Selective antibacterial peptides: a review on their polarity

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Selective antibacterial peptides usually contain 10-45 amino acid residues, most of them have positive charge to fold into amphipathic conformations and the cecropin type of linear peptides without cysteine. These peptides are featured by having high toxicity to bacteria membrane, low toxicity to mammal cells and not adopting an α-helical structure in aqueous solution. The peptide-membrane interaction has been studied for decades but so far the mechanism ruling it is still unknown. The aim of this paper is to summarize existing information about the correlation between polarity profile and selective antibacterial activity.

Keywords: selective antibacterial peptides; antibacterial activity; polarity profile; amphipathic conformations

Abbreviations: A, amphipathicity; Ala, alanine; APD2, Antimicrobial peptide database (http://aps.unmc.edu/AP/database/query_input.php) [1] accessed December-2012 & July, 2011; Arg, arginine; Asn, asparagine; Asp, aspartic acid; CL, cardiolipin; Cys, cysteine; Gln, glutamine; Glu, glutamic acid; Gly, glycine; H, hydrophobicity; His, histidine; HM, helical hydrophobic moment; HPD, host defence peptides; Ilu, isoleucine; IP, isoelectric point; Leu, leucine; Lys, lycine; Met, methionine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; Phe, phenylalanine; P, polarity; Pro, proline; PubMed, US National Library of Medicine. National Institutes of Health (http://www.nlm.nih.gov/bsd/policy/cit_format.html) accessed February-2013; Q, charge; SAP, selective antibacterial peptides; Ser, serine; SM, sphingomyelin; SWISS-PROT+TrEMBL European Bioinformatics Institutes (http://www.ebi.ac.uk/uniprot/) accessed November-2002; Thr, threonine; TI, therapeutic index; Trp, tryptophan; Tyr, tyrosine; Val, valine; WWW, World Wide Web; AMP, antimicrobial peptides.

1. Characterization of selective antibacterial peptides

Resistance to antibiotics has become a global health problem, therefore it is imperative to develop new antibiotics to treat illnesses. Antimicrobial peptides (AMP) are an evolutionary component of the innate immune response and are found in most living organisms from prokaryotes to humans as their principal defense system [2, 3].

Research and clinical interest [4] have turn to AMP as a new family of antibiotics representing another therapeutic option to treat infections caused by multi-drug-resistant bacteria. The diversity of AMP discovered is so great that it has been broadly categorized by their secondary structure [5, 6]. They have been classified in four major groups: α-helical, β-sheet, loop and extended peptides [7], the first two are the most common in nature. There are also thousands of synthetic variations produced which are classified the same way.

In this group there is a subgroup of peptides containing 4-35 amino acid residues, we called it Selective Antibacterial Peptides (SAP); they are featured by permeating the plasma membrane of target bacteria without causing extensive damage to host membrane [8]. This antimicrobial peptide group with selective toxicity has linear molecules with potential to adopt an amphipathic α-helical conformation while lacking secondary structure in watery solution. The interaction of lipid bilayers in the membrane induces the amphipathic α-helical structure, which seems to be basic for antimicrobial activity. The membrane integrity can be disrupted by the formation of pore like structures [9, 10] or a more general alteration [11]. A second structural group includes peptides with several intramolecular disulfide bonds that stabilize a conformation containing amphipathic β-sheets. These peptides seem to carry out its antimicrobial activity primarily through membrane disruption. The other two less common groups include extended peptides rich in one or two amino acids. They have over 2172 naturally occurring cationic antimicrobial peptides which structural and functional properties are recently reviewed [12, 13]. Much of the effort has being directed to design similar peptides to those occurring naturally that have important antimicrobial activity coupled with low toxicity toward host cells. The peptide effect on mammalian cells integrity is usually measured by Hemolysis of the red blood cells.

Scientific literature identifies several QSAR algorithms [14] that efficiently identify SAP using physicochemical differences in peptide membrane i.e. Charge (Q) [15], Amphipathicity (A) [16], Helical hydrophobic moment (HM) [17], Hydrophobicity (H) [18], Isoelectric point (IP) [19], Polar angle (ɵ) [20], and Polarity (P) [21]. The aim of this review is to show how these properties correspond to the peptide polarity profile and how this property can effectively recognize this important group.

1.1 Introduction and biological activity

The membrane mechanisms to identify SAP and their pathogen action towards bacteria are two subjects that will be studied and discussed here as they are interrelated. SAP improve their toxic effectiveness but they do not evolve isolated, bacteria evolve with them and improve their mechanism to remain unnoticed. This evolutionary symbiosis partially explains peptide toxicity, which is experimentally measured by its therapeutic index [22], i.e. the peptide amount required to toxically affect bacteria.

Biomembranes are made of proteins and phospholipids and in some organisms there are also sterols and glycerides which shape their surface. Their main component is a phospholipid bilayer which is amphipathic, it forms hydrophobic and hydrophilic domains, however, prokaryotic and eukaryotic biomembranes differ considerably based on their percentage composition [23]. Human cells such as erythrocytes have membranes rich in PC, PE and SW, compared to non-human cells membranes that frequently have less PE and more SW. In contrast, bacterial membrane is much more electronegative and has higher amounts of PG and CL whilst human membrane has almost no PG and CL.

1.2. Mean net charge (Q)

Cationicity is very important in antibacterial activity for the electrostatic attraction between peptide and membrane anionicity or negative charge, this is caused by its high content of acidic phospholipids PG, PS and CL. Cationicity and peptide antibacterial activity correlate [15, 24, 25], though the correlation between these two variables [25] is not always linear, it can be direct, indirect or inverse. Neither is it that at greater cationicity greater the peptide selectivity or toxicity. Most SAP are featured by a mean net charge from 0.2 to 0.5 or higher.

1.3. Amphipathicity (A)

Antibacterial peptides generally adopt amphipathic structures under multiple conformations interacting with bacterial membranes [16]. One of the simplest conformations is the α-helical amphipathic characteristic in SAP with three or four residues. Peptide conformation directly correlates with hydrophobic domain capability of segregation and peptide toxicity. Amphipathicity also evidences the contrast between peptide hydrophobic and hydrophilic domains.

1.4. Helical hydrophobic moment (HM)

The hydrophobic moment measures peptide amphipathicity, it is the sum of the side chain hydrophobicities in the helix of n amino acids. The numerical hydrophobicity associated to the kind of side chain gives the length of its corresponding vector and its direction is determined by the orientation of the side chain according to the helix axis. A high value of HM means the helix is amphiphilic perpendicular to its axis. The peptide hydrophobic moment directly correlates with its permeabilizing and hemolytic activities towards the bacterial membrane. A peptide is considered SAP if its value ranges from 0.4 to 0.6 [2].

1.5. Mean hydrophobicity (H)

Peptide hydrophobicity mean shows the number of peptide hydrophobic residues. It is the mean of hydrophobicities of the amino acids normalized to 1 over all amino acids of the peptide. Hydrophobicity is necessary to adequately permeabilize bacterial membrane. Peptide hydrophobicity and toxicity directly correlate in mammal cells membrane, however both characteristics inversely correlate with the lack of antibacterial specificity, for that reason many slightly hydrophobic SAP improve their pathogen action towards bacterial membrane [23]. The algorithm was given by the technical department of the Swiss Institute of Bioinformatics SWISS-PROT+ TrEMBL databases [26]. The value range considered was from 0.35 to 0.55 [22].

1.6. Isoelectric point (IP)

The Isoelectric point is the pH at which the hybrid ion concentration in a protein is at its maximum level and the net movement of solute molecules in an electric field is non existent [19]. In SAP the observed range is rather high from 10.8 to 11.8 [22].

1.7. Polar Angle (ɵ)

Polar angle measures the relative proportion of the polar and non polar faces in the peptide α-helical conformation [20], in which one of the faces has hydrophobic amino acids and the other has charged amino acids of let us say 180°, consequently an increase in the hydrophobicity face will reduce the polar angle. A reduced polar angle inversely correlates with the peptide ability to permeabilize bacterial membrane [27]. This peptide tendency to adopt the α-helical structure can be measured with a computational program called AGADIR [21]. If the peptide has less than 10 amino acids in length the range to consider it SAP will have to be from 0 to 10.0 [22], for longer peptides it is not an effective SAP discriminator [28].

1.8. Polarity

Peptide polarity is related to the polar characteristics of the amino acids forming it. An amino acid differs by its lateral chain or R radical of random structure determining its identity. There are hundreds of radicals in hundreds of different amino acids but only 20 are part of the proteins and have specific codons in the genetic code. These amino acids are usually classified according to the properties of their side chain [29] shown in Table 1.

Table 1 Twenty proteinogenic amino acid classification differentiated by their side-chain according to their polarity charge.

Symbol	Category	3-letter code amino acid
P-	Polar. Polar amino acids with negative charge have more carboxyl groups than amino groups making them acidic. The amino acids, which have negative charge on the R group are placed in this category. They are called as dicarboxylic mono-amino acids.	Asp, Glu, Tyr
N	Neutral. These amino acids do not have any charge on the R group. These amino acids participate in hydrogen bonding of protein structure.	Cys, Gly, Asn, Gln, Ser, Thr
P+	Basic. Polar amino acids with positive charge have more amino groups as compared to carboxyl groups making it basic. The amino acids, which have positive charge on the R group are placed in this category.	His, Lys, Arg
NP	Non polar. These amino acids have equal number of amino and carboxyl groups and are neutral. These amino acids are hydrophobic and have no charge on the R group.	Ala, Phe, Ilu, Leu, Met, Pro, Val, Trp

Peptide polarity is determined building a matrix called Polarity matrix P[*i,j*], its rows and columns have four polarity groups in this order (P+,P-,N,NP) from these four categories we construct a 16 element matrix where elements [*i,j*] from matrix P[*i,j*] represent 16 interaction possibilities between the groups. We built this matrix adding to the peptide sequence the number of incidences from left to right in amino acid pairs one at the time until the end [6]. To consider it SAP the value range for matrix P[*i,j*] should be (i,j) = (1,4) or (4,4) corresponding to interactions [P+,NP] and [NP–NP] respectively.

1.9. Physicochemical equivalence

Physicochemical characteristics define SAP under two equivalent criteria: (i) IP in range 10.8 to 11.8, HM in range 0.4 to 0.6 and AGADIR (equivalent to ϴ) in range 0 to 10.0. AGADIR property [22] is not particularly discriminatory when the peptide is greater than 10 amino acids in length, in that case AGADIR can be substituted by Q in range 0.2 to 0.5 and H in range 0.35 to 0.55, which means the peptide is natively unfolded [30–43]. (ii) P where the higher relative frequency must be in elements [P+,NP] and [NP–NP] (see Section 1.8). The correspondence between (i) and (ii) does not inform selectivity, which is supposed to take place with the electrostatic interaction between peptide and bacterial membrane [15, 27]. Since the peptide does not form an α-helical structure in watery solution, it leads us to think selectivity information lies in the peptide itself and not in the electrostatic exchange with the membrane [30] which takes place at a later stage. In this regard, the peptide linear structure will include "inherited information" with its pathogenic preferences towards the bacterial membrane.

2. Natively unfolded peptides and non-aggregation activity

Uversky [30–45] compared 102 natively unfolded proteins with 275 folded proteins selected from the SWISS-PROT+TrEMBL databases [26], for having no disulphide bonds, no interaction with ligans and a length between 50 and 100 residues. In a linear graph the two groups of proteins have their Q and H quite separated in the X and Y axis. Besides having high Q and lower H, the intrinsically unstructured proteins also have a lower number of aggregating sequences; they use the strategies of folded proteins to avoid aggregation, which seem to be dictated by the peptide linear sequence [46–48]. Thus aggregation is an evolutionary advantage for SAP, especially in the first stages, as it encourages peptide-membrane interaction so they can adopt a structure related to its pathogenic function and disaggregate.

3. Polarity profile and selectivity

A prevailing opinion is that selectivity originates from electrostatic attraction of the cationic peptide to the anionic bacterial membrane [49], as the majority of antibacterial peptides are positively charged at physiological pH. This opinion is consistent with present findings however, electrostatics can not be the only contributing factor because many cationic peptides also disrupt neutrally charged mammalian cells at higher concentrations. Consequently, more subtle peptide properties, including its hydrophobic moment [50], oligomerization state [51], and/or the specific type and

orientation of larger residues dictated by the sequence, may play a role determining the extent of peptide insertion or disruption of membrane integrity. For instance, on human erythrocytes [52] monomeric peptides were practically free from antibacterial activity, while their pentameric covalently attached oligomers were highly active.

4. Polarity and Chirality

The interaction between chirality of lipids and cholesterol has been widely studied. Some studies have shown that the two dipalmitoyl-PC enantiomers interact indistinctively with cholesterol; however, PC has only one chiral centre at C-2 position of the glycerol moiety, whereas *ent*-cholesterol differs at several chiral centres from cholesterol. Yet, the two enantiomers of cholesterol seem to interact identically with dipalmitoyl-PC [53], but for certain molar ratios of egg sphingomyelin and cholesterol, it has been found that there are differences between mixtures of cholesterol and e*nt*-Cholesterol when using monolayers [53, 54].

Most recent work questions these finding [55] as e*nt*-Cholesterol is an enantiomer of cholesterol, i.e. the exact mirror image, instead of being a diastereoisomer with different configuration in some of the chiral centres. As a result pure cholesterol and *ent*-cholesterol should have the same physical and chemical properties (except for optical rotation), though the two compounds may differ in their interaction with other chiral molecules as phospholipids or chiral peptides. The conclusion is that there is no enantioselectivity in sterol-lipid interactions [56].

The above results of membrane disruption caused by peptides to membrane components show they do not respond to peptide chirality. On the other hand our results indicate that peptide and protein membrane interaction capable of inducing the formation of cholesterol rich domains are affected by small changes in the physical properties of the membrane caused by variations in cholesterol chirality [53, 54]; therefore, peptide-induced segregation of cholesterol into domains is strongly affected by cholesterol chirality, though the peptide-lipid interactions involved are not very specific.

5. Can Polarity develop from functional taxonomy?

A practical peptide classification using QSAR algorithms has been aimed mainly to search matches of experimentally found peptides and classifying them by toxic action in several databases. One of the best cured databases is APD2 [1] which has the following subclassifications: Gram+ ONLY, Gram– ONLY, Gram+/Gram–, viruses, HIV, fungi, protists, parasites, insects, carcinogenic cells, mammalian cells, sperms and SAP. A step on this direction is the classification using the polarity profile in APD2; another alternative is to find peptide classification and taxonomy in digital magazines published by PUBMED [22–24, 44]. Both approaches are being developed with computational programs APAP [14] and Polarity profile [28].

6. Perspectives and trends

Future Therapeutics.-Antibacterial peptides have vast potential as agents in our search for new therapeutics for topical and systemic administration. They represent a good option against pathogenic microbes and they are also important as immunomodulatory agents.

Among their main interesting features are their broad spectra activity with rapid action, the low potential for pathogen resistance on repeated exposure, and the low toxicity for eukaryotic cells. Many SAP are immunomodulatory, but have limited immunogenicity [57]. Their small size makes them easy to synthesize but the substantial cost of peptide synthesis must still be overcome.

Some studies show the construction of synthetic host defense peptides (HDP) without any direct antimicrobial activity, these peptides are still efficient protecting against bacterial infection [58]. These results indicate direct antimicrobial activity is not necessary for protection against infection. Nevertheless, all in vivo interactions and all immunomodulatory effects must be thoroughly evaluated in order to assess safety before the systematic administration of SAP.

These immunomodulatory and anti-infective SAP/HDP have great potential for therapeutic use provided there is more knowledge and understanding about them. Cationic peptides will probably be able to avoid issues of antibacterial resistance because they do not eliminate microbes directly. It has been found that SAP of evolutionary distant origins are functionally very similar, and the understanding of the design of lower invertebrate peptides will be of great help to develop new antibiotics, host defense and/or immunomodulatory agents in humans.

Paradigms. - There are two different approaches to identify SAP: target-based and activity-based. The first one requires knowledge about the target molecule. The second is oriented to find patterns associated to the activity of the SAP. From a mathematical and computational standpoint, there are methods that relate chemical structure with biological activity that can be classified in two main groups: supervised learning and non-supervised learning [59, 63]. These methods vary on their mathematical computational degree of complexity; they were developed few decades ago and represent the first important effort to consolidate the Bioinformatics field [60–62]. In the next decades this

discipline will probably be combined with Robotics [63–65] to build biological robots [66] capable "to learn" from the World Wide Web (WWW) [67] exhaustive transversal analysis.

As many other disciplines, the consolidation of this science will come in time with the credit given to the regularity of the phenomenon studied. The methods to identify SAP to elaborate pharmaceutical drugs are not the exception to this procedure. Nowadays we know computational limitations are not an obstacle, the real difficulty lies in the efficiency of the methods used and the acknowledgment given to the phenomenon regularity [68, 69].

Regularity is a pattern observed when studying the dynamics of a phenomenon, to determine the moments where it changes and gives way to a new regularity. Those moments are not obvious as they underlie in the structure studied. Regularity is applied from subatomic particle waves to astronomic distances in the universe.

How can we build Biological robots of 10^{-9} meters size to identify regularities in the interaction membrane-peptide? Undoubtedly mathematical-computational methods aimed to detect and predict SAP will have to feature: (i) A greater mathematical orientation to recognize regularities from WWW in peptide sequences of different lengths; (ii) Must be parallelizable [70, 71] to be able to run in clusters [72] and grids [73] for GPU/FPGA [74–77], with an average processing speed of 2^{40} bytes per second for peptide sequence, it must be capable of discriminating relevant data and avoid storing unnecessary information, but learning from it; (iii) they must be capable of using the primary peptide sequence.

Biological robots will be the result of the knowledge we are getting from peptide identification and they will benefit from the properties of new materials at molecular scale so they can efficiently interact with peptides. In this light, robots will be hybrid peptides at molecular scale. Nanotechnology today is a reality and it is applied in the manufacturing of polymers or plastics found now in industry.

7. Conclusions

During the past two decades evident bacterial drug resistance has created an urgent need for new types of antibiotics. Even if a major epidemic has not yet hit and the panel of traditional antibiotics can still manage drug resistant pathogens, SAP seem to represent one of the most promising alternatives in future strategies for dealing with this threat.

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