RESEARCH ARTICLE

The Formation of Oxytocin Dimers is Suppressed by the Zinc–Aspartate–Oxytocin Complex

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ABSTRACT: The aim of this study was to investigate the effect of divalent metal ions (Ca, Mg^{2+}, and Zn^{2+}) on the stability of oxytocin in aspartate buffer (pH 4.5) and to determine their interaction with the peptide in aqueous solution. Reversed-phase high-performance liquid chromatography and high-performance size-exclusion chromatography measurements indicated that after 4 weeks of storage at 55°C, all tested divalent metal ions improved the stability of oxytocin in aspartate-buffered solutions (pH 4.5). However, the stabilizing effects of Zn^{2+} were by far superior compared with Ca^{2+} and Mg^{2+}. Liquid chromatography–tandem mass spectrometry showed that the combination of aspartate and Zn^{2+} in particular suppressed the formation of peptide dimers. As shown by isothermal titration calorimetry, Zn^{2+} interacted with oxytocin in the presence of aspartate buffer, whereas Ca^{2+} or Mg^{2+} did not. In conclusion, the stability of oxytocin in the aspartate-buffered solution is strongly improved in the presence of Zn^{2+}, and the stabilization effect is correlated with the ability of the divalent metal ions in aspartate buffer to interact with oxytocin. The reported results are discussed in relation to the possible mode of interactions among the peptide, Zn^{2+}, and buffer components leading to the observed stabilization effects. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

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