

Supporting Text

Dot Blotting

Column fractions were analyzed by a dot blot assay, with Avidin-AP, as described (1) and/or by immunostaining with anti-SOD1 antibody. At high ($>1 \mu\text{M}$) protein concentrations, column fractions were diluted before blotting. For example, for a $30\text{-}\mu\text{M}$ protein sample, $5 \mu\text{l}$ of the column fraction (volume $250 \mu\text{l}$) was diluted into a volume of $300 \mu\text{l}$ with the flow buffer, and $100 \mu\text{l}$ of the diluted sample was blotted. For immunostaining, the membrane was blocked for 1 h with 5% BSA, then incubated for 1 h with rabbit anti-SOD1 (Abcam, diluted 4,000-fold in PBS-Tween, 3% BSA), followed by another hour of anti-rabbit-AP secondary antibody (Sigma, diluted 40,000-fold in PBS-Tween, 1% BSA). After two 10-min washes with PBS-Tween (0.1%) and one 10-min wash with Tris-buffered saline, the membrane was treated with Amersham Pharmacia ECF reagent for 5-20 min. The resulting signal was detected by a phosphorimager and quantitated with the software IMAGEQUANT. Background correction was applied by local averaging of the spot intensities and was used as an estimate of the baseline. It was found that while the relative intensity of spots on the dot blot for a given set of column fractions was highly reproducible, their absolute values varied from blot to blot. Therefore, for comparison of dot-blot elution profiles obtained at different time points, the spot intensities were normalized by the maximum value detected on a given blot.

Estimation of Rate Constants and Numerical Integration

The constants k_m^- , k_m^+ and k_{agg} in Eqs. 1 and 3 were estimated from the data in Fig. 3 A-E. (i) k_m^- : The changes in SOD1 dimer and monomer concentrations under dialysis conditions were used to estimate $k_m^- = 1.2 \times 10^{-4} \text{ s}^{-1}$. As shown in Fig. 3E, in the initial 6,300 s of the reaction (900-7,200 s), D_{holo} decreased from 25 to 21 μM . Based on $K_d = 1 \mu\text{M}$, M_{holo} is expected to concomitantly decrease from 5 to 4.5 μM , whereas the observed total monomer ($M_{\text{holo}} + M_{\text{apo}}$) increased from 5 to 8.1 μM . Therefore, $M_{\text{apo}} = 8.1 - 4.5 = 3.6 \mu\text{M}$ was formed during this period from $M_{\text{holo}} = 5 \mu\text{M}$ in a first-order process: $3.6 \times 10^{-6} = k_m^- \cdot 5 \times 10^{-6} \cdot 6,300$. (ii) k_{agg} : The apparent second-order rate constant for aggregation, $k_{\text{agg}} = 0.12 \times 10^3 \text{ M}^{-1} \cdot \text{s}^{-1}$, was estimated from the observed 9 μM aggregation in 10,800 s (7,200-18,000 s; Fig. 3E), from a nearly constant $M_{\text{apo}} = 3.6 \mu\text{M}$ during this period: $9 \times 10^{-6} = 0.5 \cdot k_{\text{agg}} \cdot (3.6 \times 10^{-6})^2 \cdot 10,800$. (iii) k_m^+ : The value $k_m^+ = 5.2 \times 10^4 \text{ M}^{-1} \cdot \text{s}^{-1}$ was obtained by taking into account the observation (Fig. 3 A and B) that aggregation was more rapid with 1 μM SOD1 than with 30 μM SOD1. With 1 μM SOD1, there was 0.6 μM aggregation in 24 h, and based on $k_{\text{agg}} = 0.12 \times 10^3 \text{ M}^{-1} \cdot \text{s}^{-1}$, it was calculated that aggregation occurred from an apo-monomer concentration $M_{\text{apo}} = 0.28 \mu\text{M}$. Thus, the equilibrium composition of 1 μM SOD1 was $D_{\text{holo}} = 0.39 \mu\text{M}$, $M_{\text{holo}} = 0.33 \mu\text{M}$, and $M_{\text{apo}} = 0.28 \mu\text{M}$, yielding an equilibrium constant for Zn-loss $K_m = \frac{M_{\text{apo}} \cdot \text{Zn}}{M_{\text{holo}}} = 2.3 \times 10^{-7} \text{ M}$;

the value of K_m and $k_m^- = 1.2 \times 10^{-4} \text{ s}^{-1}$ were used to estimate k_m^+ , and the estimated values of rate constants were used for numerical simulation.

The differential master equations corresponding to the aggregation reaction under dialysis conditions are:

$$\left\{ \begin{array}{l} \dot{D}_{\text{holo}} = -k_{\text{off}} D_{\text{holo}} + \frac{1}{2} k_{\text{on}} M_{\text{holo}}^2 \\ \dot{M}_{\text{holo}} = 2k_{\text{off}} D_{\text{holo}} - k_{\text{on}} M_{\text{holo}}^2 - k_{\text{m}}^- M_{\text{holo}} + k_{\text{m}}^+ M_{\text{apo}} Zn \\ \dot{M}_{\text{apo}} = k_{\text{m}}^- M_{\text{holo}} - k_{\text{m}}^+ M_{\text{apo}} Zn - k_{\text{agg}} M_{\text{apo}}^2 \\ \dot{A} = \frac{1}{2} k_{\text{agg}} M_{\text{apo}}^2 \\ \dot{Zn} = -k_{\text{dialysis}} Zn - k_{\text{m}}^+ M_{\text{apo}} Zn + k_{\text{m}}^- M_{\text{holo}} \\ \left[A + \frac{M_{\text{holo}}}{2} + \frac{M_{\text{apo}}}{2} + \frac{Zn}{2} + D_{\text{holo}} \right] (t) = D_{\text{holo}} (0) \end{array} \right.$$

where the symbols are as defined in Eq. 3. In the absence of dialysis, the rate of loss of Zn, $\dot{Zn} = 0$. These equations were solved numerically and a time step of 10^{-2} was sufficient for the convergence of the solution.

SOD1 Dimer Dissociation Under Aggregation-Promoting Conditions Is Reversible

The reversibility of dimer dissociation at pH 3.5 was evaluated by SEC. A SOD1 sample at 6.5 μM eluted as a mixture of approximately 73% dimer and 27% monomer (Fig. 6B, black curve). When this sample was concentrated ≈ 4 -fold to a final concentration of 24.1 μM , it eluted as a mixture of $\approx 87\%$ dimer and 13% monomer (Fig. 6B, blue curve). The fraction of protein eluting as monomer reduced 14% upon concentration, showing that the dimer was re-formed from the monomer. Thus, SOD1 dimer dissociation at pH 3.5 is reversible.

The Arg-79 – Asp-101 Salt Linkage

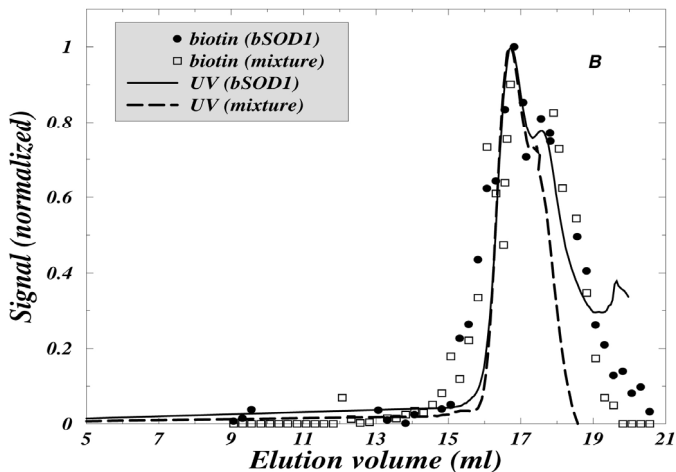
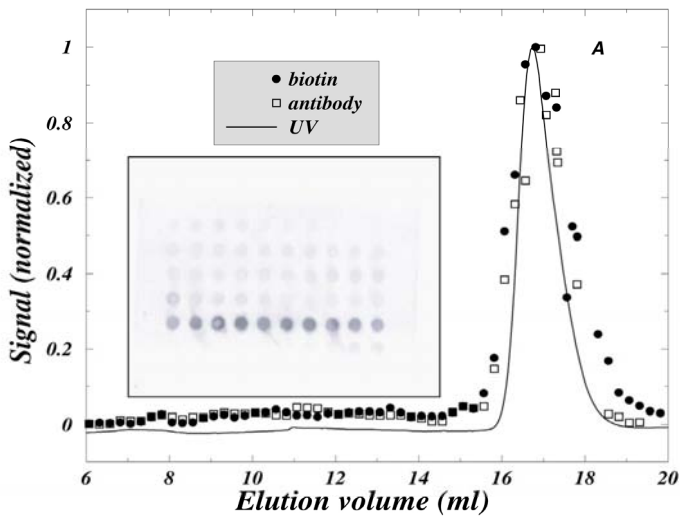
We propose that the salt linkage governing dimer dissociation rate is between Arg-79 and Asp-101 (numbering corresponding to human SOD1) based on the observations that: (i) a structure-based alignment of 76 SOD1 sequences obtained from the FSSP database (2) showed that this salt linkage Arg-79 – Asp-101 is 100% evolutionarily conserved in the eukaryotic SOD1 fold (Fig. 7), while being absent in prokaryotic SOD1, which has a structurally distinct dimer interface, and (ii) Arg-79 and Asp-101 are located in the Zn loop and an edge-strand of the β -barrel of SOD1, respectively, and their interaction is crucial for the stability and the folding kinetics of SOD1 (3). It is likely that the titration of the Arg-79 – Asp-101 salt linkage promotes greater disorder in the Zn-binding region, thereby disrupting its interactions with the β -barrel and the disorder is propagated in the molecule leading to dimer dissociation. The mutation of Asp-101 to Gly or Asn is implicated in FALS (www.alsod.org) and the disordered Zn loop of metal-deficient SOD1 has previously been found to be a site for SOD1 oligomerization (4). Therefore, we propose that salt linkages in SOD1 are crucial for maintaining SOD1 dimer structural integrity, and that the disruption of these charge-charge interactions promotes misfolding into an aggregation-competent state.

1. Caplow, M. & Fee, L. (2002) *Mol. Biol. Cell* **13**, 2120-2131.
2. Holm, L. & Sander, C. (1996) *Nucleic Acids. Res.* **24**, 206-209.
3. Khare, S. D., Ding, F. & Dokholyan, N. V. (2003) *J. Mol. Biol.* **334**, 515-525.
4. Elam, J. S., Taylor, A. B., Strange, R., Antonyuk, S., Doucette, P. A., Rodriguez, J. A., Hasnain, S. S., Hayward, L. J., Valentine, J. S., Yeates, T. O., *et al.* (2003) *Nat. Struct. Biol.* **10**, 461-467.

Fig. 5. SOD1 dimer dissociation equilibrium detected by SEC. (A) Size exclusion profiles of SOD1 sample (30 μ M, pH 3.5) a 1:4 mixture of the biontinylated and unmodified protein (bSOD1-SOD1), obtained by UV-absorbance and two dot-blotting methods: antibody to SOD1, and biotin detection; a typical dot-blot for this sample, stained by anti-SOD1 antibody, is shown (*Inset*). (B) Size exclusion profiles of pure bSOD1 and a bSOD1-SOD1 under identical conditions (5 μ M, pH 3.5, 20 minute incubation) showing that biontinylated SOD1 has identical chromatographic properties as the unmodified SOD1.

Fig. 6. SOD1 dimer dissociation under aggregation-promoting conditions is reversible (A) Column calibration at pH 3.5. SOD1 (30 μ M) at pH 3.5 eluted at 16.5 ml and 17.3 ml, before and after incubation with 10 mM EDTA. These samples were known to be primarily dimeric and primarily monomeric respectively, from AUC (Table 1). Calibration at pH 3.5 was done as above because a set of globular protein standards at pH 3.5 was not available. (B) The reversibility of SOD1 dimer dissociation. The elution profiles correspond to 6.5 μ M SOD1 (black curve), and 24.1 μ M SOD1 (blue curve) which was obtained by concentrating the 6.5- μ M sample. The fraction of monomer reduced upon concentration, indicating that dimer was formed from the monomer.

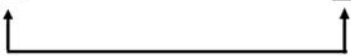
Fig. 7. A portion of the sequence alignment of 76 eukaryotic SOD1 sequences obtained from the FSSP database (ftp://ftp.embl-heidelberg.de/pub/databases/protein_extras/fssp/) showing the conserved salt-linkage between Arg-79 and Asp-101. Positions with 100% and >50% conservation have yellow and grey backgrounds respectively.



<i>SODC_HUMAN</i>	KDEE	R	H	V	G	D	L	G	N	V	T	A	D	K	D	G	V	A	D	V	S	I	E	D	S	V	I	S	L	S	G	D	H	C	I	I	G	R	T	L	V	V	H
<i>SODC_RABIT</i>	KDEE	R	H	V	G	D	L	G	N	V	T	A	G	S	N	G	V	A	D	V	L	I	E	D	S	V	I	S	L	S	G	D	M	S	V	I	G	R	T	L	V	V	H
<i>SODC_PIG</i>	KDQE	R	H	V	G	D	L	G	N	V	T	A	G	K	D	G	V	A	T	V	Y	I	E	D	S	V	I	A	L	S	G	D	H	S	I	I	G	R	T	M	V	V	H
<i>SODC_BOVIN</i>	KDEE	R	H	V	G	D	L	G	N	V	T	A	D	K	N	G	V	A	I	V	D	I	V	D	P	L	I	S	L	S	G	E	Y	S	I	I	G	R	T	M	V	V	H
<i>SODC_SHEEP</i>	KDEE	R	H	V	G	D	L	G	N	V	K	A	D	K	N	G	V	A	I	V	D	I	V	D	P	L	I	S	L	S	G	E	Y	S	I	I	G	R	T	M	V	V	H
<i>SODC_HORSE</i>	KDEE	R	H	V	G	D	L	G	N	V	T	A	D	E	N	G	K	A	D	V	D	M	K	D	S	V	I	S	L	S	G	K	H	S	I	I	G	R	T	M	V	V	H
<i>SODC_CHICK</i>	KDAD	R	H	V	G	D	L	G	N	V	T	A	-	K	G	G	V	A	E	V	E	I	E	D	S	V	I	S	L	T	G	P	H	C	I	I	G	R	T	M	V	V	H
<i>SODC_XENLA</i>	KDAD	R	H	V	G	D	L	G	N	V	T	A	E	-	G	G	V	A	Q	F	K	F	T	D	P	Q	I	S	L	K	G	E	R	S	I	I	G	R	T	A	V	V	H
<i>SODC_MAIZE</i>	EDED	R	H	A	G	D	L	G	N	V	T	A	G	E	D	G	V	V	N	V	N	I	T	D	S	Q	I	P	L	A	G	P	H	S	I	I	G	R	A	V	V	V	H

75

120



Salt-bridge

