Aspartate buffer and divalent metal ions affect oxytocin in aqueous solution and protect it from degradation

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ABSTRACT

Oxytocin is a peptide drug used to induce labor and prevent bleeding after childbirth. Due to its instability, transport and storage of oxytocin formulations under tropical conditions is problematic. In a previous study, we have found that the stability of oxytocin in aspartate buffered formulation is improved by the addition of divalent metal ions (unpublished results). The stabilizing effect of Zn2+ was by far superior compared to that of Mg2+. In addition, it was found that stabilization correlated well with the ability of the divalent metal ions to interact with oxytocin in aspartate buffer. Furthermore, LC–MS (MS) measurements indicated that the combination of aspartate buffer and Zn2+ in particular suppressed intermolecular degradation reactions near the Cys1–5 disulfide bridge. These results lead to the hypothesis that in aspartate buffer, Zn2+ changes the conformation of oxytocin in such a way that the Cys1–5 disulfide bridge is shielded from its environment thereby suppressing intermolecular reactions involving this region of the molecule. To verify this hypothesis, we investigate here the conformation of oxytocin in aspartate buffer in the presence of Mg2+ or Zn2+, using 2D NOESY, TOCSY, 1H–13C HSQC and 1H–15N HSQC NMR spectroscopy. Almost all 1H, 13C and 15N resonances of oxytocin could be assigned using HSQC spectroscopy, without the need for 13C or 15N enrichment. 1H–13C and 1H–15N HSQC spectra showed that aspartate buffer alone induces minor changes in oxytocin in D2O, with the largest chemical shift changes observed for Cys1. Zn2+ causes more extensive changes in oxytocin in aqueous solution than Mg2+. Our findings suggest that the carboxylate group of aspartate neutralizes the positive charge of the N-terminus of Cys1, allowing the interactions with Zn2+ to become more favorable. These interactions may explain the protection of the disulfide bridge against intermolecular reactions that lead to dimerization.

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1. Introduction

Oxytocin is a nonapeptide hormone secreted by the posterior lobe of the pituitary gland, and is involved in the control of labor and bleeding cessation after child birth (Maughan et al., 2006). The peptide consists of nine amino acids (Cys–Tyr–Ile–Gln–Asn–Cys–Pro–Leu–Gly–NH2) with an internal disulfide bridge, and an amidated C-terminus (du Vigneaud et al., 1953). Oxytocin is the preferred drug to prevent postpartum hemorrhage and is commonly formulated in aqueous solution for parenteral administration (Gard et al., 2002). The instability of oxytocin in aqueous solution under harsh circumstances, particularly under tropical conditions, presents a significant challenge to pharmaceutical scientists (Hawe et al., 2009). The instability of oxytocin in aqueous solution has been reported in several studies (Hogerzeil et al., 1993; Trissel et al., 2006). It has been found that the degradation rate strongly depends on the pH of the formulation, with the highest stability reported at pH 4.5 (Hawe et al., 2009). Several studies have been aimed at the improvement of the stability of oxytocin in aqueous solution (Avanti et al., 2011; Hawe et al., 2009). The most recent finding is that the use of divalent metal ions, in combination with certain buffers, strongly increases the stability of oxytocin in aqueous solution (Avanti et al., 2012).

Previously, we have shown that Zn2+ in combination with aspartate buffer strongly stabilizes oxytocin in aqueous solutions, whereas Ca2+ and Mg2+ only have minor effects. The stabilization

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