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Evaluation of the stability of oxytocin and vasopressin under oxidative stress conditions

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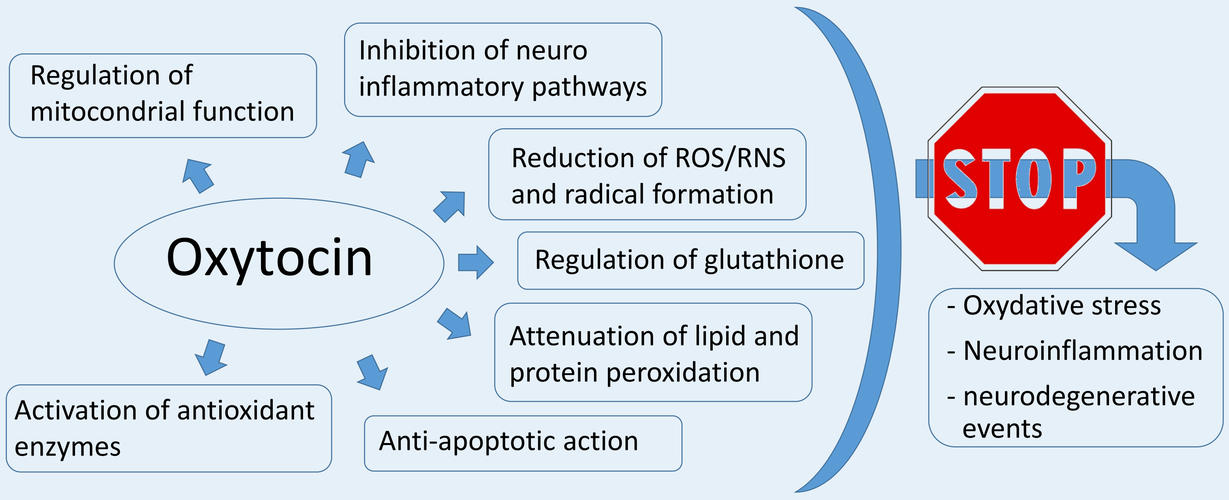
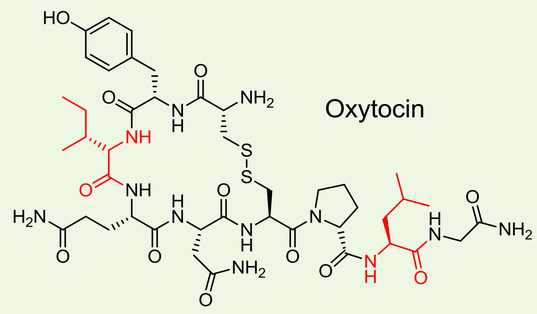
Introduction

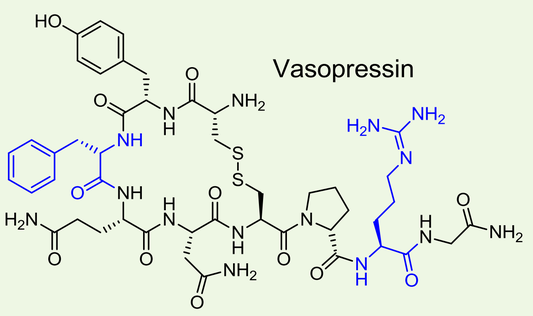
Oxytocin and vasopressin are neuropeptides produced in the hypothalamus that play a well-known role in social and emotional behavior, as well as in the regulation of body fluids tonicity. In recent studies, oxytocin was suggested to act as a neuroprotective factor by inhibiting neuro inflammation, oxidative stress, free radical formation and neuronal apoptosis, preventing mitochondrial dysfunction, activating free radical scavengers and several antioxidant enzymes. [1] Herein, we investigated the stability of oxytocin and vasopressin under oxidative stress conditions, such as UV irradiation, treatment with ROS and lipoxidation byproducts, similarly to our previous study on other neuropeptides.[2]



Synthesis

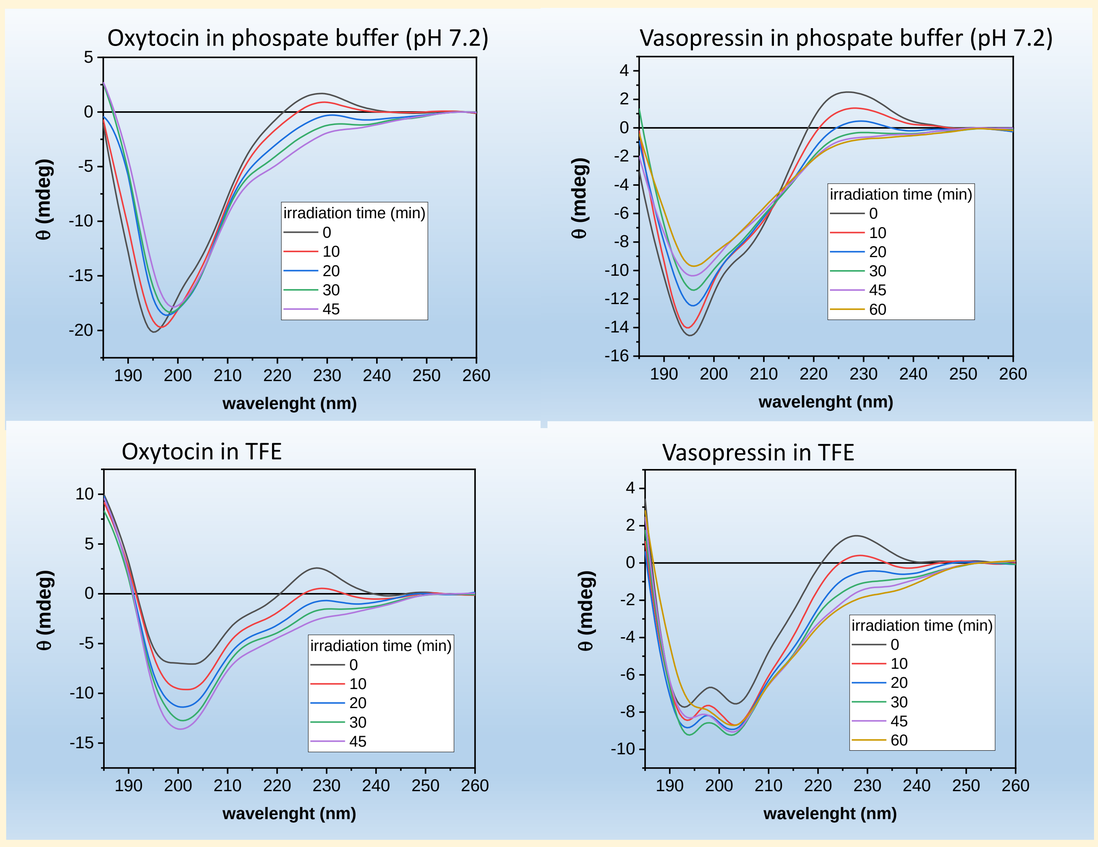
Oxytocin and vasopressin are cyclic nonapeptides with similar sequence of CYIQNCPLG and CYFQNCPRG respectively. The cysteine residues cyclize the structure through a disulfide bond and the C-terminus is found as a primary amide. Both peptides were prepared by solid phase peptide synthesis from rink amide resin and Fmoc-protected aminoacids. Activation of each residues was achieved with HBTU/HOBt and Fmoc deprotection was performed with piperidine. After cleavage and deprotection, the disulfide bridge is spontaneously obtained through oxidative folding.





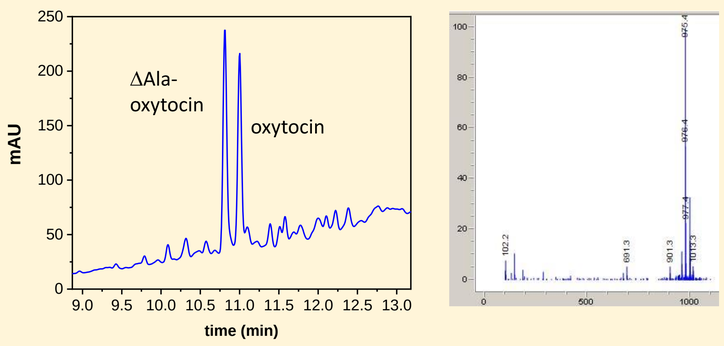
Reactivity under irradiation

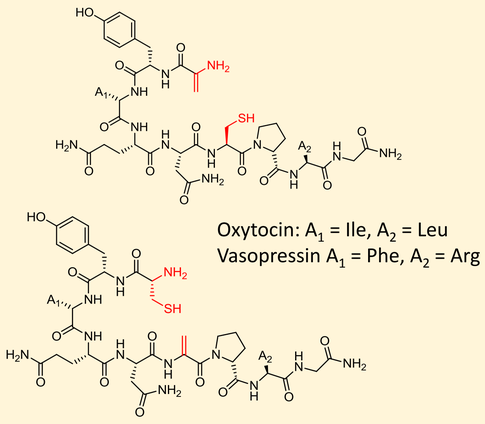
Both peptides present a positive CD band at 226 - 228 nm and a negative band at 195 nm. Vasopressin display another negative band at 203 nm in TFE. The most evident spectral change after UV irradiation (254 nm) is the disappearance of the positive band in both aqueous (phosphate buffer) and organic (trifluoroethanol) environments, which is related to the opening of the disulfide bridge.



HPLC-MS analysis evidenced the formation of ΔAla -oxytocin and -vasopressin as the main irradiation products, together with a complex mixture of degradation and oligomerization by-products. We are determining wherever the desulfurization is a radical process caused by the ROS generated upon UV irradiation, or a photochemical reaction.

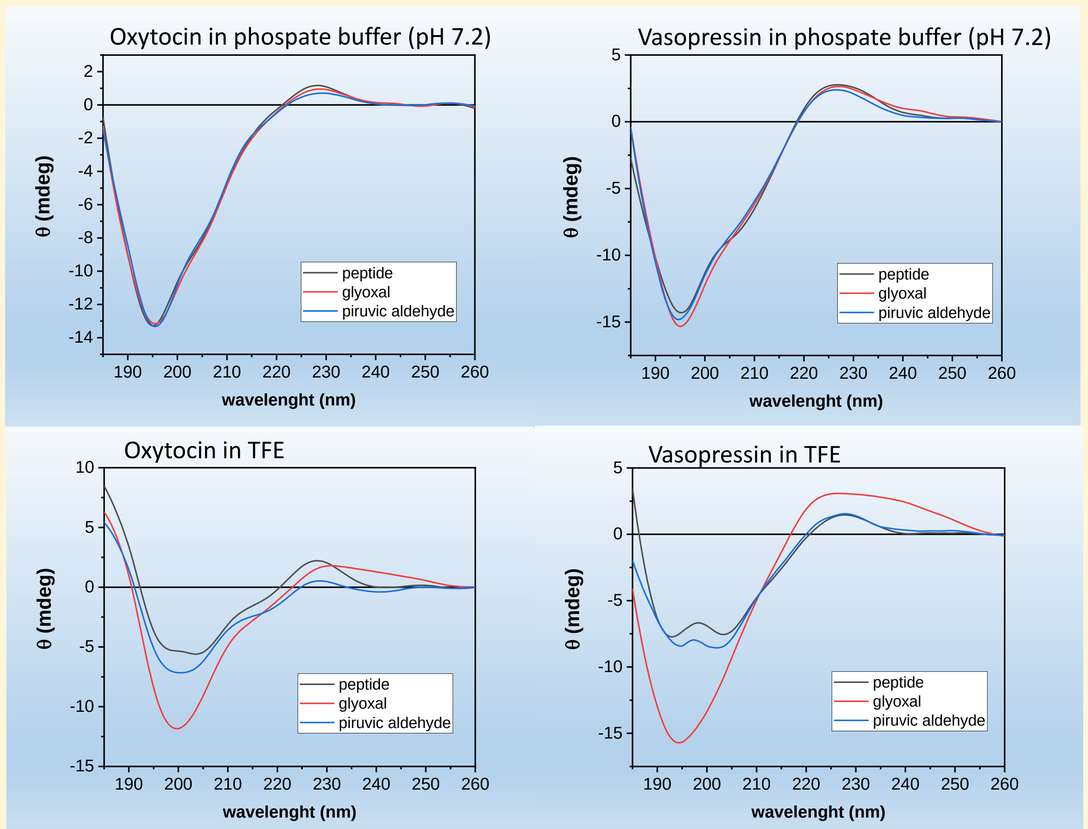
We are also optimizing the desulfurization process in order to clearly define in which Cys residue is occurring the β-elimination process *via* NMR investigations. It is worth noting that ΔAla oxytocin was previously suggested to act as an intermediate in the thermal degradation of oxytocin formulations but it was not directly detected. [3]

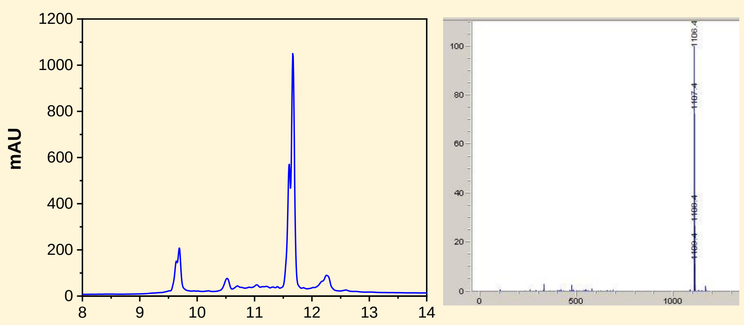


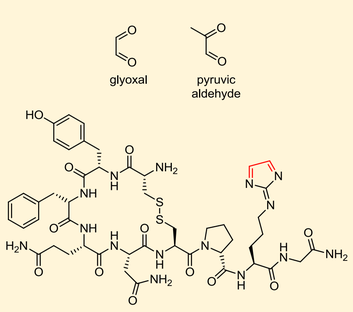


Reactivity with aldehydes

We tested the reactivity of our peptides with glyoxal and pyruvic aldehyde, which can be found in cells and tissues as lipid oxidation by-products. Although a significant change of CD spectra can be observed in organic media (TFE), HPLC-MS analysis evidenced the formation of imine condensation products also in aqueous media. Glyoxal exhibited the highest reactivity towards condensation reactions with respect to pyruvic aldehyde. Indeed, the condensation on arginine side chain resulted the major adduct with vasopressin, while oxytocin provided a condensation product currently under investigation.







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[3] K. Wisniewski, J. Finnman, M. Flipo, R. Galyean, C. D. Schteingart, Pept. Sci. 2013, 100, 408-421.