





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

Christina Avanti^{a, b, 1, 2},  , Nur Alia Oktaviani^{c, 1}, Wouter L.J. Hinrichs^a, Henderik W. Frijlink^a, Frans A.A. Mulder^{c, d}

^a Department of Pharmaceutical Technology and Biopharmacy, University of Groningen, Antonius Deusinglaan 1, 9713 AV, Groningen, The Netherlands

^b Department of Pharmaceutics, Faculty of Pharmacy, University of Surabaya (Ubaya), Surabaya, Indonesia

^c Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

^d Department of Chemistry and Interdisciplinary Nanoscience Center iNANO, University of Aarhus, Langelandsgade 140, DK-8000 Aarhus C, Denmark

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that the stability of oxytocin in aspartate buffered formulation is improved by the addition of divalent metal ions (unpublished results).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 Zn^{2+} was by far superior compared to that of Mg^{2+} . 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 Zn^{2+} in particular suppressed intermolecular degradation reactions near the Cys^{1,6} disulfide bridge. These results lead to the hypothesis that in aspartate buffer, Zn^{2+} changes the conformation of oxytocin in such a way that the Cys^{1,6} 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 Mg^{2+} or Zn^{2+} , using 2D NOESY, TOCSY, 1H - ^{13}C HSQC and 1H - ^{15}N HSQC NMR spectroscopy. Almost all 1H , ^{13}C and ^{15}N resonances of oxytocin could be assigned using HSQC spectroscopy, without the need for ^{13}C or ^{15}N enrichment. 1H - ^{13}C and 1H - ^{15}N HSQC spectra showed that aspartate buffer alone induces minor changes in oxytocin in D_2O , with the largest chemical shift changes observed for Cys¹. Zn^{2+} causes more extensive changes in oxytocin in aqueous solution than Mg^{2+} . Our findings suggest that the carboxylate group of aspartate neutralizes the positive charge of the N-terminus of Cys¹, allowing the interactions with Zn^{2+} to become more favorable. These interactions may explain the protection of the disulfide bridge against intermolecular reactions that lead to dimerization.