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From synthetic poly-α-amino acids to bioactive peptides and peptidomimetics‡

EVARISTO PEGGION*
Institute of Biomolecular Chemistry, CNR, Department of Chemistry, University of Padova, 35131 Padova, Italy

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In this article I would like to summarize the evolution of our research in Padova and to show how strong was the impact and stimulus of the collaboration with Murray Goodman on the research of our group.

In 1958 Ephraim Katchalsky and Michael Sela published an important review on poly-α-amino acids [1]. The study of this authoritative review stimulated the interest of our research group, headed by Prof. Ernesto Scoffone, in this new field. We followed the work of different groups, including that of Murray Goodman in Brooklyn, NY, who was making outstanding contributions to the field. At that time poly-α-amino acids represented the bridge between classical polymers and biological macromolecules. After the first meeting on poly-α-amino acids held in Madison, Wisconsin, in 1961, the prestigious scientific journal *BIOPOLYMERS* was founded (the founding Editors were Elkan Blout and Ephraim Katchalski). Later, Murray became the Editor-in-Chief for the Journal and remained in the position until he passed away.

His first visit to Padova was in 1962. From that meeting started the scientific and personal interactions between Ernesto Scoffone and Murray.

In the 1960s a major controversial point was the mechanism of polymerization of N-carboxyanhydrides initiated by tertiary amines or strong aprotic bases. The elucidation of the polymerization mechanism required intensive and competitive efforts from different groups. Important contributions came from the groups of E. Katchalski [1], C.H. Bamford [2], M. Goodman [3], M. Szwarc [4] and our group in Padova [5]. These combined efforts led to the formulation of a general polymerization mechanism which was summarized in a review article in *Pure and Applied Chemistry* by Murray and myself in 1981 [6].

Starting from the early 1960s, major research interest was devoted to the conformational properties of poly-α-amino acids. Studies from different groups allowed the establishment of a correlation between chiroptical properties and ordered conformations. A much debated problem was the contribution of the aromatic side-chain chromophores of Tyr, Trp and Phe to the optical activity of ordered peptide conformations. In 1968 Goodman and coworkers demonstrated that the introduction of a nitro group in the *para* position of the phenyl groups of poly-β-benzyl-L-aspartate reverses the screw sense of the helical structure from left- to right-handed [7]. When I joined Murray's group at the Polytechnic Institute of Brooklyn in 1965, my research programme was directed to the synthesis and conformational studies of poly-*p*-amino-L-phenylalanine. The polymer was characterized in aqueous solution by ORD and CD. A pH-induced conformational transition from a random coil to an ordered structure was detected [8]. With the techniques available at that time it was not possible to establish the nature of the ordered structure. Ten years later, taking into account the theoretical CD studies by R. W. Woody [9], in collaboration with Murray we demonstrated that at room temperature the ordered conformation of poly-*p*-amino-L-phenylalanine is the α-helix, while above 40 °C the predominant conformation is a β-sheet structure [10]. After my return to Padova (October 1966) we continued our research on the conformational properties of poly-α-amino acids containing aromatic side-chain chromophores. In particular, we determined the conformation in solution of poly-L-tyrosine, poly-L-tryptophan, poly-L-histidine and poly-L-phenylalanine [11]. We found that the CD properties of charge-free random copolymers of L-lysine and L-tyrosine in water containing 90% 2,2,2-trifluoroethanol (TFE) change in a monotonic way upon increasing the tyrosine content in the peptide chain. These findings indicated that the helical structure of poly-L-lysine is not modified upon increasing the percentage of aromatic residues in the chain and the consequent monotonic change of the CD pattern reflects the contribution of the aromatic chromophore. Using the same approach we also demonstrated that poly-L-tyrosine in pure water assumes a β-sheet structure.

By studying the conformational properties of copolymers of γ-ethyl-L-glutamate and L-tryptophan we also demonstrated that the conformation of poly-L-tryptophan in TFE is the right-handed α-helix. The CD

* Correspondence to: Dr Evaristo Peggion, Department of Chemistry, University of Padova, Via Marzolo 1, 35131 Padova, Italy;
e-mail: evaristo.peggion@unipd.it

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spectrum of the aromatic polypeptide is characterized by two very strong CD bands of opposite sign, with the cross-over point at 222 nm, coincident with the absorption band of the indole side-chain chromophore. These findings indicated that the side chains in the helical structure are arranged in a way that allows exciton coupling of the indole transitions. The helical structure of the polymer was confirmed by our x-ray diffraction studies on oriented fibres of the polymer [12], and very recently by FTIR absorption studies by Pande *et al.* [13].

By studying random copolymers of L-phenylalanine and N-carbobenzoxy-L-lysine we also demonstrated that poly-L-phenylalanine is in the right-handed α-helical structure in tetrahydrofuran, while in water the β-sheet structure is predominant.

Our collaboration with Murray continued on N-substituted poly-α-amino acids. In particular our efforts were directed to the synthesis and conformational studies of poly-(γ-ethyl, N-methyl-L-glutamate) and poly-(N-methyl-L-glutamic acid) [14,15]. By CD and NMR measurements it was found that these N-substituted polypeptides, in spite of the absence of intramolecular hydrogen bonds, form a very stable helical structure with all *trans* peptide bonds. This structure is very similar to that of poly-(N-methyl-L-alanine), described by Goodman and coworkers [16] on the basis of conformational energy calculations and theoretical and experimental CD results. Interestingly enough, the conformation of poly-(N-methyl-L-glutamic acid) is identical to that of the parent ester polymers and, most important, this structure is insensitive to the extent of ionization of the carboxyl groups in the side chains [17]. Part of this work was carried out during my stay as Visiting Professor in Murray's laboratory in San Diego, CA, in 1979.

After my return to Padova our research interest moved to peptide hormones of the gastrin family. Again, the encouragement of Murray to investigate structure–function relationships of these bioactive peptides was essential to establish a collaboration between our group and the group of Prof. E. Wünsch and L. Moroder in Munich. One interesting result of this collaboration was obtained from the study of minigastrin analogues in which the glutamic acid sequence at the *N*-terminal portion of minigastrin was elongated from 1 to 5 residues:

$$\text{H-Leu-(Glu)}_5\text{-Ala-Tyr-Gly-Trp-Nle-Asp-Phe-NH}_2$$

The *C*-terminal tetrapeptide amide Trp-Nle-Asp-Phe-NH_2 is essential for the physiological action of all gastrins. Upon chain elongation an increase of the α-helical conformation takes place which parallels the increase of biological potency [18]. On the basis of CD and NMR studies we concluded that the bioactive conformation of minigastrin in TFE–water solvent mixtures is U-shaped with an α-helical segment in the -Glu_5- sequence (at the *N*-terminus), a β-bend in the central part of the sequence, and a C_7-conformation at the *C*-terminus [19].

In the 1990s our research activities were directed to the study of the structure–function relationships of bioactive peptides from insect venoms. In particular, we studied the bombolitins, heptadecapeptides present in the bumble bee venom, which form amphipathic helices and are responsible for the activation of phospholipase A_2 and the resulting inflammatory response to a bee sting. We demonstrated that the activity of the bombolitins and of many synthetic analogues is dependent solely on the amphipathic helical structure and on the interaction with lipids. Preliminary results of this work were presented in the Murray Goodman Symposium held in San Diego in 1993 (in honour of his 65th birthday). As usual, Murray was following our work with interest and invited us to contribute a review article to *Biopolymers (Peptide Science)* which was published in 1997 [20].

In 1993 we started a collaborative research programme with Michael Chorev and Mike Rosenblatt of the Harvard Medical School, Boston, MA, on structure–function relations of parathyroid hormones (PTH). This very productive collaboration, still continuing, was strongly encouraged by Murray, who was always following the research activities of his former students and coworkers through discussions, suggestions and proposals. The most recent results on bioactive *N*-terminal undecapeptides derived from PTH were presented at the 18th American Peptide Symposium in 2003 [21]. In his last visit to Padova (November 2003) Murray delivered an outstanding lecture, demonstrating once more how his work was a stream of innovative advancement in bioorganic chemistry and related areas.

Murray had a strong impact on the peptide research in Padova. I am deeply grateful to him for his suggestions and critical evaluation of our research activities. I miss the illuminating scientist, the superb educator and the mentor of many of my students and post-doctoral fellows in Brooklyn and San Diego, and particularly a very dear and unforgettable friend.

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