SUPPORTING INFORMATION :

Spatially resolved protein hydrogen exchange measured by MALDI in-source decay

Kasper D. Rand,[1,2], Nicolai Bache[2], Morten M. Nedertoft[2] & Thomas J.D. Jørgensen[2*]*

1. Department of Pharmaceutics and Analytical Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen, DK-2100 Copenhagen Ø, Denmark 2. Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark.

*Correspondence to: K.D.R (kdr@farma.ku.dk) or T.J.D.J (tjdj@bmb.sdu.dk).

Experimental section

Materials. Horse heart cytochrome c was purchased from Sigma (St. Louis, MO, USA) and used without further purification. D_2O (99.9 atom % D) was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Ammonium acetate-d_7 (99 atom % D) was obtained from Isotec-Sigma-Aldrich (St. Louis, MO, USA). The MALDI matrices, 2,5-dihydroxybenzoic acid (DHB) and sinapinic acid (3,5-dimethoxy-4-hydroxycinnamic acid) was obtained from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were of the highest grade commercially available.

Hydrogen/Deuterium exchange experiments. Horse heart cytochrome c (oxidized) was fully deuterated by dissolution (5 mM) into D_2O, lyophilized and redissolved in deuterated buffer (ammonium acetate-d_7, 50 mM, pH 6.1, uncorrected value) followed by incubation for 3 hrs at 60˚C.[1] Native state D-to-H exchange of backbone amides in cytochrome c was carried out by a 50-fold dilution of 5 µL fully deuterated cytochrome c into ^1H_2O buffer (10 mM ammonium acetate, pH 7.0) followed by incubation for 20 min at 20˚C. Subsequently, the isotopic exchange reaction of backbone amide

hydrogens was quenched by the addition of 6.5 µL 2.5 % (v/v) aqueous trifluoroacetic acid (TFA) to 250 µL exchange buffer resulting in a pH of 2.5. To determine the deuterium loss that inevitably occurs at quench conditions (i.e., back exchange), a 100% D control was prepared by a 50-fold dilution of fully deuterated cytochrome c into acidified $^{1}H_2O$ buffer (10 mM ammonium acetate, pH 2.5). The samples were frozen immediately and stored in dry-ice until MS analysis.

Sample Preparation and MALDI ISD Mass Spectrometry. A Bruker anchorChip™ MALDI sample plate with a spot-diameter of 400 µm was precoated at room temperature with 0.5 µL 20 mg/mL sinapinic acid in 100% acetone. The rapid evaporation of acetone resulted in the formation of thin microcrystalline layer of sinapinic acid. The precoated MALDI sample plate was placed in a sealed container and cooled to 4 °C. The container limits the amount of water condensation when the plate is handled afterwards and this in turn minimizes the pump-down time of the vacuum interface of the MALDI mass spectrometer when the plate is loaded. Furthermore, the low temperature of the sample plate minimizes the amount of backexchange.[2] The precoated crystalline layer improves the MALDI crystallization process for the sample presumably by providing many seeding sites for crystal growth when the solvent rapidly evaporates *in vacuo*.

Quenched cytochrome c samples (100 µM) were manually thawed and 0.5 µL was quickly applied on to the cooled (4 °C) MALDI sample plate together with 0.5 µL of a cooled solution of 20 mg/mL sinapinic acid in 70 % (v/v) acetonitrile, 0.05 % (v/v) aqueous trifluoroacetic acid (TFA). Subsequently, the plate was rapidly loaded into the ion source of the MALDI instrument. MALDI ISD mass spectra were acquired on an Ultraflex TOF/TOF (Bruker, Germany) equipped with a 337-nm nitrogen laser. The ISD spectra were acquired in positive linear mode using a 25-kV acceleration voltage, a low mass gate set to 1500 Da and 150 ns ion extraction delay. To induce ISD, the laser was operated at 10 Hz with the laser fluence set to 12% above the threshold for ion production and 1200 laser shots were accumulated per spectrum using a random laser pattern. Sample spots of nondeuterated cytochrome c were placed adjacent to the sample spots of deuterated cytochrome c on the MALDI target plate. Peak tables with average masses of the various ions were generated by the software FlexAnalysis v. 2.4

(Bruker, Germany) and exported to an Excel spreadsheet to calculate the deuterium content of the c-ions.

In a limited number of experiments, an alternate MALDI matrix was used consisting of 0.25 µL 20 mg/mL 2,5-dihydroxybenzoic acid in 50 % (v/v) acetonitrile, 0.1 % (v/v) aqueous trifluoroacetic acid (TFA), which was precoated on the MALDI sample plate and allowed to dry at room temperature. This matrix gave rise to the formation of highly heterogeneous crystals and a higher laser fluence was needed to induce fragmentation (50% above the threshold for ion production). These factors contributed to inferior reproducibility and reduced signal-to-noise in resulting ISD spectra of cytochrome c recorded using this matrix.

Data analysis. The deuterium content of c fragment ions was determined from the difference between the average mass of the deuterated fragment ion and the average mass of the corresponding nondeuterated fragment ion. To calculate the theoretical deuterium content of a c fragment ion in the case of 100% hydrogen scrambling, the deuterium content of cytochrome c (precursor ion) was divided by the number of its exchangeable sites (i.e., N- and O-linked hydrogen atoms), and this ratio was then multiplied with the number of exchangeable sites in the c fragment ion.

References

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(2) Mandell, J. G.; Falick, A. M.; Komives, E. A. *Anal. Chem.* 1998, *70*, 3987-3995.