Available online at www.sciencedirect.com
SCIENCE DIRECT
International Journal of Mass Spectrometry 253 (2006) 249–255
International Journal of Mass Spectrometry
www.elsevier.com/locate/ijms

Segmental charge distributions of Cytochrome *c* on transfer into the gas phase

K. Breuker *

Institute of Organic Chemistry and Center for Molecular Biosciences Innsbruck (CMBI), University of Innsbruck, Innrain 52a, A-6020 Innsbruck, Austria

Received 21 November 2005; received in revised form 6 April 2006; accepted 11 April 2006
Available online 26 May 2006

Dedicated to Prof. Bernhard Kräutler on the occasion of his 60th birthday.

Abstract

Segmental charge distributions of Cytochrome *c* ions in the transition from solution to gas phase are studied by native electron capture dissociation (NECD). The data suggest that the solution charge distribution of native Cytochrome *c* is partially preserved during the electrospray ionization process. Segments with charge values different from those in solution correspond to protein regions that are the first to unfold on transfer into the gas phase, consistent with an increased gas phase basicity of, and facile proton transfer to, the newly exposed sites. Changes in the charge distribution at elevated temperatures indicate further unfolding, as well as proton transfer as a result of the increased electrostatic interactions in a gas phase environment.

Keywords: Electrospray ionization; Proton transfer

1. Introduction

Protein ions generated by electrospray ionization (ESI) [1] carry multiple charges. For ESI in positive ion mode, the number of charges depends on the structure of the protein in solution, with compact conformations resulting in narrow distributions at lower charge values, and open conformations resulting in broader distributions at higher charge values [2]. Although the protein net charge is evident directly from the *m*/*z* spectrum, the distribution of charges within a protein ion emerging from an ESI droplet is so far unknown. Here the charge distributions within Cytochrome *c* ions in the transition from solution to gas phase are studied by native electron capture dissociation (NECD) [3,4]. Cytochrome *c*, a small electron transfer protein, was also used in a number of studies that discussed the effect of surface accessible area, number of exposed residues, and number of basic versus acidic residues on the net charge of its ESI ions [5]. Equine Cytochrome *c* has 24 basic (R: 2, K: 19, H: 3) and 12 acidic (D: 3, E: 9) residues; the N-terminus is acetylated and the C-terminus carboxylated. Its heme group, covalently bound to the protein chain via thioether linkages at C14 and C17, has two propionate functionalities and an iron center in the Fe^{II} or Fe^{III} state. In aqueous solution at pH 5, equine (Fe^{III})Cytochrome *c* exists in its native structure [6].

In electron capture dissociation (ECD), multiply protonated protein ions stored in the trapped ion cell of a Fourier transform-ion cyclotron resonance (FT-ICR) mass spectrometer react with low-energy electrons to form $\boldsymbol{c}$, $\boldsymbol{z}^{\bullet}$ (∼90%) and $\boldsymbol{a}^{\bullet}$, $\boldsymbol{y}$ product ion pairs [7]. Although $\boldsymbol{a}^{\bullet}$ ions can also be formed by 157 nm photodissociation [8], and $\boldsymbol{y}$ ions (along with $\boldsymbol{b}$ ions) by conventional dissociation methods (e.g., collisionally activated, CAD, or infrared multiphoton dissociation, IRMPD) [9], the $\boldsymbol{c}$, $\boldsymbol{z}^{\bullet}$ ions are unique products of reaction with electrons. The observation of $\boldsymbol{c}$ ions, along with $\boldsymbol{y}$ but no $\boldsymbol{b}$ ions, in ESI spectra of Cytochrome *c* without added electrons was therefore unexpected [3]. The following model of native electron capture dissociation (NECD) was proposed to account for the formation of $\boldsymbol{c}$ and $\boldsymbol{y}$ ions [3]. NECD of (Fe^{III})Cytochrome *c* requires solution concentrations sufficiently high (∼75 μM) for the formation of noncovalently bound homodimers [3]. ESI of such an aqueous dimer solution produces homodimer ions that enter the FT-ICR MS via a heated metal capillary for desolvation, where both

* Tel.: +43 512 507 5240; fax: +43 512 507 2892.
E-mail address: kbreuker@gmx.net.

1387-3806/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2006.04.004

monomers unfold, but one of them (monomer I) faster than the other (monomer II) [4]. This causes proton transfer from the more compact monomer II to the more unfolded monomer I, and induces a substantial charge asymmetry [10]. Whenever the charge asymmetry is sufficiently high (~2:1) [4], two electrons are transferred from monomer II to the heme of monomer I, one reducing the heme iron and the other causing protein backbone cleavage (NECD) next to residues in contact with the heme [3,4]. Thus the NECD fragment ions from a given backbone cleavage site indicate intact noncovalent bonding between the residue next to this cleavage site and the heme, whereas the "missing" cleavages identify regions where the native structure is lost on transfer into the gas phase. For equine (Fe^{III})Cytochrome *c*, the NECD data at different capillary temperatures revealed a sequential unfolding mechanism, with the terminal helices and the 18–34 Ω-loop unfolding first [4]. The order of unfolding of the native (Fe^{III})Cytochrome *c* structure on transfer into the gas phase determined by NECD was found to be essentially the reverse of the order of unfolding in solution [4]. Although the extent of protein hydration at the time when NECD occurs is not known, this implies that the Cytochrome *c* dimers undergoing NECD cannot be fully solvated.

2. Experimental

This study was performed on a 6 T Fourier transform-ion cyclotron resonance (FT-ICR) mass spectrometer described previously [7b]. Ions generated by nano-electrospray in ambient air enter the differentially pumped vacuum system through a heated metal capillary and are transferred into the trapped ion cell by quadrupole ion guides. The capillary temperature was measured at the orifice; no attempt was made to measure the temperature inside the capillary as it cannot be assumed that the protein ions thermalize with the capillary temperature. Electrospray ionization (flow 200–500 nl/min, 1 kV spray potential) utilized emitters (~5 μm inner diameter) made from borosilicate capillaries with a pipette puller (Sutter Instrument Co., Novato, CA). The distance between the emitter and the metal capillary orifice was ~0.5 mm. A platinum wire was inserted into the spray solution from the back of the emitter for application of the spray potential. Equine Cytochrome *c* (Sigma, St. Louis, MO) was dissolved in nanopure water to a final concentration of 75 μM, stirred on a vortex mixer, and stored at 4 °C for 3 months. No buffer was added to the protein solution, as this could affect the charge distributions of the protein ions formed by ESI [11]. All NECD data discussed here were from the same (Fe^{III})Cytochrome *c* solution (all "dimer B", [4]) at pH 5. For each spectrum except that in Fig. 4, a new ESI emitter was loaded with protein solution and the spectrum recorded within 3 min after initiation of the electrospray. The spectrum in Fig. 4 is an average of 32 scans, for which the electrospray lasted 20 min. Mass spectral interpretation utilized the automated THRASH program [12]. Relative ion abundance values were calculated from signal heights divided by the ions charge value, as signal height scales inversely with charge for FT-ICR detection in the low-pressure limit. Average charge values, n_0, were calculated as arithmetic mean values from relative abundance values, $A(n)$, and charge values, n, as $n_0 = \sum nA(n)/\sum A(n)$.

3. Results and discussion

Fig. 1 shows a representative NECD spectrum obtained by nano-electrospray of the aqueous (Fe^{III})Cytochrome *c* solution

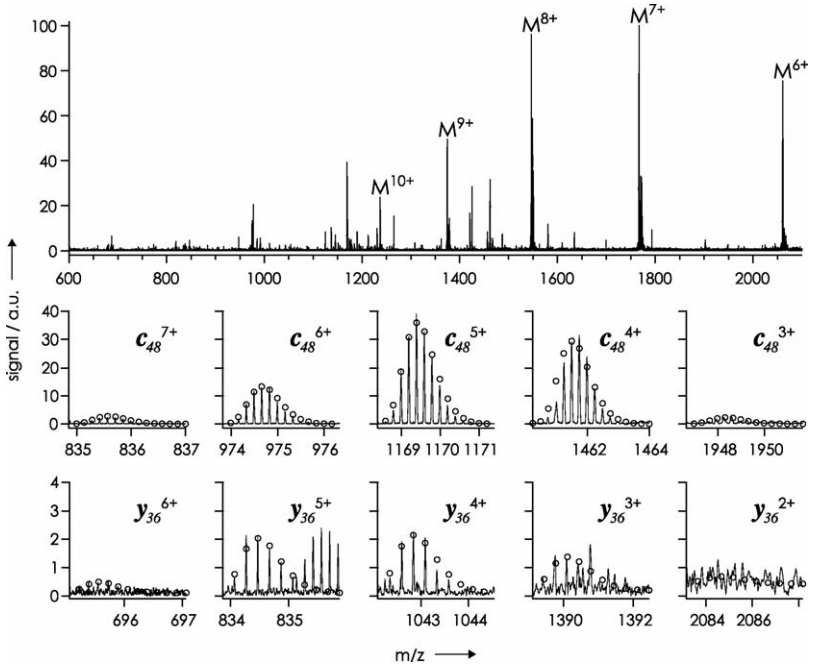

Fig. 1. NECD spectrum of (Fe^{III})Cytochrome *c* from ESI of an aqueous solution (75 μM, pH 5), spray duration 3 min, capillary temperature 38 °C. Bottom traces show enlarged *m/z* ranges for c_{48} for charge values $n = 3$–7 and y_{36} for $n = 2$–6, with calculated isotopic profiles for $[(Fe^{II})c_{48} + nH]^{n+}$ and $(y_{36} + nH)^{n+}$ shown as open circles.