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Historical perspective of peptidomics

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ABSTRACT

Peptides have been studied for over 100 years, but for most of this time the focus was on a specific peptide or peptides, and not on the general peptidome of a biological sample. In the 1990s, mass spectrometry techniques were developed for the analysis of proteins, usually after digestion into peptides. The field of peptidomics started soon after proteomics and has grown to over 600 publications that use the word "peptidomic" or "peptidomics". Although peptidomics is related to proteomics, there are fundamental differences. In this review, we discuss these differences along with the history of the field of peptidomics.

1. Peptide research prior to peptidomics

1.1. *Early discovery of peptide hormones*

Bioactive peptides have been studied for over 100 years, ever since Bayliss and Starling found in 1902 that a substance secreted by the intestine stimulated the pancreas to secrete digestive enzymes [1]. This substance was named secretin and the class of signaling molecules carried by the bloodstream was named hormones. Once secretin was purified and found to be a peptide, its amino acid sequence was determined. Many other peptide hormones were discovered using a similar approach in which a bioactive substance was purified to homogeneity and sequenced, and several of these discoveries resulted in Nobel Prizes for the novel groundbreaking scientific insights how the body communicates internally, locally between cells and especially via the blood stream between distant cells and entire organ systems. Similar methods were used to discover peptide neurotransmitters and peptidergic neuromodulators, collectively termed neuropeptides. While these studies involved the discovery of peptides, they were not designated as peptidomics because they followed a single bioactive moiety and did not attempt to characterize all of the abundant peptides present in a biological sample, which was far too complex for the tools available prior to the 21st century.

Despite the limited tools, several researchers characterized peptides present in biological samples without primarily

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focusing on biological activity; such "peptide-first" approaches are a hallmark of peptidomics studies. In the early 1980s, Mutt, Tatemoto, and colleagues purified peptides from pig intestine. They replaced bioassays by screening for the presence of a C-terminal amide group, recognizing at that time that C-terminal amidation is a common feature of peptide hormones. Using this approach, Mutt and Tatemoto identified a handful of peptides that were subsequently found to be neuropeptides and/or peptide hormones, such as neuropeptide Y, peptide YY, galanin, and others [2–4]. A decade later, Sigafoos, Viveros, and colleagues purified peptides from chromaffin granules, the peptide-rich secretory vesicles of the adrenal medulla [5]. In addition to finding several novel forms of chromogranins A and B, they found four novel peptides with no homology to any known peptides. A search of these peptides in the current databases show that one corresponds to the C-terminal region of neuroendocrine secretory protein 55, another corresponds to the N-terminal region of prohormone convertase 2, and the other two peptides correspond to proSAAS-derived peptides named KEP and little SAAS.

Being inspired by their work, and also in close collaboration with the Swedish group, the Heidelberg-based group of Forssmann isolated peptides of the atrial natriuretic peptide family from porcine, bovine and equine heart tissue [6]. Driven by the observation that tissue extraction can lead to biologically inactive pre-forms of peptide hormones (prohormones) Forssmann subsequently attempted to systematically address circulating human peptides from blood in a quest to comprehensively discover circulating peptide hormones, and also to identify the circulating (processed) forms of already known peptides. At that time HPLC and significantly improved and diversified chromatography media became available. Peptides from 800 L of human urine and later from tens of thousands of liters of human blood filtrate (obtained from patients with end-stage renal disease) were extracted and purified [7]. Using a systematic sequencing approach on this large-scale human peptide bank, a new human defensin [8] and a chemokine [9] were purified and identified followed by their biological characterization. The analysis of the purified products involved mass spectrometry, but finally relied still on Edman sequencing for identification. Mass spectrometry was already used to study peptides in the 1990s, but due to limitations in the techniques, the studies were primarily aimed at detecting known neuropeptides or their degradation fragments [10–12].

1.2. *Isolation of neuropeptide processing-intermediates*

Early applications using mass spectrometry to detect peptides especially from body fluids but also from tissue extracts regularly reported thousands of peptides to be present in the samples. Upon closer inspection, the vast majority of these peptides were protein degradation fragments [13–15] leading to an uncertainty about the scientific value of this type of "peptide mapping" excursions. One solution to this problem was to purify biologically meaningful peptides by enriching for neuropeptide processing intermediates using an affinity column that bound these intermediates and not the majority of protein degradation fragments. Most neuropeptides and peptide hormones are produced from precursor proteins by endopeptidase cleavage at sites containing multiple Lys and/or Arg residues followed by exopeptidase removal of these amino acids from the C-terminus of the processing intermediates. The enzyme primarily responsible for the second step is carboxypeptidase E, which was identified in the early 1980s [16,17]. Carboxypeptidase E is mutated in the *fat* mouse, leading to a dramatic increase in peptides containing C-terminal Lys and/or Arg residues [18,19]. Extracts from brains of *fat* mice were purified on an anhydrotrypsin resin to bind peptides containing C-terminal basic residues, eluted, and analyzed by liquid chromatography–mass spectrometry (LC–MS). This resulted in the identification of many known neuropeptides as well as several novel peptides from a precursor named proSAAS [20,21]. As with the discovery of neuropeptide Y by Mutt and Tatemoto, the finding of novel peptides in mouse brain did not provide clues as to the function, and only after extensive additional research has it been determined that some of the proSAAS-derived peptides play a role in feeding/body weight regulation and reward pathways [22–25].

2. Technological origin and developments

2.1. *Definition of peptidomics and comparison to proteomics*

While some of the above studies could be considered peptidomics, the term was coined at a scientific meeting organized by Micromass in the late nineties (Shrigley Hall, Cheshire, UK) [26]. It was almost simultaneously trademarked by BioVisioN, a Hannover based biotech company (see below). Afterwards, the two executive founders scientifically introduced it in February 2000 at an ABRF conference organized by Aebersold (Bellevue, Washington, USA) [27]. The term first appeared in several full scientific papers in 2001 [28–33].

Peptidomics is defined as the comprehensive characterization of peptides present in a biological sample. Since its first use, the term peptidomics (or peptidomic) has been used in over 400 publications listed on PubMed (Fig. 1). Including the term "peptidome" together with peptidomic(s), PubMed searches reveal over 600 publications (Fig. 1). Peptidomics is a specific subdiscipline of proteomics, the characterization of proteins in a biological sample, which has considerably more publications listed in PubMed (Fig. 1). A few years after introduction, already a few special issues were dedicated to peptide analysis and peptidomics (e.g. [34,35]). Moreover, several books have been published specifically targeting this new research area (e.g. [36,37]).

Although peptidomics and proteomics are related, there are fundamental differences in the conceptual as well as subsequent analytical strategies in both these fields. The first comprehensive proteomics technology used on a worldwide, large scale basis has been 2-D polyacrylamide gel electrophoresis [38]. The beauty of this technology was that proteins were fractionated by isoelectric point on one dimension and apparent molecular weight on a second dimension, thereby allowing for the detection of thousands of proteins on a single gel. Post-translational modifications that altered the isoelectric point or electrophoretic mobility could be detected. However, further work was required to sequence