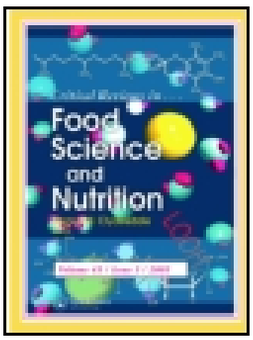

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REVIEW

Aptamers: an emerging class of bioaffinity ligands in bioactive peptide applications

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ABSTRACT

The food and health applications of bioactive peptides have grown remarkably in the past few decades. Current elucidations have shown that bioactive peptides have unique structural arrangement of amino acids, conferring distinct functionalities, and molecular affinity characteristics. However, whereas interest in the biological potency of bioactive peptides has grown, cost-effective techniques for monitoring the structural changes in these peptides and how these changes affect the biological properties have not grown at the same rate. Due to the high binding affinity of aptamers for other biomolecules, they have a huge potential for use in tracking the structural, conformational, and compositional changes in bioactive peptides. This review provides an overview of bioactive peptides and their essential structure–activity relationship. The review further highlights on the types and methods of synthesis of aptamers before the discussion of the prospects, merits, and challenges in the use of aptamers for bioaffinity interactions with bioactive peptides.

KEYWORDS

Bioactive peptides; Structure–activity relationship; Physiological responses; SELEX; Nucleic acid aptamers; Peptide aptamers; Affinity interactions

Introduction

With concomitant perspectives on both complementary health promotion and disease prevention mechanisms, recent interests in bioactive proteins and peptides are warranted (Agyei and Danquah 2011; Udenigwe and Aluko 2012; Korhonen and Pihlanto 2006). There are diverse investigations so far, ranging from process optimization strategies (Agyei et al. 2016; Gnasegaran et al. 2017), to continuous ongoing examinations targeting the development of the technologies to predict and/or identify the inherent functional components (Udenigwe 2014), and these highlight the exceptional credibility of bioactive peptides as therapeutic biomolecules. The aforementioned domains have generated tremendous research and commercial interests, ultimately leading to an extraordinary global market demand for bioactive peptides. To date, a wide array of biological properties such as antimicrobial, antihypertensive, cytomodulatory, and antiappetizing, amongst others, have been associated with the externally isolated bioactive peptides (Agyei and Danquah 2011; Udenigwe and Aluko 2012). Probably the most influential impact of bioactive peptides has been their exceptional abilities to target and alleviate symptoms pertaining to critical ailments that impair or degenerate the human physiological systems including metabolic syndrome, cardiovascular diseases, and cancers (Agyei, Potumarthi, and Danquah 2015; Agyei and Danquah 2012; Ricci-Cabello, Olalla Herrera, and Artacho 2012; Egger and Ménard 2017).

Due to the health benefits associated with bioactive peptides, hordes of high-end technologies have been employed for characterization of the structures and biological potencies of these peptides. A detailed account of these techniques, such as X-ray diffraction, small-angle neutron scattering, and surface plasmon resonance (SPR), amongst others, may be traced to the pioneering book (Vesely 2006). These techniques have been instrumental in effectively deciphering interesting factoids regarding the primary and secondary structures of proteins and peptides, and these have enhanced understanding of the factors that influence the biological and binding characteristics of proteins and peptides (Kaur, Garg, and Raghava 2007). However, in spite of the aforesaid advantages, these high-throughput procedures require expertise in handling both samples and instruments, are prohibitively expensive, and do not pose as an attractive option for small and medium scale commercial end users (Agyei et al. 2017). The focus has, therefore, shifted toward utilization of techniques that would overcome these shortcomings, and yet be cost-effective and proficient enough, to be amenable to a broad spectrum of end users.

In this context, the significance and applicability of the SELEX (Systematic Evolution of Ligands by Exponential enrichment) technique is well recognized. For an in-depth

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understanding of the SELEX technology, interested readers may refer to the recent reviews (Wu and Kwon 2016; Tan et al. 2016; Sun and Zu 2015; Wang et al. 2018; Bayat et al. 2018). The technology has almost signaled the onset of a new era with the generation of specific aptamers for ligand detection. Generation of aptamers through SELEX and their applications in broad domains such as targeted delivery of therapeutic agents, diagnosis, and evolution of the next generation of biomarkers, amongst others, have been under consideration for nearly two decades. However, only recently, the superiority of aptamers-mediated targeted identification facilitated by the SELEX technology, over conventional antibodies, has been highlighted (Sun and Zu 2015).

Contextually to the aim of the current article, immobilized aptameric ligand detection via affinity regulation of the prominent structural and practical features of bioactive peptides (Acquah et al. 2015; Agyei et al. 2017; Agyei et al. 2018) is at the forefront of bioactive peptide research. This is mostly based on the principle of mimicking the structures of internal "motifs" (Tan et al. 2016), as demonstrated by the biologically active peptides isolated from food proteins. There are several advantages associated with biophysical characteristics of the aptamers, which include improved binding affinities through *in vitro* alterations, extended chemical and thermal stabilities, and propensity to accept either probes or "reporter" molecules without letting their binding properties get affected significantly (McKeague et al. 2015; Tan et al. 2017). All these enhance the prospects of using aptamers for the structural characterization and affinity-based regulation of the bioactive peptides (McKeague et al. 2014; Maehashi et al. 2007). The current review extends the discussions on these areas while focusing on regulation of structural and functional properties of bioactive peptides using aptameric ligands, including laying out agendas that could be considered critical for this research area. The article is organized as follows. In the next section, we provide an overview of the structural and functional features of the bioactive peptides. Section 3 deals with a discussion on the SELEX technology, including the current state of the art. The focus of Section 4 is to elucidate the bioaffinity attributes of the aptamers. In Section 5, the challenges and outlooks related to the solicitations of aptamers in structural scrutinizing of bioactive peptides are presented.

Bioactive peptides: overview

Bioactive peptides are short (2–20 units) and low molecular weight (<6000 Da) peptides, mostly derived from food proteins and exhibiting essential biological activities (Meisel and FitzGerald 2003; Ejike et al. 2017; Aluko 2018). These peptides are commonly present in inactive forms within the sequence of the parent (precursor) protein molecule; however, they are liberated by means of gastrointestinal digestion, *in vitro* enzymatic (e.g. trypsin, pepsin, chymotrypsin) and food processing (e.g. microbial fermentation) processes. The liberation process renders the resulting peptide highly bioavailable, target-specific, and less allergenic in comparison with their native proteins (Agyei, Potumarthi, and Danquah 2015). Upon liberation, bioactive peptides elicit several physiological responses associated with cardiovascular, gastrointestinal, immunological, neurological, and other hormonal activities of the human system. As a result, bioactive peptide research has been an area of great interest for health, pharmaceutical and nutraceutical demands. Accordingly, there has been a lot of recent specialized reviews covering bioactive peptides from sources such as salivary proteins (Saitoh et al. 2017), marine sources (Kim and Wijesekara 2010; Ejike et al. 2017), plant RuBisCo (Di Stefano et al. 2018), collagen and gelatin (Gómez-Guillén et al. 2011) and, most dominantly, milk and whey proteins (Mohanty et al. 2016; Nielsen et al. 2017). Due to the vast array of scholarly discussions in the extant literature on bioactive peptides, this section focused on the necessary structural prerequisites for their biofunctional properties.

Structure–function characteristics of bioactive peptides

Structure–function elucidation of biologically active peptides is a growing field of research. The structural characteristics of bioactive peptides dictate the numerous functional attributes reported in the current scientific literature. The primary sequence of the peptide in association with its secondary and tertiary structural motifs determines the functionality (Agyei and Danquah 2011). Slight differences in molecular weight, amino acid chain length, or composition of a bioactive peptide are significant to improve, reduce, or nullify the functional behavior (Yao, Agyei, and Udenigwe 2018; Madureira et al. 2010). Furthermore, disparities in peptide conformation synergistically or antagonistically influence the bioactivity of the peptide (Hernández-Ledesma et al. 2005). As a result, the physiological behaviors of certain peptides relate to specific structural, conformational, and compositional integrity (Udenigwe and Fogliano 2017). Some examples of structure–function characteristics of bioactive peptides are given below.

Antihypertensive characteristics

The antihypertensive characteristics of bioactive peptides have been extensively researched. Generally, peptides with antihypertensive attributes are mostly competitive inhibitors of vasoactive enzymes such as angiotensin converting enzyme (ACE) and endothelin converting enzyme-1 (ECE-1). Inhibition via noncompetitive pathways is also feasible (Qian, Je, and Kim 2007; Suetsuna and Nakano 2000). ACE is a peptidyl dipeptidase that modulates hemodynamic activities in animals and humans. It catalyzes the transformation of angiotensin I to angiotensin II, which stimulates the release of aldosterone and the inactivation of bradykinin (vasodilator) to elicit hemodynamic responses. ECE-1 on the other hand mediates the *in vivo* proteolytic release of endothelins which are potent vasoconstrictors. Therefore, the inhibition of ACE and ECE in the renin angiotensin system is known to offer a therapeutic relief of hypertension.

Casokinins and lactokinins from casein and whey, respectively, are the predominant food-derived peptides with

antihypertensive characteristics. Important structure–activity relationships have been identified in most bioactive peptides. For instance, peptides that inhibit ACEs are mostly identified as short and low molecular weight amino acid sequences. The inhibitive interaction of ACE occurs as a result of the presence of aromatic or aliphatic hydrophobic amino acids at C-terminal (as in the case of Ile-Pro-Pro and Val-Pro-Pro) (Nongonierma and FitzGerald 2016). The hydrophobicity of antihypertensive peptides facilitates the binding and blocking of the N-terminal catalytic site of ACE (Moskowitz 2002). Reasonably, the catalytic pocket of ACE can hardly interact with large peptides (Gómez-Guillén et al. 2011). Besides, short-chain peptides are easily assimilated in comparison to large oligopeptides or proteins. According to Pripp et al. (2004), long-chain peptides (>6 residues) could experience a suppression of the C-terminal amino acid influence on ACE inhibition due to steric hindrances. Also, it has been reported that the existence of Lys, Pro, His, or Arg at the C-terminus of peptides highly influences the ACE inhibitive behavior (Meisel and FitzGerald 2003; Mohanty et al. 2016). The presence of N-terminally associated aliphatic amino acids such as leucine, valine, and isoleucine also improves the inhibition of ACE (Lee and Hur 2017).

Antioxidative characteristics

On the other hand, the antioxidative characteristics of peptides have been linked with aromatic amino acid residues capable of donating protons (or hydrogen) to electron-deficient species such as free radicals and reactive oxygen species (Sarmadi and Ismail 2010). Free radical species cause detrimental oxidative stresses to cells, and this may lead to deterioration or death of cells, tissues, and organs. As a result, the antioxidant activity of bioactive peptides is hypothesized as a consequence of the ability of these peptides to (1) scavenge free radical, (2) inhibit lipid peroxidation, and (3) chelate transition metal ions (Sarmadi and Ismail 2010).

Similarly, a key characteristic of antioxidative peptides is their hydrophobic nature that promotes their solubility in lipids and transport across lipid-like membranes (Kim et al. 2001). According to Chen et al. (1996), the length of an antioxidative peptide is about 5–16 amino acid residues. Most antioxidative peptides possess hydrophobic residues at either the N-terminus or the C-terminus to increase their interaction with free radicals and fatty acids (Li and Li 2013). It has been reported that the antioxidative characteristics of peptides, specifically from collagen and gelatin, are dependent on the Gly-Pro-Hyp unique repetition of amino acid units in their architecture (Kim and Mendis 2006; Kim et al. 2001). In marine-derived bioactive peptides, the high contents of Pro and Gly render them highly antioxidative (Ngo et al. 2012). Further, while His- and Try-containing peptides help in chelating electron-deficient free radical species (Li and Li 2013), Hyp-containing peptides have excellent absorptive and metabolizing essentialities (Gómez-Guillén et al. 2011). Also, the presence of Pro-Pro at the C-terminal of a biologically active peptide makes it resistant to digestion by gastrointestinal peptidases (Nakamura et al. 1995; FitzGerald and Meisel 2000). Trp, Met, Cys, and Lys are also amino acids known for antioxidative characteristics (Stadtman and Levine 2003). These features control the delivery and potency of antioxidative peptides.

Antimicrobial characteristics

Bioactive peptides with antimicrobial functions usually have cationic and amphiphilic characteristics that enable the interaction with the negatively charged outer membrane of microbes to initiate the antimicrobial effect (Mohanty et al. 2016). Antimicrobial peptides are mostly longer than 11 amino acid residues and bear a net positive charge at the C-terminus (Nielsen et al. 2017). The net positive charge is critical for its antimicrobial behavior (Alvarez-Ordóñez et al. 2013). The presence of an unblocked N-terminus as well as Lys, Arg, or His residues on the C-terminus confers a positive charge state to the peptide (Schrader, Schulz-Knappe, and Fricker 2014). Moreover, the hydrophobic and cationic residues putatively promote the solubility and penetrability of antimicrobial peptides across the lipid-like membranes of bacteria (both Gram positive and Gram negative), fungi, and enveloped viruses (Anjum et al. 2017).

Mineral-binding characteristics

Conspicuously, peptides with mineral binding abilities are mostly phosphopeptides with varied amino acid compositions which dictate the magnitude of mineral chelation (Clare and Swaisgood 2000). For example, casein phosphopeptides are capable of chelating minerals such as calcium and iron because of the presence of three phosphoserine clusters associated with two glutamic acid residues (Walters, Esfandi, and Tsopmo 2018). Lactoferrin is a prominent, high-affinity iron-binding glycoprotein derived from whey with the ability to sequestrate minerals. It is characterized by folded interacting N- and C-terminal lobes which confer its functional properties (Baker and Baker 2005). The ability to sequestrate minerals also makes lactoferrin a possible bacteriostatic biomolecule for suppressing the mineral-dependent growth of bacteria. It is worth mentioning that lactoferrin also exhibits latent anti-inflammatory and immunoregulatory function upon proteolytic digestion (Nuijens, van Berkel, and Schanbacher 1996).

Immunomodulatory, antithrombotic, and opioid characteristics

Immunomodulatory attributes of peptides within the human body is largely nonspecific (Agyei and Danquah 2012). Peptides with immunomodulating effects reportedly control the proliferation of cells and the stimulation of humoral defense mechanisms such as antibodies, lymphocytes, cytokines, and macrophages. Antithrombotic peptides (e.g. casoplatelins) disturb platelet functions via the inhibition of fibrinogen-platelet binding (Clare and Swaisgood 2000). On the other hand, opioid peptides regulate certain functions in the intestines, central or peripheral nervous system to elicit responses such as insulin secretion, regulation of electrolyte

transport, regulation of body temperature, calmness, satiety, and whetting of appetite (Mohanty et al. 2016). Through receptor–ligand interaction, opioid peptides such as α-, β-lactorphin, and β-lactotensin also could demonstrate antihypertensive functions, unlike the inhibition of ACE (Madureira et al. 2010). Opioid peptides commonly have aromatic residues (e.g. tyrosine or phenylalanine) representing the second, third, or fourth unit, with a tyrosine residue of a negative potential usually at the amino acid N-terminal (Meisel 1997). These characteristics allow opioid peptides to fit well within the binding pockets of their receptors and interact effectively (Clare and Swaisgood 2000). The aforementioned structure–activity traits in bioactive peptides make it feasible for them to be monitored for structural changes that impact on the biological properties of the peptides.

Anticancer characteristics

The exact mechanism of action for peptides with anticancer activities is not fully understood. Nevertheless, structural features such as the presence of hydrophobic amino acids, net charge, and chain length are pivotal in conferring anticancer activities in bioactive peptides. For instance, the presence of aromatic hydrophobic amino acids such as proline, alanine, tryptophan, glycine, leucine, and tyrosine have been observed to be present in anticancer peptides to prevent cell proliferations (Wang and Zhang 2017; Chi et al. 2015). Shorter chain lengths of peptides, low molecular weight coupled with hydrophobicity of amino acid residues, further enhance the ability of peptides to penetrate through cell membranes and exhibiting higher cytotoxicity against tumor cells (Song et al. 2014; Chi et al. 2015; Hung et al. 2014).

Types of aptamers

DNA/RNA-based aptamers

Oligonucleotides have emerged as essential precursors for the synthesis of bioaffinity ligands with great potentials. This emergence centers on the capacity of the oligonucleotide molecule to selectively hinder critical biological processes such as gene transcription and the expression of malignant cells. For instance, aptamers are short-chain oligonucleotides with the capacity to recognize minor conformational differences in biological molecules with appreciable specificity. The high specificity and selectivity of aptamers even render them suitable for use as biological drugs and drug delivery vectors in therapeutic applications (Acquah et al. 2016). In contemporary research, aptamers are confirmed as suitable substitutes for antibodies in traditional animal-based antibody applications, including the probing of DNA damage and repair processes (McKeague 2017).

DNA and RNA-based aptamers are the most common forms of aptamers known. They are predominantly single-stranded and synthetic nucleic acids capable of targeting and binding their cognate targets, such as surface proteins, peptides, biological receptors, and biomarkers, with high specificity and selectivity (Orava, Cicmil, and Gariépy 2010; Santosh and Yadava 2014). The development and selection procedure of nucleic acid aptamers mainly involves the alteration of nucleotide bases or the reformation of the phosphodiester backbone to confer the aptamer a resistance against chemical and enzymatic degradations (Kopylov and Spiridonova 2000). The selection method, especially the *in vitro*, is unaffected by cell physiology and promoted by bulky library size. The reformation of the phosphodiester backbone is putatively more beneficial in DNA-aptamers than the RNA-aptamers. Both DNA and RNA-based aptamers assume a highly ordered, robust, 3-D structure. The DNA and RNA-based aptamers have no notable differences in their performance in terms of target selectivity and binding affinity. However, the DNA aptamers possess higher chemical robustness than the RNA aptamers, but the latter have a varied range of structural configurations as a result of their more adaptable characteristics (Radom et al. 2013; Toh et al. 2015). The essential base pairing features and 3-D forming abilities of RNA-based aptamers coupled with their facile nucleotide modification and intracellular expression possibilities make the aptamer well-characterized for targeting ligands (Radom et al. 2013). Nonetheless, the application of RNA aptamers faces more challenges compared to DNA aptamers. For instance, the RNA sequences are very vulnerable to nuclease-mediated hydrolysis as compared to their DNA counterparts (Santosh and Yadava 2014; Radom et al. 2013). Also, the construction of RNA oligonucleotide libraries in the SELEX—a technique for synthesizing aptamers—is expensive due to an additional transcription step. For the DNA-based aptamers, the SELEX procedure is much simpler with inexpensive initial DNA libraries. Also, unlike RNA-based aptamers, DNA aptamers are more stable and the synthesis procedure obviates the use of reverse transcription in their selection. Therefore, DNA aptamers have become preferable for use as therapeutic targeting ligands or delivery vectors.

Peptide aptamers

Peptide aptamers are a recent development in the field of aptamers. Unlike the nucleic acid-based counterparts, peptide aptamers are 'doubly inhibited' flexible amino acid sequences with the termini bonded to an inactive scaffold (Mascini, Palchetti, and Tombelli 2012). Peptide-based aptamers have a wide range of essential functional groups which are deficient in nucleic acids. The critical functional groups have electrostatic and/or hydrogen bond attributes which endow the peptide aptamers with excellent interactive and target affinity competencies. The development and selection procedure of peptide aptamers involve the generation of libraries and subsequent amplification of amino acid sequence followed by a separation procedure that makes use of high affinity target which subsequently gets eluted (Song, Lee, and Ban 2012). Herein, a rigid and rationalistic selection protocol is employed which considers the scaffold selection, the length of the peptides, and the marker count to render the peptide aptamer a resistance against DNAse-and RNAse-mediated lysis (Mascini, Palchetti, and

Tombelli 2012). The ensuing aptameric molecule is known to demonstrate excellent molecular, structural, conformational, and performance characteristics as reviewed by Reverdatto, Burz, and Shekhtman (2015).

Several reports have indicated that peptide aptamers exhibit better specific target affinity characteristics in comparison with the DNA- and RNA-based aptamers (Reverdatto, Burz, and Shekhtman 2015; Hamdi and Colas 2012). The aforesaid is believed to be as a result of the superior acid–base behavior and rigid backbone of peptides relative to DNA/RNA. For instance, DNA/RNA aptamers' selection procedures are negatively affected by RNAse actions and often yield poor response to protein and membrane constituents (Cotten et al. 2011). The process, moreover, suffers from limitations concerning post-translational modifications and possible forfeiture of peptide attaching agents (Crawford, Woodman, and Ko Ferrigno 2003). Also, despite the need for large quantities of refined target, the *in vitro* selected nucleic acid aptamers mostly suffer poor cellular acceptance (Baines and Colas 2006; Dassie and Giangrande 2013). However, the selection process of peptide aptamers obviates these challenges to a large extent through proper protein folding and glycosylation steps as well as the synthesis of appropriate disulfide bond and facilitation of less competition amongst the peptide binders (Crawford, Woodman, and Ko Ferrigno 2003). One distinct characteristic of the peptide aptamers is that they can be mounted on microarrays, which may subsequently be used to identify and enumerate proteins, even from complex samples (Colas 2008). However, according to Mascini, Palchetti, and Tombelli (2012), it is difficult to lay a strong dais in favor of peptide aptamers over the DNA- and RNA-based aptamers. In fact, Reverdatto, Burz, and Shekhtman (2015) remarked that both the peptide and nucleic acid aptamers possess considerable performance and application similarities, and that high-throughput selection and statistical interventions practically enhance the latter. To this point, although the peptide aptamers have received a better feedback from the scientific community, it is currently not straightforward to outrightly disregard their nucleic acid counterparts.

Technologies for the preparation of nucleic acid aptamers

SELEX technology

Aptamers can be synthesized artificially via an *in vitro* chemical process named as systematic evolution of ligands by exponential enrichment (SELEX). SELEX is an iterative and repetitive selection cycle comprising of three sequential steps: incubations, selections, and amplifications of the target. Basically, SELEX mimics evolution via iterative cycles and it starts with an initial library containing a pool of randomized sequences, which provides a high diversity of different aptamers toward a given target (Tan et al. 2016; Radom et al. 2013). There are varieties of SELEX modifications with various functions. These modifications are often made in terms of the initial library pool, selection stringency, and PCR amplification in order to improve the SELEX process and production of more specific aptamers with high affinity toward a wide range of targets including cells, ions, tissues, peptides, and proteins (Acquah et al. 2018; Tan et al. 2016).

Modifications in SELEX technology

In general, aptamers that target proteins are synthesized via the *in vitro* protein-based SELEX using a purified protein as the target. The enrichment of a specific pool of aptamers can be easily obtained during the selection step if the target protein owns a stable conformational structure. Limited variety of aptamers with high affinity toward specific peptides and proteins have been successfully generated via SELEX. This includes the MUC1 peptides (Ferreira, Matthews, and Missailidis 2006), protein tyrosine phosphatase 1B (Townshend et al. 2010), and prostate-specific membrane antigen (Lupold et al. 2012). Some SELEX modifications are utilized to generate desired aptamers targeting peptides or proteins with high efficacies. For instance, affinity chromatography-based SELEX selects only specific aptamers with binding affinity toward a given protein or peptide target. This works by immobilizing the target molecules using glutathione S-transferase or His-tag onto the agarose-based beads in a column (Song, Lee, and Ban 2012). Nitrocellulose membrane filtration-based SELEX is another SELEX modification used widely to separate and select aptamer-bound protein target. It possesses nonspecific affinity toward both proteins and amino acids due to the hydrophobic adsorption. Therefore, it is employed in both atomic force microscopy and Western blots in order to immobilize and separate protein molecules from other components effectively (Challa, Tzipori, and Sheoran 2014). In addition, covalent or crosslinking-based SELEX is one of the techniques aimed to identify and select desired aptamers with reactive groups which specifically bind to their target protein via covalent bonds (Yang, Li, and Gorenstein 2011).

The efficacy of a conventional or protein-based SELEX is greatly dependent on highly purified target molecules as well as previous information of the conformation of peptides or proteins. As a result, it is not efficient in selecting aptamers toward proteins with nonnative conformational structure. In order to overcome this limitation, cell-SELEX that uses whole living cells as targets during the selection process has been applied. The step of protein purification is not required in cell-SELEX which helps to preserve and maintain the native folding as well as conformation of targeted cell-surface transmembrane proteins, protein receptors, or biomarkers (Ye et al. 2012). Also, specific cell-targeting aptamers produced from cell-SELEX can enhance the discovery of novel biomarkers for membrane proteins on disease cells. This is certainly important to improve the current medical diagnosis and therapies.

Besides that, there are non-SELEX methods available as new approaches for the synthesis of aptamers. Non-SELEX is significantly different from SELEX technology due to the absence of PCR amplification in the SELEX iterative cycles (Tabarzad and Jafari 2016). This unique characteristic of non-SELEX technologies makes them as an ideal method to

select aptamers against protein and peptide targets with higher efficacy. All these SELEX modifications are ideal techniques for the synthesis of desired aptamers targeting various proteins and peptides.

Bioaffinity traits of aptamers

Protein and peptide molecules have unique sequences based on the arrangement of their amino acids, which in turn enables inter- and intra-molecular affinity interactions. These unique sequence arrangements ensure variability in their functional properties in relation to physiological and pathological processes. Aptamers are short single-stranded DNAs or RNAs, which are chemically synthesized via the robust, repetitive, and amplification SELEX technique for an infinite range of molecular targets. They have molecular interaction with their cognate targets such as ions, cells, drugs, proteins, and bioactive peptides via their 3-D secondary structure and unique sequences (Sharma et al. 2016; Song et al. 2008). Some of the unique secondary shapes of aptamers that can be formed include stems, internal loops, hairpins, pseudoknots, and G-quadruplexes (Teng et al. 2016; Yang et al. 2013). Gelinas, Davies, and Janjic (2016) highlighted that 14 out of the 16 aptamer–protein complexes have a double helix motif mostly capped with hairpin loops. Insights into the structural rules governing the interactions between aptamer–protein complexes are necessary to form high specific binding (Teng et al. 2016; Yang et al. 2013). A noncovalent bond of attraction such as van der Waals, electrostatic, hydrogen bonding, and hydrophobic stacking exists between aptamers and their targets (Yang et al. 2013).

Aptamers, a new class of bioaffinity probes have superior binding kinetics and biophysical features over antibodies (Vasilescu et al. 2016; Tan et al. 2017). They have dissociation constants within the nanomolar to picomolar range (Acquah et al. 2018). Aptamers can be easily engineered via pre- and/or post-modification techniques with chemomolecular features such as reporter molecules and specific functional groups (Maier and Levy 2016; Gao et al. 2016). They are also economical, thermally stable, reusable, have minimal batch-to-batch variability, a prolong shelf-life, devoid of ethical issues, and have a shorter production time (Dong et al. 2014; Hori et al. 2018). The chemical synthesis of aptamers, therefore, makes them readily adjustable for different targets and applications (Tan et al. 2017; Zhou, Liu, and Jiang 2018).

The biophysical structure of aptamers includes a 5′ and 3′ moiety that enables easy modification and immobilization onto varied supports. Owing to the thermal stability and reusability of aptamers, their immobilization onto suitable matrices further allows for the development of varied biosensing devices, known as aptasensors, with high specificity and sensitivity (Mukama et al. 2017; Acquah et al. 2018). Their small-size-to-volume ratio leads to the formation of sensors with high ligand densities. Immobilization of aptamers can be possible through a host of chemistries such as physical adsorption, chemisorption, biocoating, covalent, and noncovalent bonding (Mukama et al. 2017).

Prospects of aptamers for structural monitoring of bioactive peptides

It is no secret that the establishment of monoclonal- and polyclonal-based therapies have been able to advance the situation from a virtual 'no optimism' to a real 'considerable confidence' one. This is evident from the range of commercially approved antibodies by the FDA and the number of antibodies that are still in the pipeline. However, strictly from the point of view of the quality of the commercially available antibodies, it is still a long haul to achieve (Groff, Brown, and Clippinger 2015). The most significant problem that mar the utilization of maximum potential of these therapeutic agents lies in their highly deprived, or in some cases, negligible target specificity. Additionally, issues regarding reproducibility of antibodies derived especially from animals have not received a universal acclaim. In fact, there has been considerable debates in recent literature regarding the justification of spending too much resources, both temporal and monetary, and whether the 'once-hailed blessings to grace the drug therapy domain' seriously deserve the hype and live up to the expectations (Baker 2015; Bradbury and Plückthun 2015a). It has been reported that only 49% of the marketable and animal-originated antibodies could be authenticated to aptly identify their only targets (Berglund et al. 2008), and that is around a staggering US $1.6 billion worth of unproductive research concerning these antibodies have simply gone down the drain (Baker 2015; Bradbury and Plückthun 2015b). All these have not been well appreciated by the community and searches are perpetual, for better agents that would mitigate the loopholes. Although not foolproof, the advent of substantially improved recombinant antibodies and aptamers has been able to alleviate the state to an impressive extent (Groff, Brown, and Clippinger 2015; Ku et al. 2015). What is heartening is that the reported advantages pertaining to the aptamers have been touted to be just the tip of the iceberg and that the community is grossly unacquainted with the prolonged assortment of concomitant welfares (Groff, Brown, and Clippinger 2015).

Till date, there are very few studies investigating the propensity of aptamers as bioaffinity probes to detect bioactive peptides and in their structure–function characterization processes. The unique inherent biophysical traits and demonstrated binding performance of aptamers (Deng et al. 2014; Nimjee et al. 2017) implies that they can be utilized effectively for the detection, enrichment, isolation, and/or production of bioactive peptides with highly acceptable pharmaceutical grades. The reason for heightened research interest in bioactive peptides is their ability to induce physiological responses (Agyei and Danquah 2011). It must be noted that there are many commercial entities, as listed in Table 1, actively engaging in the commercialization of aptamers and their associated assays due to the high prospects of aptamers.

In addition, there is a wide range of portable aptasensors currently under development, which can be adapted for bioactive peptide detection. These aptasensors can be broadly grouped into electrochemical, optical, and mass-based (van

den Kieboom et al. 2015; Song et al. 2008). Similarly, research into aptamer-based chromatographic separations (Acquah et al. 2015; Du et al. 2015) can also be a promising field in advancing the isolation and enrichment of bioactive peptides.

According to Tabarzad and Jafari (2016), most research on aptamer-proteins and peptide interactions have largely been demonstrated through the use of thrombin and lysozyme biomolecules as the model targets. The successes in these apta-assays can be applicable by extension and customized for bioactive peptide molecules, as shown in Figure 1. In addition, due to the versatility of SELEX techniques, aptamers can be synthesized and engineered for newly discovered bioactive molecules.

An exciting prospect in the synthesis and application of aptamers is in the detection of biomarkers associated with bioactive proteins. The World Health Organization in conjunction with the United Nations Environment Programme and International Labor Organization defines biomarkers as 'any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease' (WHO 2001). For instance, casein which is an example of milk protein with bioactive peptides is responsible for inducing functionalities such as angiotensin-converting enzyme inhibitory (ACE-I), immunomodulatory, antihypertensive, antimicrobial, antioxidative, and opioid activity (Nguyen et al. 2015; Korhonen and Pihlanto 2006). Although β-casein A2 variant, a Type A2 milk, is less susceptible to proteolytic degradation resulting in the formation of only β-casomorphin-9A2 peptide, β-casein A1 undergoes a different cycle of proteolytic degradation. This is mainly due to the presence of histidine in the latter (Ng-Kwai-Hang, Monardes, and Hayes 1990). It has been reported that the breakdown of β-casein A1 variant of milk produces β-casomorphin-9A1 which in turn further degrades into β-casomorphin-7 (Sodhi et al. 2012). Consequently, β-casomorphin-7 has been identified as a biomarker linked with diseases such as type I diabetes, arteriosclerosis, autism, coronary heart disease, and schizophrenia (Tailford 2003; Laugesen and Elliott 2003; Parashar and Saini 2015). In light of this Parashar, Rajput, and Sharma (2015) recently developed fifteen (15) ssDNA aptamers with low dissociation constants ranging from 7.7 to 156.7 nM for the detection of β-casomorphin-7. The

Table 1. Examples of companies engaging in active aptamer research for clinical approval.

Company	Location
Aptamer Group	United Kingdom
Aptagen	United States of America
Aptamer Sciences	South Korea
Base Pair Biotechnologies	United States of America
Gilead Sciences	United States of America
NOXXON Pharma	Germany
Ophthotech Corporation	United States of America
SomaLogic Incorporation	United States of America

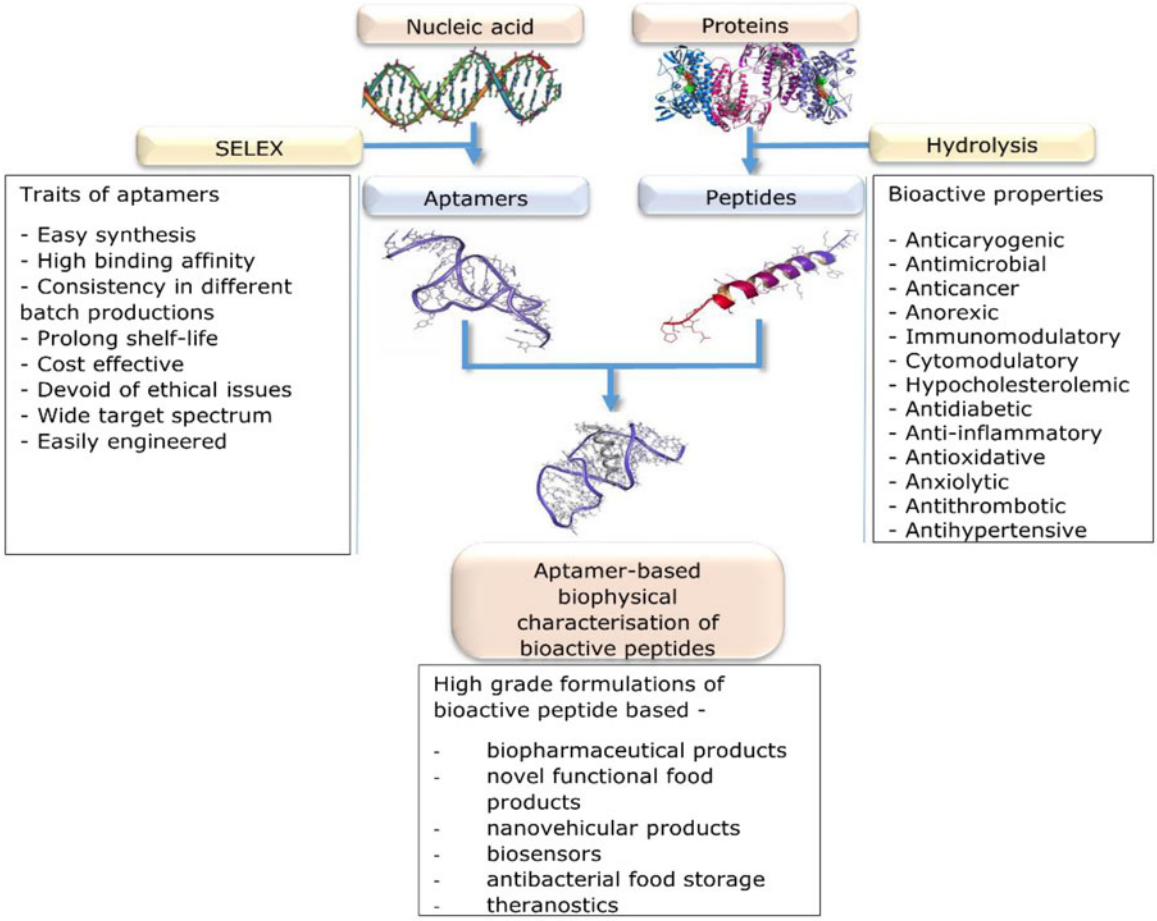

Figure 1. Integration of aptameric ligand sensing into the development and structure monitoring of high grade bioactive peptides.

synthesized aptamers were successfully used to develop an aptasensor known as enzyme-linked aptamer-sorbent assay for the detection of β-casomorphin-7 in urine, plasma, and β-casein digest (Parashar, Rajput, and Sharma 2015).

Challenges, potential solutions, and prospects

Despite the prospects of aptameric sensing in bioactive peptides research, there are a number of challenges in their synthesis and applications. First, selection of aptamers via the traditional SELEX approach is challenging for bioactive peptides due to the expectation of a 'no effect' on the structure and functionality of the peptides. However, advances in high-resolution spectroscopy such as Förster resonance energy transfer (FRET) spectroscopy for biomolecular interaction analysis have shown a proven history of potentially surmounting this perceived challenge (Obeng et al. 2016). For instance, FRET with its complementation (e.g. fluorescence lifetime imaging, FLIM; high-resolution photoactivation localization microscopy, PALM; fluorescence correlation spectroscopy, FCS; fluorescence cross-correlation spectroscopy) could potentially help in the preliminary screening of the expected interaction between candidate aptamers and the bioactive peptide in a model environment to ascertain and optimize the specificity while maintaining the structural and functional integrity of the peptide. That said, a number of non-SELEX methods have recently been described to be suitable for the selection of aptamers specific for protein and peptide molecules (Gelinas, Davies, and Janjic 2016; Reverdatto, Burz, and Shekhtman 2015; Tabarzad and Jafari 2016).

Second, owing to the limited studies investigating the binding performance of aptamers against peptide molecules, there are no approved standards in their application (Acquah et al. 2015). To date, the Macugen remains the only FDA-approved aptameric drug (Ruckman et al. 1998), although several others are under different phases of clinical trials (Parashar 2016). The conjugation of aptameric drugs onto bioactive peptides will not only help study changes in the properties of the peptides but could also lead to the generation of a novel molecule with improved or multiple biological properties. There is, therefore, the need for standards and regulations on the application of aptamers in this area, especially in the cases where novel bioactive conjugates are created from the binding interaction of peptides and aptamers.

Lastly, although aptamers can be easily engineered by incorporating new functional groups and are reusable, immobilization of aptamers onto supports often leads to a reduction in their binding affinity. There is also the challenge of steric hindrances in support-aptamer-bioactive peptide systems. Advances in conjugation chemistry to develop suitable techniques that can be used to immobilize aptamers with little or no decrease in binding capacity will help address this challenge. Since many biological activities, for example binding, adhesion, and charge transfer, are controlled by interfacial dynamics (Salamon, Macleod, and Tollin 1997), the application of plasmonic techniques, specifically SPR spectroscopy, could be a potential tool to understand and resolve the perceived challenges. For instance, the SPR spectroscopy could be used to preinvestigate the solid–liquid interfacial characteristics pertaining to folding and conformational dynamics, specificity and selectivity, and the binding rate, capacity, and isotherms during immobilization. This could be done in complementation with single-molecule fluorescence resonance energy transfer (smFRET). Similar studies of biomolecular interactions in other applications have led to the development of several biosensors (Homola 2008), and other high-throughput screening devices (Lausted et al. 2009; Maynard et al. 2009) for clinical, pharmaceutical, and other industrial uses. Overall, advances in aptamers technologies will strengthen their applications in the discovery and development of bioactive peptides, leading to growth in both fields and outcomes in functional food and health sectors.

Disclosure statement

The authors declare no conflict of interests.

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