International Journal of Mass Spectrometry 330–332 (2012) 302–309

Contents lists available at SciVerse ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Hydrogen/deuterium exchange reflects binding of human centrin 2 to Ca^{2+} and Xeroderma pigmentosum Group C peptide: An example of EX1 kinetics

Justin B. Sperry[a,b], Zachary C. Ryan[c,d], Rajiv Kumar[c,d], Michael L. Gross[b,*]

[a] Analytical Research and Development, Pfizer Inc., Chesterfield, MO 63017, United States
[b] Department of Chemistry, Washington University in St. Louis, St. Louis, MO 63130, United States
[c] Department of Medicine, Mayo Clinic, Rochester, MN 55905, United States
[d] Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN 55905, United States

ARTICLE INFO

Article history:
Received 26 July 2012
Received in revised form 16 October 2012
Accepted 17 October 2012
Available online 27 October 2012

Keywords:
Hydrogen/deuterium exchange
EX1 kinetic
Protein
Protein/peptide interaction
Centrin 2
Xeroderma pigmentosum

ABSTRACT

Xeroderma pigmentosum (XP) is a genetic disease affecting 1 in 10,000–100,000 and predisposes people to early-age skin cancer, a disease that is increasing. Those with XP have decreased ability to repair UV-induced DNA damage, leading to increased susceptibility of cancerous non-melanomas and melanomas. A vital, heterotrimeric protein complex is linked to the nucleotide excision repair pathway for the damaged DNA. The complex consists of XPC protein, human centrin 2, and RAD23B. One of the members, human centrin 2, is a ubiquitous, acidic, Ca^{2+}-binding protein belonging to the calmodulin superfamily. The XPC protein contains a sequence motif specific for binding to human centrin 2. We report here the Ca^{2+}-binding properties of human centrin 2 and its interaction with the XPC peptide motif. We utilized a region-specific H/D exchange protocol to localize the interaction of the XPC peptide with the C-terminal domain of centrin, the binding of which is different than that of calmodulin complexes. The binding dynamics of human centrin 2 to the XPC peptide in the absence and presence of Ca^{2+} are revealed by the observation of EX1 H/D exchange regime, indicating that a locally unfolded population exists in solution and undergoes fast H/D exchange.

1. Introduction

The incidence of skin cancer is doubling every decade owing to the aging population and increased UV exposure, caused inter alia by ozone depletion and personal habits of excessive tanning [1,2]. Approximately 1 in 10,000–100,000 people suffer from the genetic disease Xeroderma pigmentosum (XP). The XP patients have a deficiency in the ability to repair damage by ultraviolet (UV) light, thus making them predisposed to skin cancer. In fact, those with XP have a 4800-fold and 2000-fold higher probability in developing non-melanoma and melanoma skin cancers, respectively [3]. The inability of XP patients to repair UV damage has been linked to the nucleotide excision repair (NER) pathway [4]. This pathway repairs DNA damage, including UV-induced photoproducts that disrupt normal transcription and replication processes [5]. The repair by the NER pathway occurs by two sub-pathways: [1] induction by RNA polymerase to remove DNA adducts by transcription and [2] global genome (GG) repair initiated by a protein complex including Xeroderma pigmentosum group C protein (XPC), human centrin 2 (HC2), and RAD23B [6–8].

Human centrin 2 is a member of the calmodulin (CaM) superfamily of calcium-binding proteins and highly conserved in eukaryotes [9–11]. The centrin family plays numerous roles in algae, yeast, and humans and has been extensively studied [12–19]. Human centrin 2 is 50% sequence identical and nearly 85% sequence similar to calmodulin; nevertheless, their calcium-binding properties are different. Calmodulin undergoes a significant conformational change upon binding calcium (Ca^{2+}) [20] whereas human centrin 2 apparently does not [21,22]. In fact, full-length human centrin 2 can self-assemble in a Ca^{2+}-saturated solution, but the protein with an N-terminal truncation of the first 25 amino acid residues (Δ25-HC2) does not assemble [23].

The recognition activity of XPC to damaged DNA increases in the presence of human centrin 2; thus, the characterization of the complex is of importance in biomedicine and biophysics [24]. Human centrin 2 binds on the C-terminus of XPC between amino acids 847 and 863 [8], which has the sequence NWKLLAKGLLIRERLKR. The affinity of centrin to the XPC-peptide, as determined by isothermal titration calorimetry [8], is high ($K_a = 2.1 \times 10^8\ M^{-1}$) in the presence of saturating Ca^{2+}, and decreases in the absence of Ca^{2+} ($K_a = 7.8 \times 10^6\ M^{-1}$). More details are available in recent reviews on the subject of centrin 2 and XPC interactions [25,26].

We applied a region-specific H/D exchange (H/DX) protocol to probe the binding of Ca^{2+} and XPC-peptide to human centrin 2. The

* Corresponding author. Tel.: +1 314 935 4814; fax: +1 314 935 4481.
E-mail address: mgross@wustl.edu (M.L. Gross).

1387-3806/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijms.2012.10.013

```
-5GPLGS1MASNFKKANMASSSQRKRMSPKPEL26TEEQKQEIREAFDLFDADGT  45
                              GP26TEEQKQEIREAFDLFDADGT

GTIDVKELKVAMRALGFEPKKEEIKKMISEIDKEGTGKMNFGDFLTVMTQ  95
GTIDVKELKVAMRALGFEPKKEEIKKMISEIDKEGTGKMNFGDFLTVMTQ

KMSEKDTKEEILKAFKLFDDDETGKISFKNLKRVAKELGENLTDEELQEM  145
KMSEKDTKEEILKAFKLFDDDETGKISFKNLKRVAKELGENLTDEELQEM

IDEADRDGDGEVSEQEFLRIMKKTSLY                          172
IDEADRDGDGEVSEQEFLRIMKKTSLY
```

Fig. 1. The full length human centrin 2 sequence (top) compared to the delta-25 sequence (bottom). The amino acids in red were part of the start sequence in the expression vector. The amino acids in blue are those 25 which were deleted to make delta-25 human centrin 2. The numbering scheme refers to the full length human centrin 2 sequence.

protocol should reveal deuterium uptake changes in human centrin 2 upon binding Ca^{2+}. We also tested the interaction of human centrin 2 to the XPC peptide in the presence and absence of Ca^{2+}. The affinity of human centrin 2 to Ca^{2+} in the presence of XPC has not been previously reported.

This report also continues our interest in the biophysics of Ca^{2+}-binding proteins and the development and application of mass spectral methods for investigating them [27–31]. We are pleased to contribute this article in honor of Peter Armentrout who has contributed significantly and productively to physical chemistry and mass spectrometry.

2. Experimental

2.1. Materials

The Δ25-human centrin 2 (Δ25-HC2) protein (N-terminal truncation of the first 25 amino acids) was expressed in *E. coli* at the Mayo Clinic (Rochester, MN). A sequence comparison of full length versus the Δ25-HC2 protein is provided in Fig. 1. The residue numbering in this report refers to the full length protein sequence. Deuterium oxide, potassium chloride, formic acid, calcium chloride, acetonitrile, EGTA (ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetra-acetic acid), HEPES [*N*-(2-hydoxyethyl)piperazine-*N*′-(2-ethanesulfonic acid)], and HEPES sodium salt were purchased from Sigma–Aldrich (St. Louis, MO) at the highest purity available. The XPC peptide (Sequence: NWKLLAKGLLIRERLKR, 9 mg, 92.6% pure) was purchased from GenScript (Piscataway, NJ). Immobilized pepsin on agarose was purchased from Pierce (Rockford, IL). The molecular masses of Δ25-HC2 and the XPC peptide, as determined by electrospray ionization (ESI) mass spectrometry, were 17085.1 Da (theoretical average mass of 17085.4 Da) and 2106.3 Da (theoretical monoisotopic mass of 2106.3 Da), respectively.

2.2. H/D exchange kinetics

A stock solution of centrin 2 (119 μM) was prepared from by diluting a 7.31 mM solution of centrin 2 (previously prepared at Mayo Clinic) with 10 mM HEPES (pH = 7.4) and 150 mM KCl. A stock solution of a 3:1 protein:peptide complex was made by mixing 3.25 μL of the master human centrin 2 solution (7.31 mM) with 6.75 μL XPC peptide (10.7 mM) and diluted to a final centrin 2 concentration of 119 uM using 10 mM HEPES and 150 mM KCl. H/D exchange kinetics experiments were conducted with four samples: Ca^{2+}-free human centrin 2 (1 mM EGTA), Ca^{2+}-bound human centrin 2 (2 mM Ca^{2+}), Ca^{2+}-free XPC:human centrin 2 (3:1 ratio, 1 mM EGTA), and Ca^{2+}-bound XPC:human centrin 2 (3:1 ratio, 2 mM Ca^{2+}). To initiate exchange, 0.5 μL of the protein stock solutions were diluted with 20 μL of D_2O containing 10 mM HEPES and 150 mM KCl at 25 °C to give a solution that was > 97% D_2O. After the specified times, the exchange was quenched with ice cold 1.0 M HCl to give a final pH of 2.0.

To examine deuterium uptake for regions of the protein, 5 μL of immobilized pepsin on agarose was added to the quenched solution. The digestion was allowed to take place for 3 min at 0 °C, accompanied by a quick vortexing pulse every 15 s. After digestion, the mixture containing the beads was briefly centrifuged (2–3 s) so that they congregated at the bottom of the sample tube. The supernatant protein digest, including some undigested protein, was loaded on a C_{18} column (LC Packings, 1 mm × 15 mm, PepMap cartridge, Dionex Corp., Sunnyvale, CA) that was pre-equilibrated with 100 μL of 0.2% formic acid in water (0 °C). The column was washed with 300 μL of 0.2% formic acid in water (0 °C), back exchanging the labile sites of the peptides and the undigested protein. The peptides were separated with a LC gradient (5% B to 40% B in 6 min, 40% B to 75% B in 2 min, 75% B to 5% B in 0.5 min, 5% B to 40% B in 0.5 min, then back to 5% B for equilibration) at a flow rate of 40 μL/min (Solvent A: 95% water, 5% acetonitrile containing 0.3% formic acid; Solvent B: 5% water, 95% acetonitrile containing 0.3% formic acid). To minimize back exchange, the incoming/outgoing LC solvent line, injection valve, and sample loop were submersed in ice (0 °C).

2.3. LC-ESI/MS analysis with a Q-TOF mass spectrometer

All ESI mass spectra monitoring the H/DX were acquired in the positive-ion mode with a Waters (Micromass) Q-TOF Ultima (Manchester, UK) equipped with a Z-spray ESI source. The capillary voltage was 3.2 kV, cone voltage readback of 100 V, and the source and desolvation temperatures were 80 and 180 °C. The cone and desolvation gas flows were 40 and 400 L/h. The MS profile used for quadrupole transmission was from *m*/*z* 500, dwell for 5% of the scan time, ramped to *m*/*z* 1000 for 45% of the scan time, and then dwelled at *m*/*z* 1000 for 50% of the scan time. The total scan time was 2 s for ions of *m*/*z* 300–2000.

2.4. LC–ESI/MS–MS analysis of protein digest

A standard proteomic protocol was used to identify accurately the peptides produced during the pepsin digest. The results of this experiment verified the peptide masses observed in the H/DX protocol above. After 3 min of pepsin digestion, following the protocol above, the solution containing the protein digest, including some undigested protein, was loaded onto a C_{18} custom-packed column (75 μm i.d., 10 cm length). The peptides were separated over 70 min using an Eksigent NanoLC-1D (Dublin, CA) with an LC gradient from 3 to 97% acetonitrile containing 0.1% formic acid at a flow rate of 260 nL/min. The solution was sprayed directly from the column into the mass spectrometer using a PicoView PV-500 nanospray source (New Objective, Woburn, MA) attached to the LTQ-FTMS (Thermo, San Jose, CA), which afforded accurate mass measurements and product-ion sequencing by MS/MS. A full mass spectrum was recorded in the FT part of the instrument, operating at 100,000 mass resolving power at *m*/*z* 400, while data-dependent product-ion spectra of precursors selected by the FTMS were collected by the ion trap. The MS/MS experiments carried out in the LTQ utilized wide-band activation and dynamic exclusion for a duration of 30 s and a repeat number of two. The peptides were identified based on their accurate *m*/*z* values and their product-ion spectra and by searching against a protein database using Mascot (Matrix Science, Oxford, UK). The spectra of peptides utilized in this research were manually verified by de novo sequencing.

2.5. Data analysis

For H/D exchange data collected on the Q-TOF, the protein mass spectrum at each H/D exchange time point was deconvoluted using