Mass spectrometry of peptoids as peptidomimetic compounds : from sequence to artificial enzyme design

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The relationship between structure and activity has captivated scientists for the past decades. By taking inspiration from the folding of proteins and enzymes, chemists currently seek to design sequence-defined macromolecular chains that can undergo intramolecular cross-linking reactions and collapse into bioinspired nanoparticles (see Fig. 1) [1]. Over the years, non-natural compounds able to dynamically fold and unfold into well-defined secondary structures called 'foldamers' have aroused interest in many fields, *e.g.* in catalysis.

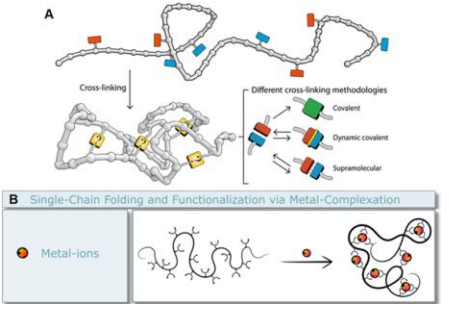

Fig. 1. (A) Collapse of a sequence-defined macromolecule via different cross-linkings. (B) metal ion induced folding of a polymer chain containing ligand moieties. Metal cations are responsible for both the collapse and catalytic activity. Adapted from [1].

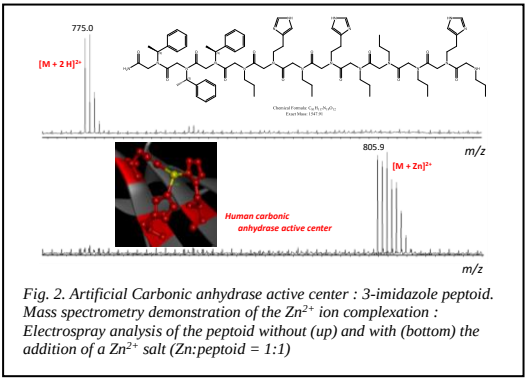

Fig. 2. Artificial Carbonic anhydrase active center : 3-imidazole peptoid. Mass spectrometry demonstration of the Zn^{2+} ion complexation : Electrospray analysis of the peptoid without (up) and with (bottom) the addition of a Zn^{2+} salt (Zn:peptoid = 1:1)

Peptoids are poly-N-substituted glycines belonging to the peptidomimetic polymer family (see Fig. 2) [2]. The combination of the side chains that are appended to the backbone nitrogen atoms in peptoids plays a key role for stabilizing the secondary structures, *i.e.*, helices, threaded-loop or ribbons, both in solution and in the solid state [2].

In associating organic synthesis with structural characterization, we are now conducting research on artificial (metallo)enzymes. These biomimetic compounds are synthetic organic (macro)molecules designed to mimic enzymatic functions toward large-scale applications. Our focus is to develop sequence-defined peptoid-based enzyme mimics. Peptoids are typically synthesized using the submonomer method [2]. This method involves the stepwise construction of the polymer

chain, allowing for the creation of tailor-made, sequence-defined structures with functional groups at selected positions. Peptoid-based catalysts are unique due to improved catalytic performance through chemogenetic optimization. This involves parallel optimization of both the direct metal surroundings (first coordination sphere) and the polymer scaffold (second coordination sphere), which are crucial for achieving (regio)(stereo)selectivity. To design the initial peptoid sequences, we draw inspiration from 3D structures of metallo-proteins archived in the Protein Data Bank (PDB). Using a ***reductionist approach***, we are creating short, heterogeneous peptoids that are intended to mimic the active sites of these proteins. Our initial research candidate is the Carbonic Anhydrase (CA) family of metalloenzymes (see Fig. 2) [3], present in many organisms and which catalyse the hydration and dehydration of CO_2. CA enzymes feature a Zn^{2+} ion at the center of their active site, coordinated to one water molecule and three histidine residues, essential for CO_2 conversion.

In recent years, by taking huge advantages from the unique combination between organic synthesis and mass spectrometry, we turned mass spectrometry into an inescapable analytical method to tackle the structure complexity of tailor-made original peptoids, *i.e.,* from the primary sequence confirmation to the 3D structure establishment, mostly by using collision-induced dissociation and ion mobility experiments, together with theoretical chemistry. In a bottom-up approach, from sequence to 3D structure, our strategy is to prepare tailor-made model peptoids to answer specific questions, such as (i) how to sequence *de novo* peptoid by tandem mass spectrometry, (ii) are solution phase secondary structures, typically the helical structures, conserved upon ionization/desolvation, (iii) are ion mobility experiments together with Molecular Dynamics simulations able to describe the gas phase structures of peptoids and to tackle subtle differences between conformers, (iv) are the gas phase complex ions generated upon electrospray ionization of peptoid/metal salt solution mimicking the 3D structures, including the coordination sphere of the metal ion (Zn^{2+}, Ni^{2+}…) of the metalloenzyme mimics and (v) can we take advantage of the online coupling between flow chemistry and mass spectrometry to detect transient intermediates of the catalytic reactions?

In the present communication, by using selected examples [4-5], the efficiency of mass spectrometry experiments for the in-depth structural characterization - ***from the primary to the ternary structures*** - of original bio-inspired peptoids will be demonstrated.

References
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