Photoacoustic calorimetry study of the conformational variation of the chignolin peptide induced by a pH jump

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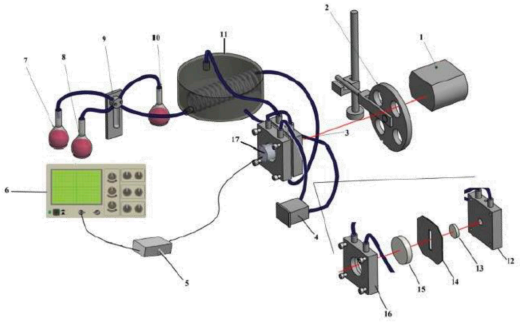
Background – Most proteins need to fold into a certain tridimensional structure, known as its native structure, to perform its biological functions. Thus, understanding all the mechanisms involved in protein folding is important to the development of models capable of predicting the impact that certain variables might have on the thermodynamics and kinetics of these processes and consequently improve the comprehension of certain diseases. The study of small peptides, such as β-hairpins, may help to understand these questions. These motifs may incorporate important catalytic residues and they can also be nucleation sites in the early stages of protein folding. All these features together make them good models for studying protein folding.

Chignolin is a decapeptide (GYDPETGTWG) designed by Honda et al.[1]. Its stability is mainly due to the number of non-covalent interactions (hydrogen bonds and hydrophobic interactions between Tyr2-Trp9 and Tyr2-Pro4), established during the folding process. CD and RMN studies carried out by Honda and collaborators, have shown that the peptide does not have any intermediate state. With the aim to study the folding mechanism of chignolin in an atomic level, computational studies have been performed. However, there are contradictory trends among them. Whereas some reports have been claiming the hydrophobic collapse model, first presented by Dinner et al.[2], Harada and Kitao, defend the zipper model developed by Muñoz et al.[3] Thus, the folding/unfolding mechanism for chignolin is still not totally unravelled, needing additional experimental assays and further computational calculations to clarify it.

Here, we intend to study the specific role of Tyr2-Trp9 in the short-length β-hairpin peptide Chignolin in aqueous media. Laser induced pH jump coupled with time-resolved photoacoustic calorimetry (TR-PAC) in a controlled temperature setup is unique for the investigation of the conformational protonation effects on this single stabilizing structure. The structural rearrangement is characterized in terms of kinetics and volume changes.

Methods – Experiments of laser-induced pH jump coupled with TR-PAC were accomplished using a homemade time-resolved photoacoustic calorimetry flow cell with temperature control and automatic injection (Figure 1). The photoacoustic pressure waves were detected using both 2.25 and 1 MHz frequency microphones. Analysis of the photoacoustic waves was performed with the CPAC software developed in our laboratory and available online (http://cpac.qui.uc.pt/) [4]. We carried out circular dichroism studies in the near and in the far-UV with the chignolin peptide.



1- Laser
2- Neutral filters
3- Photoacoustic cell
4- Thermal bath
5- Ultrasonic amplifier
6- Oscilloscope
7,8,10-Syringes
9- Pump
11- Chamber
12,16- Blocks
13- Quartz window
14- Copper foil
15- Dielectric mirror

Results and conclusions – We first performed circular dichroism studies which allowed us to conclude that at a pH over 10 there is a loss of the β-sheet structure, possibly due to deprotonation of the Tyr2 side chain, which has a pKa of approximately 10.3. Considering this, we performed two-temperature TR-PAC experiments to characterize the protonation of the amino acid model Ac-Tyr-NH_2, the protonation of the Tyr2 side chain, which is embedded in the peptide chain, and the refolding process resultant from this protonation. This approach allowed us to calculate the lifetime and the volume change of these reactions, using the software CPAC. For the model Ac-Tyr-NH_2 the rate constant associated with the protonation of its side chain was approximately 8.5×10^9 $M^{-1}s^{-1}$ and the corresponding volume changes was 8.7 mL mol^{-1}. For Tyr2 we obtained a rate constant of 2.8×10^{10} $M^{-1}s^{-1}$ and volume change of 4.9 mL mol^{-1}. For the process of refolding associated with the protonation of the side chain of Tyr2, we obtained a volume change of roughly 10.4 mL mol-1, indicating that the folding of chignolin is related to a volume expansion. Regarding the time related to this event, our experiments point out to a refolding time of ~1.15 μs. Based on these results, we presume that the hydrophobic collapse model, developed by Dinner et al. [2], is possibly the most accurate at describing the mechanism of folding of chignolin.

Acknowledgments – This work was supported by the Portuguese Science Foundation FCT (UIDB/00313/2020).

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