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Atypical behavior in the electron capture induced dissociation of biologically relevant transition metal ion complexes of the peptide hormone oxytocin

Anne J. Kleinnijenhuis [a,b], Romulus Mihalca [a], Ron M.A. Heeren [a,b], Albert J.R. Heck [b,*]

[a] *FOM Institute for Atomic and Molecular Physics (AMOLF), Kruislaan 407, 1098 SJ Amsterdam, The Netherlands*
[b] *Department of Biomolecular Mass Spectrometry, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Sorbonnelaan 16, 3584 CA Utrecht, The Netherlands*

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Abstract

Doubly protonated ions of the disulfide bond containing nonapeptide hormone oxytocin and oxytocin complexes with different transition metal ions, that have biological relevance under physiological conditions, were subjected to electron capture dissociation (ECD) to probe their structural features in the gas phase. Although, all the ECD spectra were strikingly different, typical ECD behavior was observed for complexes of the nonapeptide hormone oxytocin with Ni^{2+}, Co^{2+} and Zn^{2+}, i.e., abundant c/z'$^{\bullet}$ and a'$^{\bullet}$/y backbone cleavages and ECD characteristic S–S and S–C bond cleavages were observed. We propose that, although in the oxytocin–transition metal ion complexes the metal ions serve as the main initial capture site, the captured electron is transferred to other sites in the complex to form a hydrogen radical, which drives the subsequent typical ECD fragmentations. The complex of oxytocin with Cu^{2+} displayed noticeably different ECD behavior. The fragment ions were similar to fragment ions typically observed with low-energy collision induced dissociation (CID). We propose that the electrons captured by the oxytocin–Cu^{2+} complex might be favorably involved in reducing the Cu^{2+} metal ion to Cu^{+}. Subsequent energy redistribution would explain the observed low-energy CID-type fragmentations. Electron capture resulted also in quite different specific cleavage sites for the complexes of oxytocin with Ni^{2+}, Co^{2+} and Zn^{2+}. This is an indication for structural differences in these complexes possibly linked to their significantly different biological effects on oxytocin-receptor binding, and suggests that ECD may be used to study subtle structural differences in transition metal ion–peptide complexes.

Keywords: Oxytocin; Electron capture induced dissociation; Metal binding

1. Introduction

Nowadays tandem mass spectrometry is one of the most valuable tools used in the structure analysis of peptides and proteins [1,2]. In these experiments peptides formed after solution-phase proteolysis of proteins are brought into the gas-phase usually either by matrix assisted laser desorption ionization (MALDI) or electrospray ionization (ESI). The most sensitive approach is to embed the peptides either in acidic matrices (MALDI) or acidified aqueous solutions (ESI), which enables the efficient formation of singly or multiply protonated peptide ions. Subsequently, these protonated peptide ions are fragmented in a collision cell within the vacuum of the mass spectrometer, which induces fragmentation of the peptides and is termed collision induced dissociation (CID). Typically relatively low collision energies are used in these experiments and activation and fragmentation occurs via multiple collision events. These collision induced dissociation experiments primarily induce backbone fragmentations in the peptide, with a preference for cleavages in the "weakest" amide bond. Depending on where the charge remains in the two concomitant fragments either b or y ions are formed [2,3]. Because of this specificity tandem MS spectra of protonated peptides are relatively simple, allowing the determination of the peptide sequence with relative ease. Therefore, it is not surprising that tandem mass spectrometry is at present one of the most valuable tools used in high-throughput identification of proteins as performed in proteomics experiments.

* Corresponding author. Tel.: +31 30 2536797; fax: +31 30 2518219.
E-mail address: a.j.r.heck@chem.uu.nl (A.J.R. Heck).

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Besides the many merits of tandem mass spectrometry of (multiply) protonated peptides by low-energy collision induced dissociation, there are also some disadvantages to this approach. It is well known that peptides often do not retain their labile modifications, such as glycosylation and phosphorylation in CID. Moreover, the relatively slow process of CID may induce substantial rearrangement reactions in the precursor ions, prior to fragmentation, a fact that for instance seriously hampers the use of CID in hydrogen/deuterium exchange mass spectrometry [4]. A decade ago a new activation method was introduced to fragment biomolecular ions termed electron capture dissociation (ECD) [5,6]. In ECD multiply charged cations stored in a Fourier transform ion cyclotron resonance (FTICR) mass spectrometer are irradiated with low-energy electrons. As the multiply protonated peptide ions are even electron systems, the ECD process leads to the formation of odd-electron species or radical cations. It is now well established that part of these ions undergo very fast fragmentation reactions [7–10]. Therefore, ECD typically does not result in cleavage of the "weakest" peptide bonds, but in formation of so-called c and z'$^{\bullet}$ ions and to a minor extent also to a'$^{\bullet}$ and y ions [3,5,11]. Being a complementary tool to low-energy CID, ECD has been proven to be particularly useful in the analysis of labile post-translational modifications [12–16], because these modifications are retained on their initial position during the fast dissociative process. Successful application of ECD has been demonstrated in the analysis of many different peptide post-translational modifications, such as phosphorylation [15,17], glycosylation [13,14,18] and disulfide and monosulfide bonds [6,19,20], all areas of structural analysis in which conventional CID often fails to produce good analytical results.

Many proteins and peptides display well-defined secondary and tertiary "native" structures in solution, which are usually lost in the acidic matrices or acidified solutions used in MALDI and ESI, respectively. Such "native" structures can be influenced by co-factors, such as small nucleotides or metal ions. An example of that is the biologically important small peptide hormone oxytocin, produced mainly by the magnocellular neurons of the hypothalamus. It exerts various hormonal effects, but is mostly known for its ability to elicit the contraction of smooth muscle in the uterus during labor. It is also critically involved in higher cognitive functions such as memory and learning [21]. Oxytocin (Fig. 1) is a nonapeptide containing a disulfide bond between the Cys1 and the Cys6 residue. The presence of a specific divalent transition metal ion dramatically enhances binding of oxytocin to its cellular receptor to the nanomole range [21]. Oxytocin

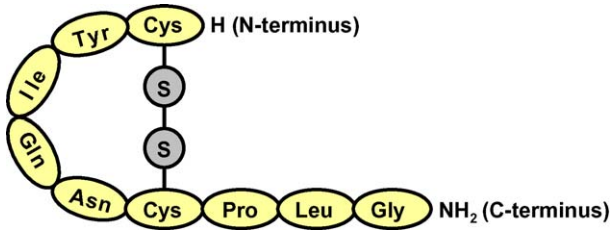

Fig. 1. Chemical structure of the nonapeptide oxytocin. At the C-terminus there is an amide instead of a carboxyl group.

binding to its receptor is potentiated in increasing order by Zn^{2+}, Ni^{2+} and Co^{2+}, but is negligible in the presence of Cu^{2+}. The affinity of bare oxytocin for transition metal ions is relatively low with dissociation constants (K_d's) in the mM to μM range [22].

Calculated molecular structures, optimized with molecular mechanics and density functional theory methods, showed that the binding of a Zn^{2+} to oxytocin results in a significant change of structure, in which the backbone carbonyl oxygens of six residues solvate the doubly charged zinc ion in an octahedral coordination shell [23]. Here we probed the structure of oxytocin–transition metal ion complexes using ECD. By binding divalent transition metal ions such as Ni^{2+}, Co^{2+}, Zn^{2+} and Cu^{2+} the oxytocin becomes automatically doubly charged in ESI, without the requirement of additional protons. We assume that the transition metal ions act as the main initial electron capture site, even though structures with the metal ion bound to a deprotonated amide bond and an additional proton in the complex cannot be ruled out. The double charge of the metal ions results in a much higher probability of electron capture compared to protons, as the electron capture cross-section is dependent on the charge squared [24]. Fast ECD cleavages in the oxytocin–transition metal ion complexes could reflect the location and/or coordination of the metal ion in the complex. Surprisingly, the ECD spectra of each different complex are spectacularly different, indicating that the electronic configuration of the transition metal ion in the complexes with oxytocin may lead to substantial structural differences in the ions, inducing very distinct fragmentations patterns.

2. Experimental

The fragmentation of oxytocin (Sigma) was studied with ECD in an FTICR mass spectrometer. Doubly protonated oxytocin ions and oxytocin ions complexed with Ni^{2+}, Co^{2+}, Zn^{2+} and Cu^{2+} ions were subjected to ECD. The ESI solution consisted typically of a 49:49:2 water:acetonitrile:acetic acid mixture and contained 20 μM oxytocin. Complexation of oxytocin to a transition metal ion was accomplished by adding the acetate salt of the metal ion (25 times in excess) to the ESI solution. The high-resolution mass spectra revealed that complexation of the oxytocin by the transition metal ions resulted in [oxytocin + M^{2+}] ions, without excess protons, indicating that formally the transition metal is the charge carrier and the oxytocin neutral. The FTICR instrument used was a modified Bruker-Spectrospin (Fällanden, Switzerland) Apex 7.0e, equipped with a 7 T superconducting magnet. The experiments were performed using an infinity cell [25]. The electron gun was an indirectly heated barium–tungsten dispenser cathode (TB-198, HeatWave Labs Inc.) placed inside the magnet approximately 30 cm behind the cell. Just above the surface of the cathode there is a copper extraction grid. The cathode was operated using a current of 1.85 A and a potential of 7.9 V, yielding a power of 14.6 W. After isolation of the ions of interest, they were exposed to low-energy electrons. During this time the grid potential was pulsed from −100 to 100 V and the cathode surface potential from 25

to −0.15 V. The trapping plates were held at a potential of 1 V. For all the ion species in this study it took quite a long time of electron irradiation, namely 10 s, to get satisfactory ECD spectra. Mainly this must have been due to poor overlap between the electron beam and the ion cloud. Argon was used as the trapping gas. Data acquisition and analysis was performed using in-house developed software and hardware [26].

3. Nomenclature

The nomenclature in this article for peptide fragment ions is based on the nomenclature proposed by Biemann [11] and Roepstorff and Fohlman [3]. When a fragment has a mass 1 Da less than the normal mass of a regular peptide fragment ion, an ' is added before the letter annotation, when it is 1 Da heavier an ' is added behind the letter. A radical is annotated by adding a dot. When a backbone bond and an S–S or S–C bond are cleaved, a regular fragment ion in our nomenclature includes a hydrogen atom bound to the resulting free sulfur or carbon atom in the charge carrying fragment. When a backbone bond and an S–C bond are cleaved +S or −S is added behind the letter, depending on whether the fragment ion contains two sulfur atoms or no sulfur atom. Every single fragment ion observed in our ECD spectra of oxytocin–transition metal ion complexes retained the metal ion. Therefore, we choose not to annotate the metal ion.

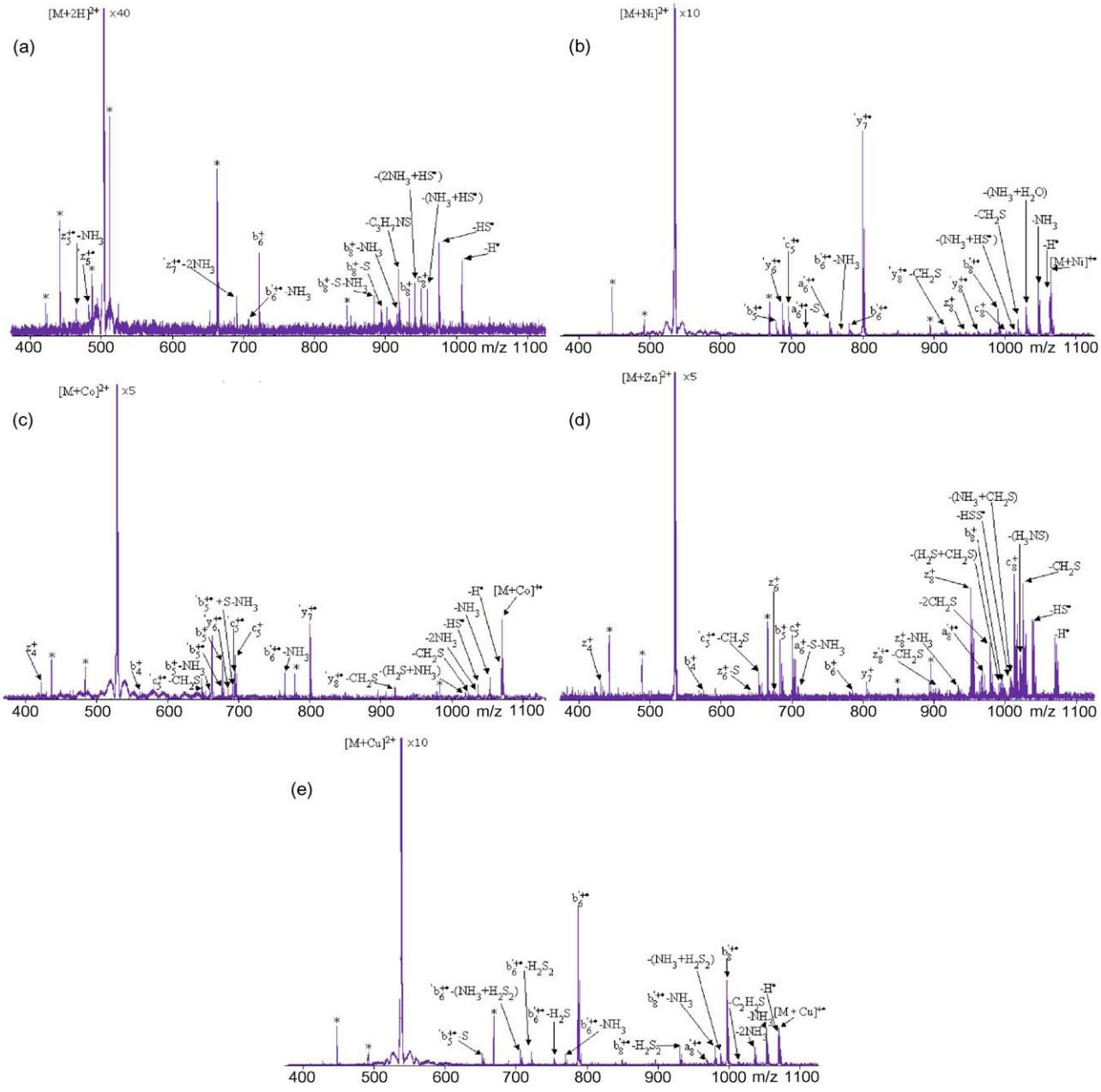

Fig. 2. ECD spectra, taken under identical experimental conditions, of: (a) [oxytocin + $2H^{2+}$], (b) [oxytocin + Ni^{2+}], (c) [oxytocin + Co^{2+}], (d) [oxytocin + Zn^{2+}] and (e) [oxytocin + Cu^{2+}] ions. The most abundant fragment ions are annotated and summarized in Table 1. The peaks indicated with * originate from "noise" signals.

4. Results

In Fig. 2 the ECD spectra of doubly protonated oxytocin ions (Fig. 2(a)) and oxytocin complexed with Ni^{2+} (Fig. 2(b)), Co^{2+} (Fig. 2(c)), Zn^{2+} (Fig. 2(d)) and Cu^{2+} ions (Fig. 2(e)) are given, which were obtained under identical experimental conditions. Additionally, the most abundant fragment ions are summarized and annotated in Table 1. In ECD of protonated peptides c and z'$^{\bullet}$ fragment ions are typically the most abundant backbone cleavages, with some minor a'$^{\bullet}$ and y fragment ions [5,27,28]. Commonly observed small neutral losses are H$^{\bullet}$ loss and NH_3 loss. In peptides containing disulfide bonds preferentially S–S and S–C bonds are cleaved, resulting in backbone cleavages near the disulfide bond [6,20,29,30].

The ECD spectra of doubly protonated oxytocin ions (Fig. 2(a) and Table 1) displayed such typical ECD fragmentations. The electron capture induced backbone cleavages resulted in c and 'z$^{\bullet}$ ions. Interestingly, also quite abundant b ions were observed, which are less common in ECD spectra of peptides, but do occur [31]. The most intense small neutral losses were HS$^{\bullet}$ loss, H$^{\bullet}$ loss, C_3H_7NS loss and NH_3 loss. Overall about 60% of the product ion intensities could be attributed to fragment ions resulting from disulfide bond related characteristic ECD cleavages (fragmentation involving cleavage of atleast one S–S or S–C bond), confirming previously reported findings that disulfide bonds are preferentially cleaved in ECD [6,19,29,30,32]. Additional minor backbone cleavage sites were mainly located in the C-terminal part of oxytocin (Fig. 3(a)).

We reiterate, as observed previously by Wei et al. [33], that in all the studied oxytocin–transition metal ion complexes the metal ions formed complexes with oxytocin in a 1:1 ratio, without extra protonation or deprotonation giving [oxytocin + M^{2+}] ions. Most strikingly, as can be seen in Fig. 2 the ECD spectra of all studied oxytocin–transition metal ion complexes deviate not only from the ECD spectra obtained for the doubly protonated oxytocin, they also show distinctive variation depending on the particular transition metal ion. In the ECD spectra of the [oxytocin + Ni^{2+}] ions (Fig. 2(b) and Table 1) radical 'y$^{\bullet}$ ions, usually a minor ECD product, were the most abundant fragment ions. Minor backbone cleavages resulted in the formation of b'$^{\bullet}$, 'c$^{\bullet}$ and a'$^{\bullet}$ ions. Abundant small neutral losses were NH_3, H_2O, H$^{\bullet}$ and CH_2S. Also minor HS$^{\bullet}$ loss was observed. Overall fragment ions resulting from disulfide bond related characteristic ECD cleavages accounted for about 60% of the product ion intensity. The most intense '$y_7^{+\bullet}$ fragment ion for instance, resulted from S–S cleavage and backbone amide bond cleavage between Tyr2 and Ile3 (see Fig. 3(b)). The assignment of this ion was confirmed with subsequent sustained off-resonance irradiation collision induced dissociation (SORI-CID, data not shown).

The ECD fragment ions of [oxytocin + Co^{2+}] (Fig. 2(c) and Table 1) were quite similar to the fragment ions observed for [oxytocin + Ni^{2+}] (Fig. 2(b)). The most intense small neutral losses were H$^{\bullet}$ loss, NH_3 loss and CH_2S loss. Also minor H_2S and HS$^{\bullet}$ losses were observed. Fragment ions resulting from disulfide bond related characteristic ECD cleavages accounted for approximately 65% of the product ion intensity. The main backbone cleavage sites in the [oxytocin + Co^{2+}] complex were

Table 1
Overview of most abundant fragment ions observed in the ECD spectra of doubly protonated oxytocin and oxytocin complexed to different transition metal ions

Parent ion	Production	PII (%)	Measured *m/z*	Calculated *m/z*
$[M+2H]^{2+}$	–H$^{\bullet}$	15.7	1007.447	1007.445
	–SH$^{\bullet}$	12.7	975.467	975.473
	b_6	11.9	723.261	723.259
	$-C_3H_7NS$	10.9	919.422	919.422
	$-(2NH_3 + SH^{\bullet})$	8.0	941.421	941.419
	b_8	6.5	933.393	933.396
	SH$^{\bullet}$ + NH_3	5.6	958.467	958.446
	'$z_7$$^{\bullet}$–$2NH_3$	5.4	691.292	691.300
	c_8	4.6	950.445	950.423
	'$z_5$$^{\bullet}$–$NH_3$	3.9	467.182	467.184
$[M+Ni]^{2+}$	'$y_7$$^{\bullet}$	38.8	798.306	798.300
	$-NH_3$	10.5	1047.346	1047.345
	$[M+Ni]^{+\bullet}$	9.2	1064.367	1064.372
	$-(NH_3 + H_2O)$	6.9	1029.354	1029.334
	–H$^{\bullet}$	6.9	1063.386	1063.364
	'$y_6$$^{\bullet}$	4.8	685.214	685.216
	b_8'$^{\bullet}$	4.6	990.333	990.323
	'$c_5$$^{\bullet}$	4.4	694.205	694.204
	$-CH_2S$	2.5	1018.407	1018.384
	a_6'$^{\bullet}$	2.2	752.189	752.191
$[M+Co]^{2+}$	'$y_7$$^{\bullet}$	23.0	799.302	799.297
	$[M+Co]^{+\bullet}$	15.9	1065.353	1065.369
	c_5	8.3	696.218	696.210
	–H$^{\bullet}$	7.4	1064.384	1064.361
	b_5	6.2	679.184	679.183
	'$c_5$$^{\bullet}$	5.8	695.212	695.202
	NH_3	5.4	1048.340	1048.342
	b_6'$^{\bullet}$–NH_3	5.2	764.171	764.157
	'$c_5$$^{\bullet}$–$CH_2S$	4.4	649.216	649.214
	'$y_8$$^{\bullet}$–$CH_2S$	3.4	916.380	916.372
$[M+Zn]^{2+}$	c_8	14.3	1012.334	1012.336
	$-CH_2S$	13.3	1024.369	1024.378
	z_8	11.0	951.336	951.338
	–SH$^{\bullet}$	10.5	1037.385	1037.386
	–H$^{\bullet}$	7.6	1069.359	1069.358
	c_5	6.6	701.209	701.206
	$-2CH_2S$	5.3	978.386	978.381
	b_5	5.1	684.183	684.179
	$-H_3NS$	3.0	1021.368	1021.367
	a_8'$^{\bullet}$	2.7	968.313	968.322
$[M+Cu]^{2+}$	b_6'$^{\bullet}$	23.0	785.187	785.181
	b_8'$^{\bullet}$	15.9	995.315	995.318
	–H$^{\bullet}$	8.3	1068.365	1068.359
	$-NH_3$	7.4	1052.339	1052.340
	b_8'$^{\bullet}$–NH_3	6.2	978.282	978.291
	b_8'$^{\bullet}$–H_2S_2	5.8	929.351	929.358
	$[M+Cu]^{+\bullet}$	5.4	1069.359	1069.367
	$-2NH_3$	5.2	1035.314	1035.313
	b_6'$^{\bullet}$–(H_3NS_2)	4.4	704.217	704.210
	$-(NH_3 + H_2S_2)$	3.4	986.386	986.380

The *m/z* values in the table correspond to the first isotope of the mentioned ion species, all the peaks were isotopically resolved. PII is the abbreviation for product ion intensity, which is the sum of the intensities of all isotopic peaks of all product ions. For all the ECD product ions from oxytocin–transition metal ion complexes the same nomenclature was used. Therefore, many ECD product ions from [oxytocin + Cu^{2+}] ions appear to be radical, whilst actually they are non-radical. When the mechanism proposed in Fig. 4 (on the right) occurs, the captured electrons are namely involved in Cu^{2+} reduction instead of H$^{\bullet}$ formation elsewhere in the complex.

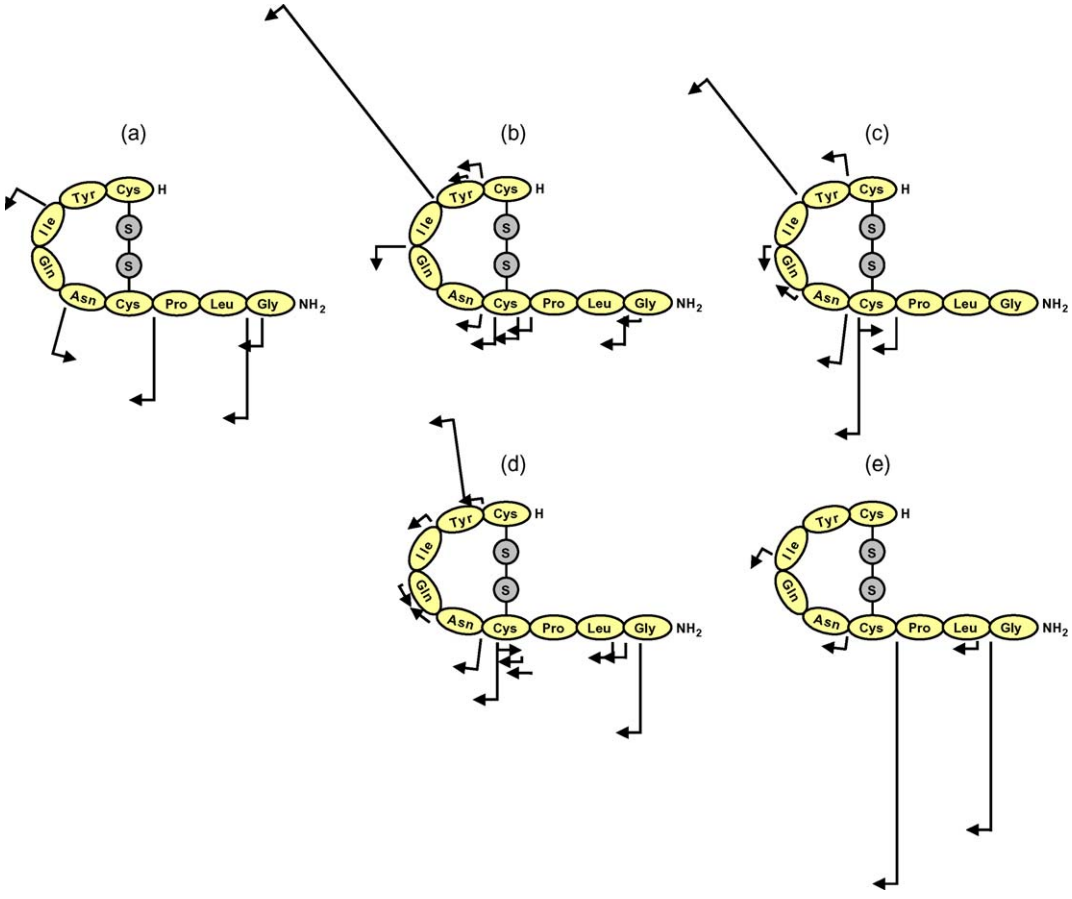

Fig. 3. Schematic picture of the preferred localization of the electron capture induced backbone cleavages in (a) [oxytocin + $2H^{2+}$], (b) [oxytocin + Ni^{2+}], (c) [oxytocin + Co^{2+}], (d) [oxytocin + Zn^{2+}] and (e) [oxytocin + Cu^{2+}] ions.

the amide bond between Tyr2 and Ile3 and the N–C_α bond in Cys6 (Fig. 3(c)).

The ECD fragment ions of [oxytocin + Zn^{2+}] (Fig. 2(d) and Table 1) showed resemblance with the fragment ions observed for [oxytocin + Co^{2+}] (Fig. 2(c)). However, the most abundant backbone fragment ions were c and z ions and minor backbone fragment ions were b and a'$^\bullet$ ions. Abundant small neutral losses were CH_2S loss, $HS^\bullet$ loss and $H^\bullet$ loss, but also less abundant NH_3, H_2S and $HSS^\bullet$ losses were observed. Approximately 70% of the product ion intensity resulted from disulfide bond related characteristic ECD cleavages. The main cleavage sites were the N–C_α bonds in Tyr2, Cys6 and Gly9 (Fig. 3(d)).

Summarizing the ECD results obtained for the oxytocin complexes with Ni^{2+}, Co^{2+} and Zn^{2+}, it can be stated that the specific transition metal ion bound plays an important role in the fragment ions formed, as locations of the backbone cleavage sites in the three complexes are strikingly different (see Table 1). However, the ECD spectra of the oxytocin complexes with Ni^{2+}, Co^{2+} and Zn^{2+} have in common that fragment ions resulting from disulfide bond related characteristic ECD cleavages are remarkably abundant. Furthermore, abundant $H^\bullet$ losses were observed from the reduced oxytocin–transition metal ion complexes. Together with the observed backbone cleavages we could therefore conclude that the oxytocin-complexes with Ni^{2+}, Co^{2+} and Zn^{2+} display quite typical ECD behavior.

In contrast, we observe that the ECD behavior of the [oxytocin + Cu^{2+}] is rather different (Fig. 2(e) and Table 1). In the ECD spectra of [oxytocin + Cu^{2+}] b'$^\bullet$ ions are the most abundant fragment ions. Remarkably, no ECD characteristic c or z'$^\bullet$ fragment ions were formed, although electron capture was observed. $H^\bullet$ loss was the only observed radical loss. Fragment ions resulting from disulfide bond related characteristic ECD cleavages accounted for only 15% of the product ion intensity. This is in clear contrast to the other studied oxytocin–transition metal ion complexes, where such ions accounted for 60–70% of the fragment ion intensity. The fragment ions observed in the ECD spectra of [oxytocin + Cu^{2+}] ions are thus rather atypical, and resemble more those typically observed in low-energy CID. In peptides low-energy CID mainly leads to the formation of backbone b and y fragments. For the [oxytocin + Cu^{2+}] complex the most favored backbone cleavage sites were the amide bonds between Cys6/Pro7 and Leu8/Gly9 (Fig. 3(e)).

5. Discussion

Oxytocin contains a disulfide bond in between two cysteines at positions 1 and 6 (see Fig. 1). Therefore, we hypothesized that this molecule is rather rigid, which may be even enhanced by transition metal ion binding. Surprisingly, we observed that the location of the cleavage sites in all the four

oxytocin–transition metal ion complexes that were subjected to ECD were remarkably different, not only from the ECD spectra obtained for the doubly protonated oxytocin, but also from each other. These large differences were a priori not expected for such a small, seemingly rigid molecule. We seek an explanation for the observed behavior in the location and the identity of the charge carrier, i.e., either the two protons or the transition metal ion. The transition metal ions all have a different electronic configuration, which may lead to different coordination towards oxytocin, resulting also in slightly different solution- and gas-phase structures. Additionally, the electron capture process may be different for the studied species, as the nature of the transition metal ion will have an effect on the released recombination energy. Moreover, the extent of charge localization/delocalization (particularly of the captured electron), may be different for the four oxytocin–transition metal ion complexes. Below we discuss the above-mentioned factors that may play a role in the observed dissimilar ECD behavior in more detail. First we focus on the similarities observed in the ECD processes.

From experimental as well as theoretical studies [6,20,29,30] it is known that S–S and S–C bonds are preferentially cleaved when ECD is performed on peptides containing disulfide bonds. Therefore, we took the abundance of S–S and S–C bond cleavages, as well as the in ECD frequently observed $H^{\bullet}$ loss and typical backbone cleavages (c/z'$^{\bullet}$ and a'$^{\bullet}$/y) [27], as a measure for "standard" ECD behavior. In ECD of doubly protonated oxytocin a high prevalence for S–S and S–C bond cleavage was observed. Fragment ions resulting from cleavage of either an S–S or an S–C bond, or both, accounted for approximately 60% of the product ion intensity. The loss of $HS^{\bullet}$ for instance, which requires the cleavage of both an S–S and an S–C bond, was very intense. Also most backbone cleavages, observed in ECD of doubly protonated oxytocin were typical for ECD, namely c and 'z$^{\bullet}$ ions. We also observed some b ions, which are typically not observed in ECD spectra of peptides. The possibility of unintended collisional activation is excluded as the ECD spectra of substance P $[M+2H]^{2+}$ ions taken under identical conditions did not show signs of collisional activation. Generally, many small neutral losses were observed. Cleavage of the disulfide bond could trigger hydrogen migration and subsequent elimination of small neutrals [34]. Overall the ECD behavior for doubly protonated oxytocin ions is quite standard and can be used to put the ECD results obtained for the oxytocin–transition metal ion complexes into perspective.

5.1. The oxytocin + Ni^{2+}, Co^{2+} and Zn^{2+} complexes display characteristic ECD behavior

Similar as in ECD of the doubly protonated ions many fragment ions in ECD of the oxytocin complexes with Ni^{2+}, Co^{2+} and Zn^{2+} resulted from disulfide bond related characteristic ECD cleavages. Other typical ECD backbone fragmentations were observed ranging from mainly radical 'y$^{\bullet}$ fragments for [oxytocin + Ni^{2+}] ions to mainly c and z fragments for [oxytocin + Zn^{2+}] ions. The ECD spectra of [oxytocin + Co^{2+}] ions displayed features of both [oxytocin + Ni^{2+}] and [oxytocin + Zn^{2+}] ions, with mainly 'y$^{\bullet}$, c and 'c$^{\bullet}$ backbone fragments. Generally, the ECD behavior of the oxytocin complexes with Ni^{2+}, Co^{2+} and Zn^{2+} leads to very specific preferences (see Fig. 2 and Table 1) in fragmentations, albeit that they are all characteristic for ECD of disulfide containing peptide ions. An interesting question is now how does electron capture take place in peptide–metal ion complexes? It has been proposed before that in peptide–metal ion complexes the metal ion serves as an initial sink for electrons (positive charge attracts negatively charged electrons). However, the electron may be subsequently transferred from the original capture site [35]. In other words, the metal ions are involved in Coulomb-assisted electron capture and subsequent intramolecular hydrogen transfer explains the typical ECD cleavages [32,36]. We indeed rationalize in our ECD experiments on the oxytocin complexes with Ni^{2+}, Co^{2+} and Zn^{2+} the dominant characteristic ECD behavior by the occurrence of subsequent electron transfer from the metal ion to the remainder of the complex. In line with this, $H^{\bullet}$ loss, which is common in ECD [5,6,37,38], was observed from all reduced oxytocin–transition metal ion complexes, that are thus able to transfer their captured electron to a proton. Because there are no extra protons in the transition metal ion complexes of oxytocin, free $H^{\bullet}$ radicals must have been formed in the complex together with a negatively charged deprotonated oxytocin bound to the doubly charged transition metal ion (as outlined in Fig. 4 on the left). It has been well-established that such free $H^{\bullet}$ radicals have a high affinity for disulfide bonds [6]. Transfer of the free $H^{\bullet}$ radicals to the disulfide bond may thus lead to the characteristic ECD behavior observed. The [oxytocin + Co^{2+}] ions contain an odd number of electrons. Therefore, after electron capture an even-electron system is formed, that still displays ECD behavior! This can be explained by assuming that the [oxytocin + Co^{2+}] complex that captures an electron actually contains two unpaired electrons. One electron is acting as an unpaired d [7] cobalt ion electron. The other electron is triggering the ECD cleavages in a manner described above.

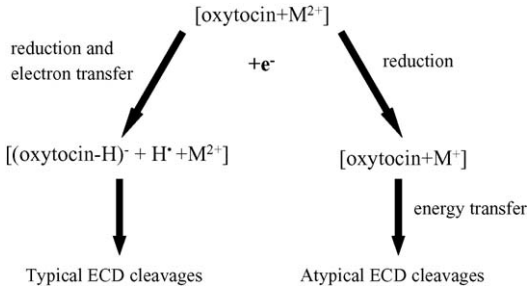

Fig. 4. Proposed mechanisms for the electron capture process in oxytocin–transition metal ion complexes. Capture of an electron can either lead to reduction of the transition metal ion (on the right), where after energy transfer to the oxytocin peptide induces low-energy peptide bond cleavages, similar to those observed in low energy CID. Alternatively, electron capture by the transition metal ion is followed by electron transfer to the oxytocin moiety where it induces the formation a free hydrogen radical in complex with the formally negatively charged deprotonated oxytocin and the doubly charged transition metal ion (on the left). The free hydrogen radical, initiates the observed characteristic ECD cleavages, preferentially by attacking the disulfide bond present in oxytocin.

5.2. The extraordinary oxytocin + Cu^{2+} complex

ECD of [oxytocin + Cu^{2+}] ions resulted in remarkably different fragmentation reactions. No c or z'• ions were observed, as backbone cleavages almost exclusively led to the formation of b'• ions, which are atypical for ECD. The disulfide bond related characteristic ECD cleavages contributed only about 15% to the total product ion intensity (see Table 1), whereas this number was close to 65% for doubly protonated oxytocin ions and the oxytocin complexes with Ni^{2+}, Co^{2+} and Zn^{2+}. Evidently, the ECD process in the [oxytocin + Cu^{2+}] complex follows a different mechanism. We argue that the electron captured by the [oxytocin + Cu^{2+}] complex hardly participates in ECD processes. The electron is initially captured by the Cu^{2+} to reduce it to Cu^{+}, i.e., the electron remains localized at the metal. A preferred use of the captured electron for reduction of the metal over transfer to the peptide can be explained by looking at the electronic structure of Cu^{+}. Cu^{+} is an electronically stable ion, with a closed d shell, in contrast to Ni^{+}, Co^{+} and Zn^{+}. If electron capture leads to the reduction of Cu^{2+} ions, excited Cu^{+} ions would be formed (see Fig. 4 on the right). Not the electron but the available recombination energy is subsequently transferred to the oxytocin peptide, leading to the breaking of the weakest peptide bonds, similar to those observed in low-energy CID. Although, there are not that many ECD studies on peptide–transition metal ion complexes the observed typical behavior observed here for the Cu^{2+} complex is not unprecedented. For instance, Kellersberger and Fabris [39] reported a relatively low abundance of electron capture induced fragmentation for the Cu^{2+} complex of a calmodulin EF-hand 1 peptide, when compared to the similar complexes with Ca^{2+} and Zn^{2+}. Related, Zubarev et al. [24] reported in an ECD study on cytochrome c ions that the region around the heme group (containing Fe^{3+}) remained relatively immune to c/z'• cleavage, when compared to stretches in the protein further away from the heme. Similarly, the electron captured near the iron in cytochrome c could be involved in reducing Fe^{3+} to Fe^{2+} not triggering typical ECD cleavages. Although, the ECD behavior of the [oxytocin + Cu^{2+}] complex is strikingly different from the other studied ions, the reduced [oxytocin + Cu^{2+}] ions did show to a minor extent H• loss. This means that also in the [oxytocin + Cu^{2+}] complex the alternative mechanism can still play a role (Fig. 4 on the left). We suggest that metal ion reduction and electron transfer to the peptide are competing processes. In complexes where reduction of the metal ion leads to the formation of another stable metal ion, such as in the case of Cu^{2+} and Fe^{3+} ions, reduction of the metal ion can be the dominant process.

5.3. Structural effects

Above we focused on the abnormal behavior observed in the ECD fragmentation of the [oxytocin + Cu^{2+}] complex, and discussed the similarities observed in the ECD spectra of the doubly protonated oxytocin and the oxytocin complexes with Ni^{2+}, Co^{2+} and Zn^{2+}. These similarities are mainly in the ECD characteristic nature of the fragment ions. As can be seen in Fig. 2 and Table 1, the ECD spectra are strikingly different when we consider the location of the cleavage sites. It has been stated that ECD can be used to localize charge carriers in ions. For instance, Kellersberger and Fabris [39] reported that metal chelation during ECD is conserved, as evidenced by the fact that transition metal ion complexes of calmodulin EF-hand 1 with Ca^{2+}, Zn^{2+} and Cu^{2+} displayed extensive fragmentations in residues involved in metal coordination. Therefore, the remarkable differences in backbone cleavage sites we observe for the oxytocin–transition metal ion complexes could point at structural differences, likely linked to the different potentiating effects these transition metal ions have on the binding of oxytocin to its receptor [22]. The earlier mentioned study by Seuthe et al. [23] on the structure of the gas-phase oxytocin complex with Zn^{2+} suggests that this complex adopts a helical shape, in which the backbone oxygen atoms of six residues (Tyr2, Ile3, Gln4, Cys6, Leu8, Gly9) solvate the Zn^{2+} ion in an octahedral coordination. This means that the Zn^{2+} ion is tightly embedded in the structure of oxytocin. As illustrated in Fig. 3(e) the [oxytocin + Zn^{2+}] ions display preferred cleavage sites at the N–C_α bonds of Gly9, Cys6 and Tyr2. These residues are all involved in coordinating Zn^{2+}. Furthermore, no c/z'• cleavages were observed in amino acids that are not involved in coordinating the Zn^{2+} ion. With these observations it seems that ECD reflects the coordination of the metal ion in the oxytocin–Zn^{2+} complex. Without further knowledge of the three-dimensional structures of the complexes of oxytocin with Ni^{2+}, $Co^{2+}$and Cu^{2+}, however, we can only speculate whether we can extrapolate this observation to the other complexes. It is, however, likely that the coordination of the metal ion, and thus the interactions of the metal ion with specific residues of oxytocin, is dissimilar for the different transition metal ion complexes. High-level theoretical calculations on the gas-phase structures of the initial and reduced oxytocin–transition metal ion complexes would be needed to validate our proposal.

6. Conclusion

Oxytocin complexes with Ni^{2+}, Co^{2+} and Zn^{2+} showed typical ECD behavior with disulfide bond related characteristic ECD cleavages accounting for 60–70% of the product ion intensity. In contrast, the oxytocin–Cu^{2+} complex showed mainly low-energy CID type cleavages. Disulfide bond related characteristic ECD cleavages accounted for only 15% of the product ion intensity. Therefore, we propose that in this complex the captured electron is more favorably involved in reduction of the Cu^{2+} ion to Cu^{+}. Subsequent transfer of the recombination energy, instead of electron transfer, would explain the atypical ECD cleavages we observed. All the oxytocin–transition metal ion complexes showed H• loss, indicating that the captured electron can be transferred to a proton. Due to the absence of free protons in the complexes this must be accompanied by the formation of a negatively charged site. The remarkable differences in the observed backbone cleavage sites could point at structural differences between the oxytocin–transition metal ion complexes.

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References

[1] R. Aebersold, M. Mann, Nature 422 (2003) 198.
[2] H. Steen, M. Mann, Nat. Rev. Mol. Cell. Biol. 5 (2004) 699.
[3] P. Roepstorff, J. Fohlman, Biomed. Mass Spectrom. 11 (1984) 601.
[4] J.A.A. Demmers, D.T.S. Rijkers, J. Haverkamp, J.A. Killian, A.J.R. Heck, J. Am. Chem. Soc. 124 (2002) 11191.
[5] R.A. Zubarev, N.L. Kelleher, F.W. McLafferty, J. Am. Chem. Soc. 120 (1998) 3265.
[6] R.A. Zubarev, N.A. Kruger, E.K. Fridriksson, M.A. Lewis, D.M. Horn, B.K. Carpenter, F.W. McLafferty, J. Am. Chem. Soc. 121 (1999) 2857.
[7] F. Turecek, E.A. Syrstad, J. Am. Chem. Soc. 125 (2003) 3353.
[8] F. Turecek, E.A. Syrstad, J.L. Seymour, X. Chen, C. Yao, J. Mass Spectrom. 38 (2003) 1093.
[9] F. Turecek, J. Am. Chem. Soc. 125 (2003) 5954.
[10] V. Bakken, T. Helgaker, E. Uggerud, Eur. J. Mass Spectrom. (Chichester, Eng) 10 (2004) 625.
[11] K. Biemann, Biomed. Environ. Mass Spectrom. 16 (1988) 99.
[12] Z. Guan, N.A. Yates, R. Bakhtiar, J. Am. Soc. Mass Spectrom. 14 (2003) 605.
[13] E. Mirgorodskaya, P. Roepstorff, R.A. Zubarev, Anal. Chem. 71 (1999) 4431.
[14] K. Hakansson, H.J. Cooper, M.R. Emmett, C.E. Costello, A.G. Marshall, C.L. Nilsson, Anal. Chem. 73 (2001) 4530.
[15] A. Stensballe, O.N. Jensen, J.V. Olsen, K.F. Haselmann, R.A. Zubarev, Rapid Commun. Mass Spectrom. 14 (2000) 1793.
[16] N.L. Kelleher, R.A. Zubarev, K. Bush, B. Furie, B.C. Furie, F.W. McLafferty, C.T. Walsh, Anal. Chem. 71 (1999) 4250.
[17] S.D. Shi, M.E. Hemling, S.A. Carr, D.M. Horn, I. Lindh, F.W. McLafferty, Anal. Chem. 73 (2001) 19.
[18] K. Hakansson, M.J. Chalmers, J.P. Quinn, M.A. McFarland, C.L. Hendrickson, A.G. Marshall, Anal. Chem. 75 (2003) 3256.
[19] O.A. Mirgorodskaya, K.F. Haselmann, F. Kjeldsen, R.A. Zubarev, Eur. J. Mass Spectrom. 9 (2003) 139.
[20] A.J. Kleinnijenhuis, M.C. Duursma, E. Breukink, R.M.A. Heeren, A.J.R. Heck, Anal. Chem. 75 (2003) 3219.
[21] C. Barberis, B. Mouillac, T. Durroux, J. Endocrin. 156 (1998) 223.
[22] A.F. Pearlmutter, M.S. Soloff, J. Biol. Chem. 254 (1979) 3899.
[23] A.B. Seuthe, D. Liu, O.T. Ehrler, X. Zhang, T. Wyttenbach, M.T. Bowers, Proceedings of the 52nd ASMS Conference on Mass Spectrometry and Allied Topics, Nashville, TN, 2004.
[24] R.A. Zubarev, D.M. Horn, E.K. Fridriksson, N.L. Kelleher, N.A. Kruger, M.A. Lewis, B.K. Carpenter, F.W. McLafferty, Anal. Chem. 72 (2000) 563.
[25] P. Caravatti, M. Allemann, Org. Mass Spectrom. 26 (1991) 514.
[26] T.H. Mize, I. Taban, M. Duursma, M. Seynen, M. Konijnenburg, A. Vijftigschild, C.V. Doornik, G.V. Rooij, R.M.A. Heeren, Int. J. Mass Spectrom. 235 (2004) 243.
[27] R.A. Zubarev, Mass Spectrom. Rev. 22 (2003) 57.
[28] R.A. Zubarev, Curr. Opin. Biotechnol. 15 (2004) 12.
[29] E. Uggerud, Int. J. Mass Spectrom. 234 (2004) 45.
[30] F. Turecek, M. Polasek, A.J. Frank, M. Sadilek, J. Am. Chem. Soc. 122 (2000) 2361.
[31] H.J. Cooper, J. Am. Soc. Mass Spectrom. 16 (2005) 1932.
[32] I. Anusiewicz, M. Jasionowski, P. Skurski, J. Simons, J. Phys. Chem. A: Mol. Spectrosc. Kinet. Environ. Gen. Theory 109 (2005) 11332.
[33] H. Wei, X. Luo, Y. Wu, Y. Yao, Z. Guo, L. Zhu, J. Chem. Soc. Dalton Trans. 22 (2000) 4196.
[34] Y.M. Fung, T.W. Chan, J. Am. Soc. Mass Spectrom. 16 (2005) 1523.
[35] R.A. Zubarev, K.F. Haselmann, B.A. Budnik, F. Kjeldsen, F. Jensen, Eur. J. Mass Spectrom. 8 (2002) 337.
[36] E.A. Syrstad, F. Turecek, J. Am. Soc. Mass Spectrom. 16 (2005) 208.
[37] N.A. Kruger, R.A. Zubarev, D.M. Horn, F.W. McLafferty, Int. J. Mass Spectrom. 185–187 (1999) 787.
[38] A.J. Kleinnijenhuis, A.J. Heck, M.C. Duursma, R.M. Heeren, J. Am. Soc. Mass Spectrom. 16 (2005) 1595.
[39] K.A. Kellersberger, D. Fabris, Proceedings of the 52nd ASMS Conference on Mass Spectrometry and Allied Topics, Nashville, TN, 2004.