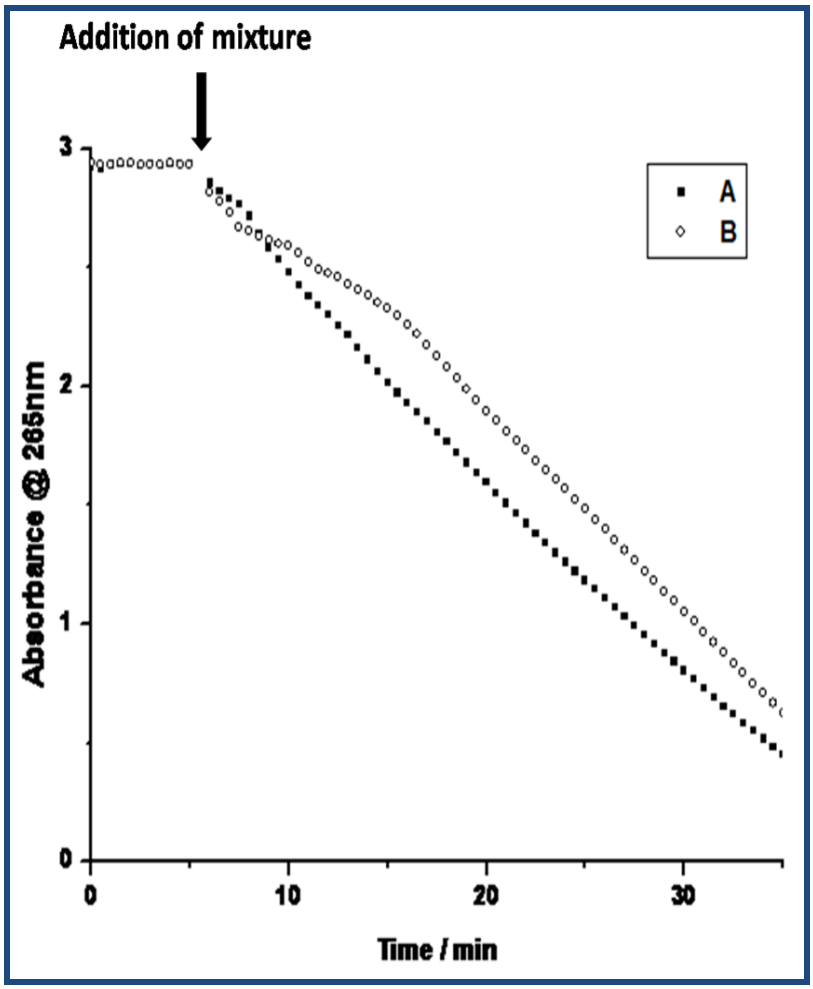

Figure S5: Ascorbate consumption; an absorbance was measured at λ=265nm as a function of the reaction time for a solution of 250μM ascorbate in 100μM ammonium acetate buffer, pH-7.4. The preformed reaction mixtures Aβ1-16-Cu(II) (A) and Aβ1-16-Cu(II)-cisplatin (B) were added after five minute measurement in the ascorbate solution. The Aβ1-16, Cu(II) and cisplatin were added to reach a final concentration of 25μM each. The experiment was performed in quadruplicates.

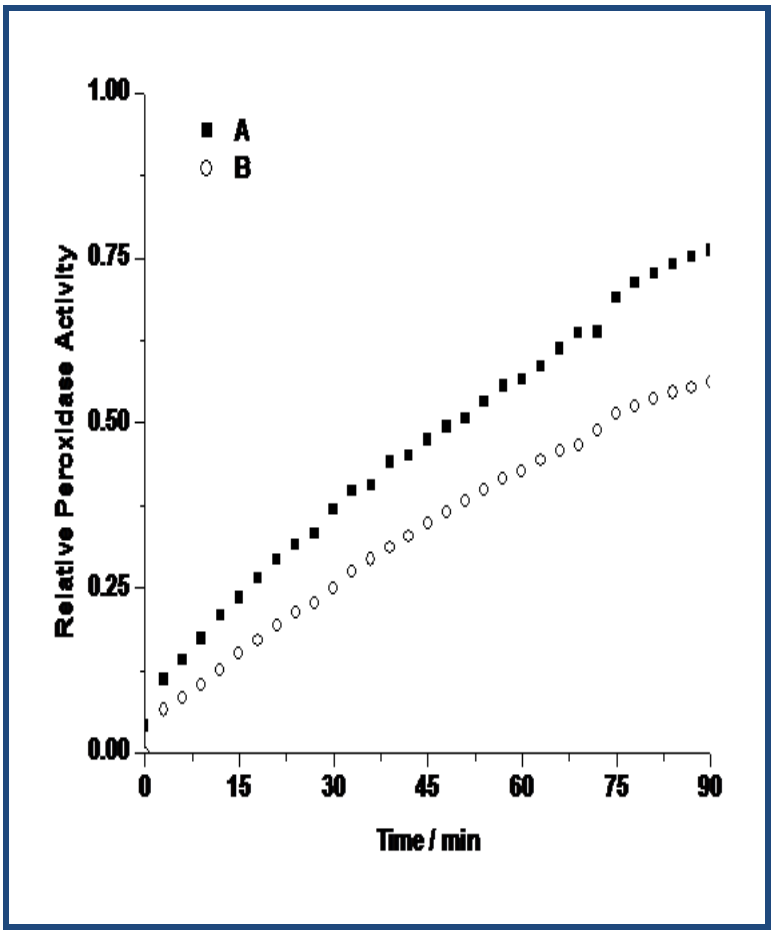

Figure S6: Peroxidase activity, monitoring the increase of 652 nm absorbance intensity, for Aβ1-16-Cu(II) (A) and Aβ1-16-Cu(II)-cisplatin (B). The experiment was performed in duplicates.

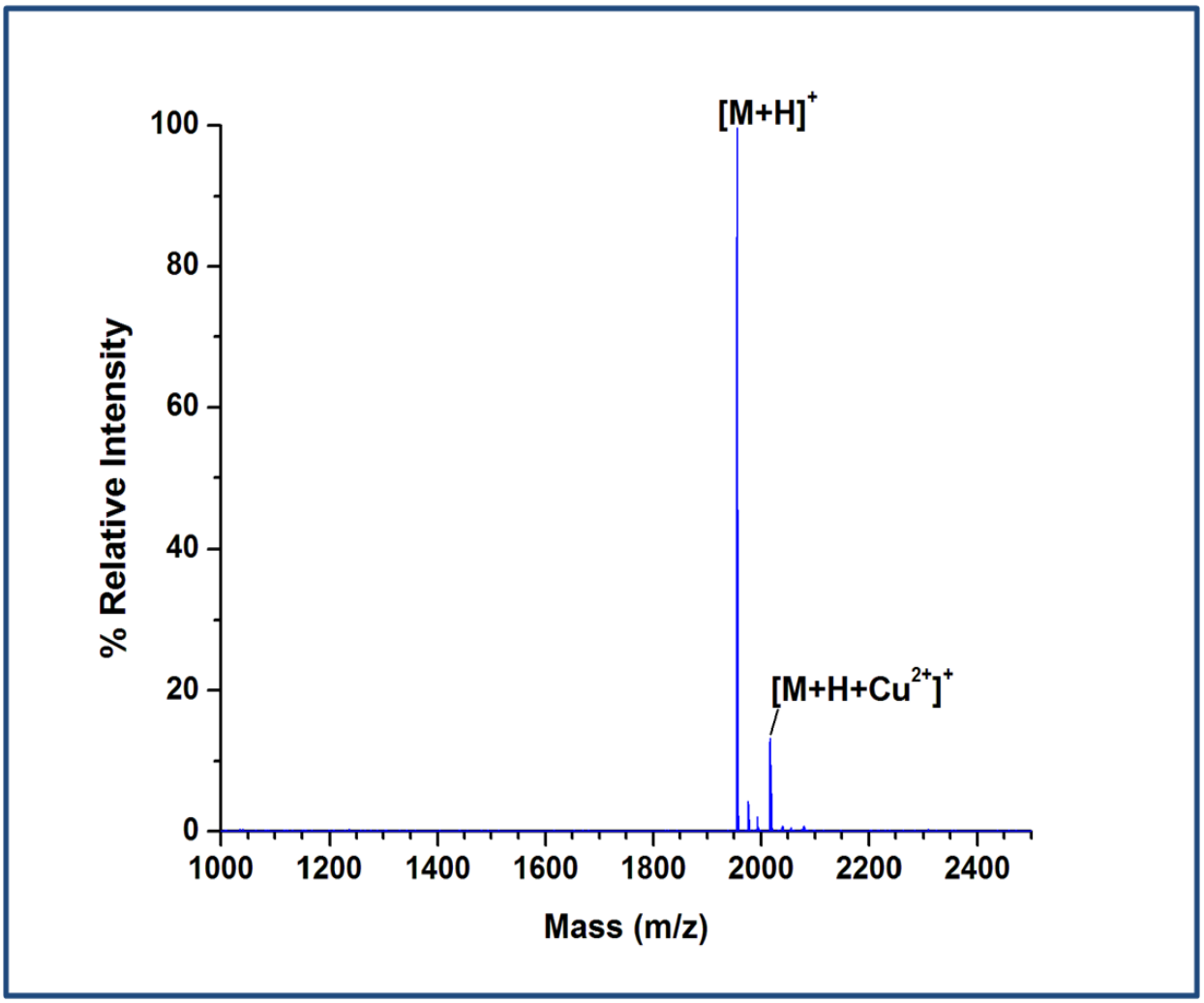

Figure S1: Mass spectrum of 500μM Aβ1-16-Cu(II) complex in the presence of 500μM cisplatin incubated for 3 hr at 4°C in 100mM ammonium acetate buffer (pH-7.4).

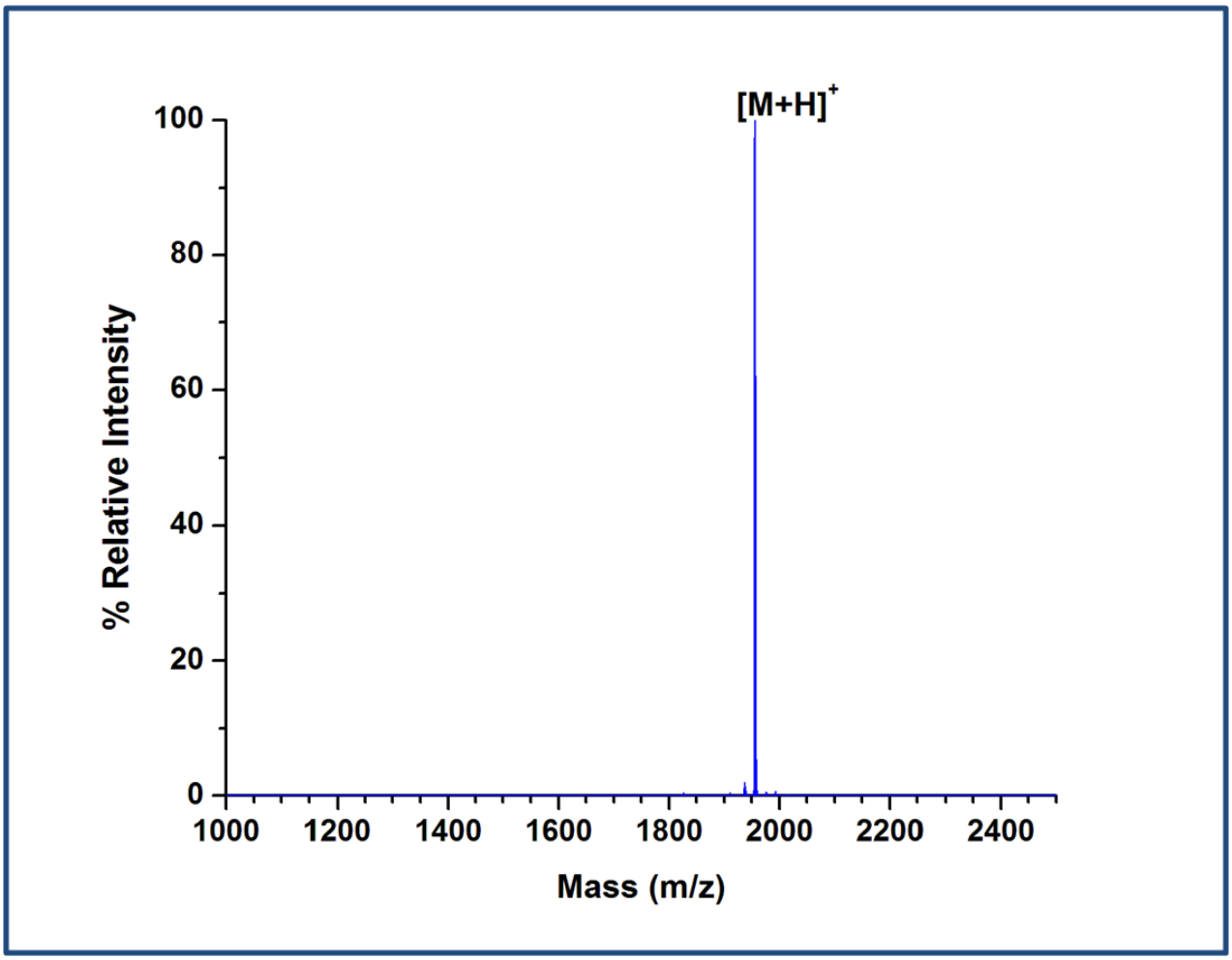

Figure S2: Mass spectrum of 500μM Aβ1-16 in the presence of 500μM cisplatin incubated for 3 hr at 4°C in 100mM ammonium acetate buffer (pH-7.4).

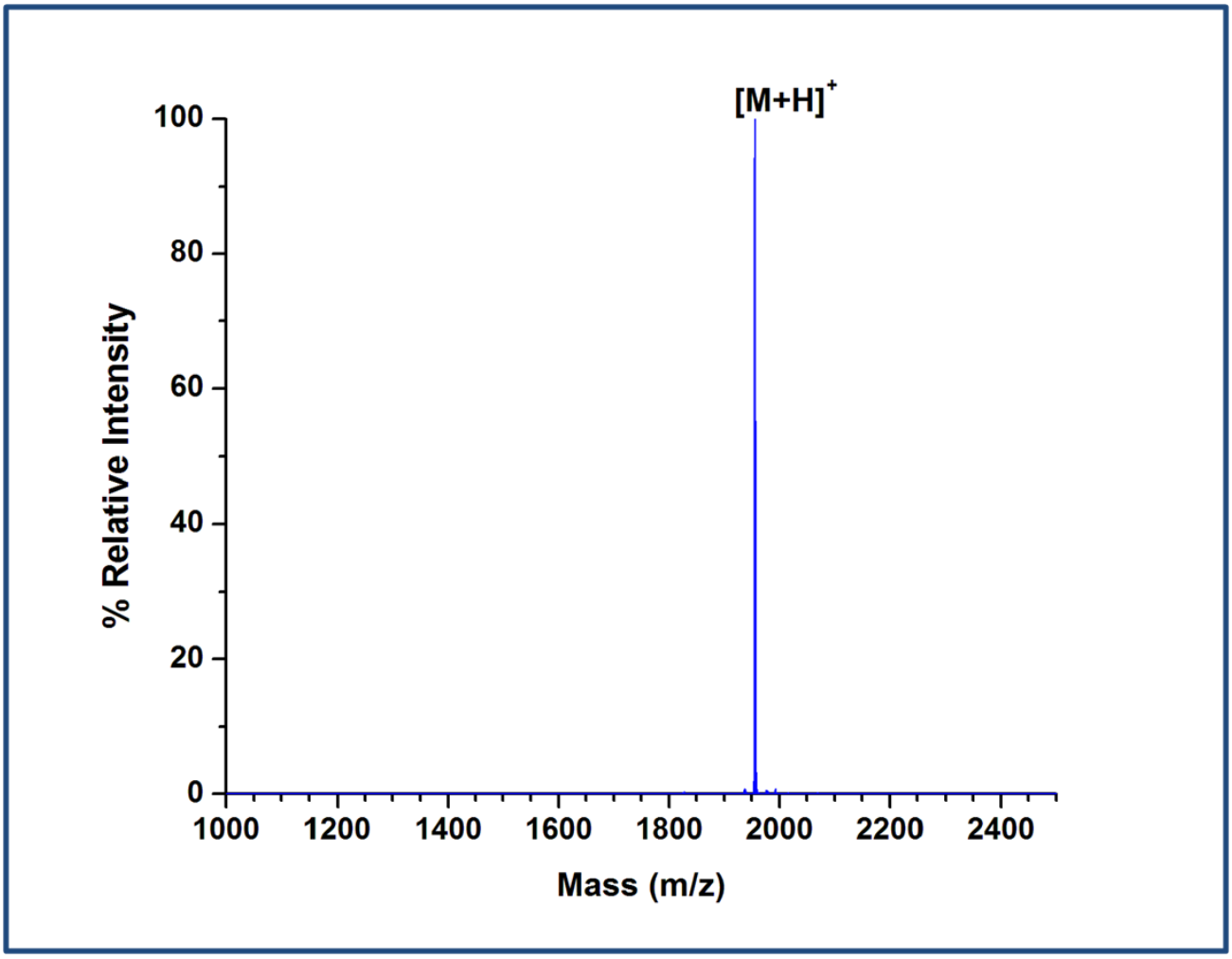

Figure S3: Mass spectrum of 500μM Aβ1-16 in the presence of 500μM cisplatin incubated for 3 hr at 4°C in 100mM ammonium acetate buffer (pH-7.4). After incubation, the reaction mixture was kept at room temperature for 30 minutes. Then 40 folds of L-ascorbic acid were added to the reaction mixture, followed by incubation for 30 minutes at 37°C with tubes open to atmospheric oxygen. The reaction was stopped by the addition of 1% (v/v) glacial acetic acid.

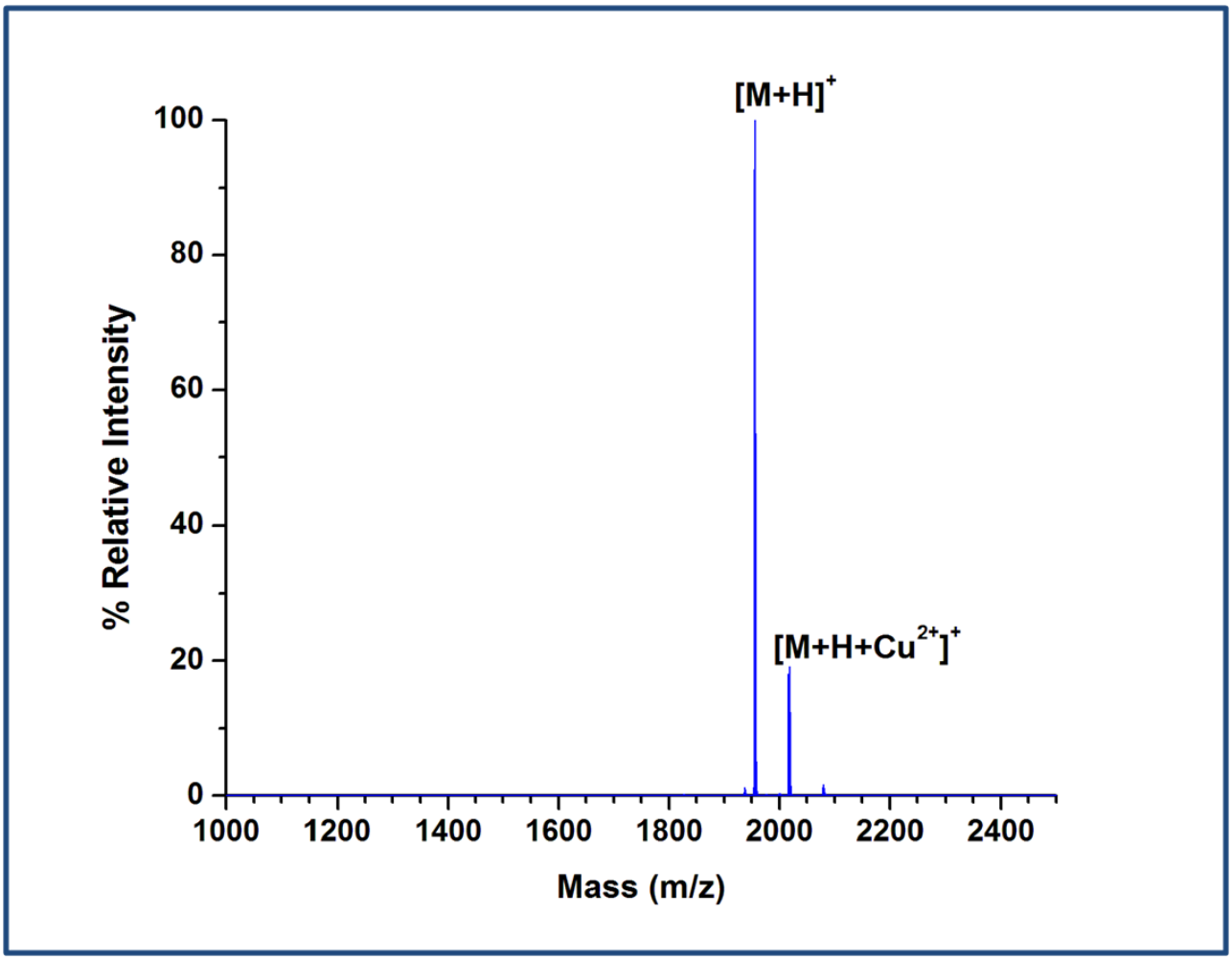

Figure S4: Mass spectrum of 500μM Aβ1-16 in the presence of 500μM cisplatin incubated for 3 hr at 4°C in 100mM ammonium acetate buffer (pH-7.4) with subsequent addition of 500μM Cu(II) followed by 3 hr incubation at 4°C.

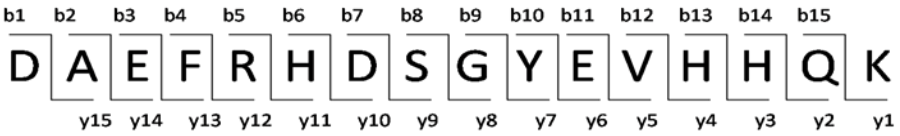

Figure S7: Fragmentation scheme for the Aβ1–16 peptide.