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Structural reorganization renders enhanced metalloprotein stability

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Electronic Supplementary Information

Materials and Methods

Rubredoxin

Desulfovibrio gigas rubredoxin (RdDg) was purified as previously described[1]. Protein concentration was determined spectrophotometrically using the visible absorption extinction coefficients ε^{376nm} = 8450 $M^{-1}.cm^{-1}$ and ε^{493nm} = 6970 $M^{-1}.cm^{-1}$ [2]. Purity was verified by the absorption ratio between 377 and 278 nm equaling 0.45. Buffer was 50 mM potassium phosphate at pH 7.

UV/visible absorption

UV/visible absorption spectra were recorded using a Shimadzu UV-1700 spectrophotometer at room temperature.

Circular dichroism

CD measurements were recorded in a Jasco J-815 spectropolarimeter equipped with a Peltier-controlled thermostated cell support. 0.1 cm (far UV) and 1 cm (near UV/visible) pathlength cuvettes were used. Thermal denaturation experiments were carried out increasing the temperature from 25 to 95°C at a heating rate of 1°C/min. Every 5°C spectra were acquired. Thermal denaturation was assessed by the CD signal variations at local spectra maxima and minima. Protein concentration was 0.1 mg/ml (17.6 µM, far UV) or 0.62 mg/ml (109 µM, near UV/visible). Far UV CD spectra were accumulated 4 times at 200 nm/min scan rate and 1 s time response. Near UV/visible spectra were accumulated 5 times at 1000 nm/min scan rate and 0.5 s time response.

ATR FT-IR spectroscopy

Attenuated total reflectance Fourier transform infrared spectroscopy (ATR FT-IR) measurements were performed using a Bruker IFS 66/S spectrometer equipped with a nitrogen-cooled MCT detector using the thermostatized Harrick BioATR cell II. Protein was concentrated to 10 mg/ml and centrifuged at 12000 g before the temperature ramp to pellet any aggregates forming while concentrating. FT-IR spectra in the amide I (1600-1700 cm^{-1}) and amide II (1500-1600 cm^{-1}) regions were recorded while temperature was increased from 20 to 94°C in a discontinuous fashion: spectra were accumulated during 1 minute (97 accumulations), temperature was raised 2°C during around 45 seconds and sample was equilibrated 15 seconds. Overall temperature change rate was 1.1°C/min. Spectral resolution was 4 cm^{-1}, scanner velocity was 20.0 kHz and aperture was 12 mm. Spectra were analyzed after vector normalization. Spectral variations at specific wavenumbers within the amide I band were assigned to secondary structure changes[3]. Deconvolution was performed as previously described[3]. Briefly, the amide I band corresponding to native rubredoxin (20ºC) was reconstituted as the sum of gaussian curves centered at second derivative minima. These bands were assigned to secondary structures according to their spectral position[3]. Then, the component bands' center position was fixed and the remaining spectra were reconstituted by adjusting the curve area and bandwidth. Secondary structure content was estimated from gaussian curve integration.

Structural analysis

The secondary structure content of RdDg was extracted from the crystal structure (2DSX)[4] using pdbsum (http://www.ebi.ac.uk/pdbsum/).

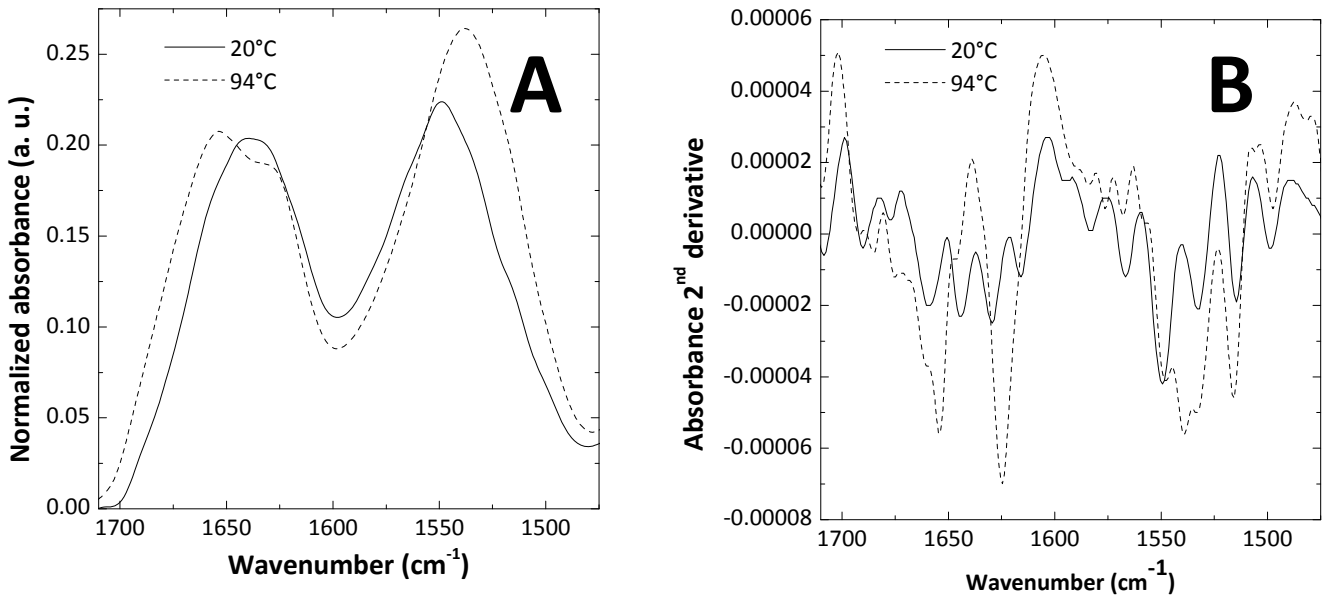
Supplementary tables

Supplementary Table S1. Band assignment for the deconvolution of FT-IR spectra.

Wavenumber (cm^{-1})	Structure
1684	β-sheets
1661	Turns
1643	Coil
1639	Coil
1644	α-helices
1625	β-sheets
1603	Mixed*

*β-sheets and amino acid side chains absorb in this region. Discarded for secondary structure quantification.

Supplementary figures



Supplementary Figure S1. FT-IR monitored thermal denaturation of rubredoxin. Amide I – amide II FT-IR spectra were recorded while increasing the temperature from 20ºC to 94ºC. (A) FT-IR absorption spectra. (B) Second derivative spectra. The spectra obtained at 20ºC (solid line) and 94ºC (dashed line) are depicted.

Notes and references

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