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A New Strategy To Stabilize Oxytocin in Aqueous Solutions: II. Suppression of Cysteine-Mediated Intermolecular Reactions by a Combination of Divalent Metal Ions and Citrate

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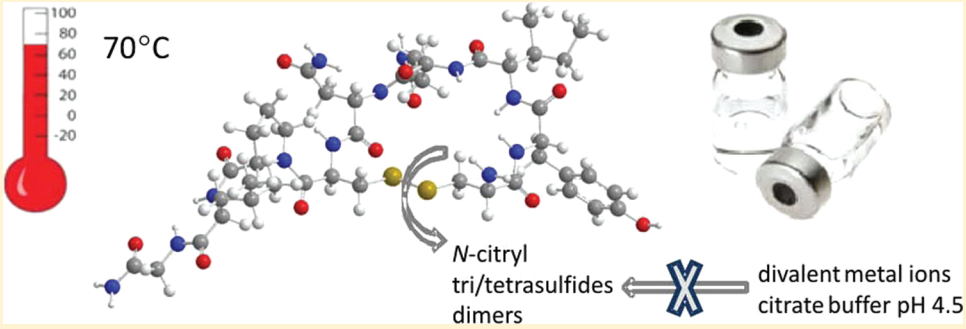
Supporting Information



ABSTRACT: A series of studies have been conducted to develop a heat-stable liquid oxytocin formulation. Oxytocin degradation products have been identified including citrate adducts formed in a formulation with citrate buffer. In a more recent study we have found that divalent metal salts in combination with citrate buffer strongly stabilize oxytocin in aqueous solutions (Avanti, C.; et al. *AAPS J.* **2011**, *13*, 284−290). The aim of the present investigation was to identify various degradation products of oxytocin in citrate-buffered solution after thermal stress at a temperature of 70 °C for 5 days and the changes in degradation pattern in the presence of divalent metal ions. Degradation products of oxytocin in the citrate buffer formulation with and without divalent metal ions were analyzed using liquid chromatography−mass spectrometry/mass spectrometry (LC−MS/MS). In the presence of divalent metal ions, almost all degradation products, in particular citrate adduct, tri- and tetrasulfides, and dimers, were greatly reduced in intensity. No significant difference in the stabilizing effect was found among the divalent metal ions Ca^{2+}, Mg^{2+}, and Zn^{2+}. The suppressed degradation products all involve the cysteine residues. We therefore postulate that cysteine-mediated intermolecular reactions are suppressed by complex formation of the divalent metal ion and citrate with oxytocin, thereby inhibiting the formation of citrate adducts and reactions of the cysteine thiol group in oxytocin.

KEYWORDS: *oxytocin, aqueous solution, degradation, citrate buffer, divalent metal ions*

1. INTRODUCTION

Oxytocin is a neurohypophyseal hormone, which was first discovered by H. H. Dale in 1909.[1,2] Oxytocin is produced by neurons of the posterior lobe of the hypophysis and pulsatively released into the periphery. In clinical practice, oxytocin has been prescribed primarily for labor induction and augmentation, control of postpartum hemorrhage and uterine hypotonicity in the third stage of labor.[3,4] Oxytocin is commonly administered by intravenous infusion.[5]

The oxytocin structure was elucidated in 1951,[6−8] and the characterization and biosynthesis of oxytocin were reported in 1953 by du Vigneaud.[9] Oxytocin consists of nine amino acids: cyclo-(Cys[1]-Tyr[2]-Ile[3]-Gln[4]-Asn[5]-Cys[6])-Pro[7]-Leu[8]-Gly[9]-NH_2 with a disulfide bridge between Cys residues 1 and 6.[10,11] The primary structure of oxytocin is shown in Figure 1. A major problem of the compound is its intrinsic instability in aqueous formulations.[12] Recently significant attention was focused on efforts to overcome the instability of oxytocin.[13,14] We have conducted several studies with the aim to develop a heat-stable oxytocin formulation.

We identified the main degradation products of oxytocin stressed at a temperature of 70 °C in various buffers, pH values and storage time,[13] as well as citryl oxytocin in citrate-buffered formulations.[14] The degradation reactions and target residues of oxytocin are indicated in Figure 1.

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