

Supporting Information

Native Mass Spectrometry-based Method for Studying the Interactions between Superoxide Dismutase and Stilbenoids

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Determination of dissociation constants of SOD1–ligand complexes

Drift scope software (Waters Corp., Manchester, U.K.) was used to extract the specific region of SOD1 dimer ions and then exported the mass spectrum for the evaluation of binding affinities.

The dissociation constants of the SOD1–ligand complexes were determined based on a direct ESI-MS approach. The protein–ligand binding equilibrium in solution can be described as follow equations:



K_{d1} and K_{d2} are dissociation constants for the 1:1 and 1:2 SOD1–ligand complexes.

$$K_{d1} = \frac{[\text{P}][\text{L}]}{[\text{PL}]} \quad (3)$$

$$K_{d2} = \frac{[\text{P}][\text{L}]^2}{[\text{PL}_2]} \quad (4)$$

It is assumed that the ionization efficiencies for the bare protein and the complex are equal, which allows the use of the ratios of the measured relative peak intensity (r) of the free protein over the complex instead of their concentrations.

$$r_1 = \frac{[\text{PL}]}{[\text{P}]_0} = \frac{I(\text{PL})}{I(\text{P}) + I(\text{PL}) + I(\text{PL}_2)} \quad (5)$$

$$r_2 = \frac{[\text{PL}_2]}{[\text{P}]_0} = \frac{I(\text{PL}_2)}{I(\text{P}) + I(\text{PL}) + I(\text{PL}_2)} \quad (6)$$

Equilibrium concentrations of protein ($[\text{P}]$) and ligand ($[\text{L}]$) can be expressed as follow equations:

$$[\text{P}] = [\text{P}]_0 - [\text{PL}] - [\text{PL}_2] = [\text{P}]_0 - r_1[\text{P}]_0 - r_2[\text{P}]_0 = [\text{P}]_0(1 - r_1 - r_2) \quad (7)$$

$$[\text{L}] = [\text{L}]_0 - [\text{PL}] - 2[\text{PL}_2] = [\text{L}]_0 - r_1[\text{P}]_0 - 2r_2[\text{P}]_0 \quad (8)$$

where $[\text{P}]_0$ and $[\text{L}]_0$ are the analytical concentrations of protein and ligand in solution, respectively.

The dissociation constants K_{d1} and K_{d2} are deduced as follows:

$$K_{d1} = \frac{[P][L]}{[PL]} = \frac{[P]_0(1-r_1-r_2)([L]_0-r_1[P]_0-2r_2[P]_0)}{r_1[P]_0} \quad (9)$$

$$= \frac{(1-r_1-r_2)([L]_0-r_1[P]_0-2r_2[P]_0)}{r_1}$$

$$K_{d2} = \frac{[P][L]^2}{[PL_2]} = \frac{[P]_0(1-r_1-r_2)([L]_0-r_1[P]_0-2r_2[P]_0)^2}{r_2[P]_0} \quad (10)$$

$$= \frac{(1-r_1-r_2)([L]_0-r_1[P]_0-2r_2[P]_0)^2}{r_2}$$

Table S1. Calculated dissociation constants for SOD1–ligand complexes.

Complexes	K_{d1} (μM)	K_{d2} (nM)
SOD1–Resveratrol	67.99 ± 5.75	5.44 ± 0.72
SOD1–Oxyresveratrol	75.57 ± 8.22	6.02 ± 0.70
SOD1–Polydatin	70.83 ± 7.45	5.47 ± 0.91
SOD1–THSG	68.46 ± 7.90	5.65 ± 0.84

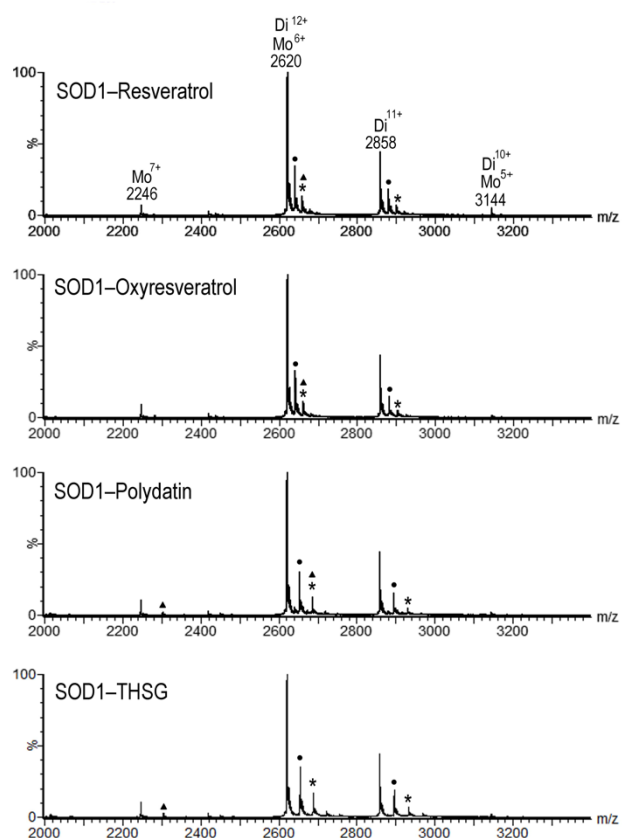


Figure S1. ESI-MS spectra of noncovalent complexes of SOD1 with stilbenoids at molar ratio 1:6 of SOD1:ligand after pre-incubation at 37 °C for 1 h. The ions correspond to m/z 2858 and 2246 were exclusively detected for Cu_2 , Zn_2 -dimeric SOD1 ($[\text{Di}]^{11+}$) and monomeric Cu, Zn-SOD1 ($[\text{Mo}]^{7+}$), respectively. The ions at m/z 2620 ($[\text{Mo}]^{6+}$ and $[\text{Di}]^{12+}$) and 3144 ($[\text{Mo}]^{5+}$ and $[\text{Di}]^{10+}$) were assigned to joint contributions of metalated monomer and dimer ions. Symbols represent experimental data for (●) mono-adducts on dimer, (*) di-adducts on dimer and (▲) mono-adducts on monomer.

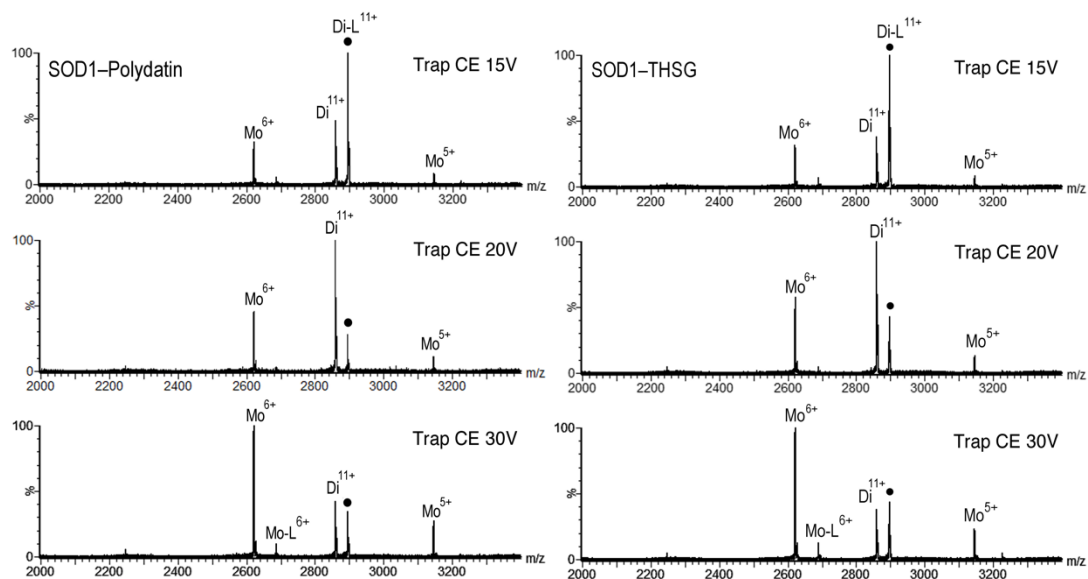


Figure S2. ESI-MS/MS spectra for SOD1–polydatin and SOD1–THSG noncovalent complexes. The 11+ charged ligand-bound SOD1 dimer ions were selected as precursor and marked with black circles (●). Data were acquired at different trap collision energies (CE). (Di = dimer, Mo = monomer)