Peptoids: Changing the Backbone of the Future of Medicine

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With ever increasing bacteria resistance antimicrobials, the world of biochemistry is a far from a static one. Because almost no antimicrobial completely kills all of the intended bacteria it is meant to, there almost always survivors. Whatever trait caused the cells to survive will be passed along to future generations and the offspring will consequentially have a good chance of surviving the drug. The lifespan of bacteria is typically less than an hour, so a new generation of the drug resistant bacteria can spring up relatively soon after implementation of the drug. Hence it can be argued that the stronger a drug is the stronger bacteria it will produce, unless of course it is strong enough to kill them all. Such is the case of Methicillin-Resistant Staphylococcus aureus (or MRSA). Staph aureus, which is found on the epidermis of 20% of people (Kluytmans, Belkum, and Verbrugh 1997), can be harmless, but certain strains have become very virulent. The treating of Staph with heavy regiments of antimicrobials has led to increasing drug resistance of certain strains to the point that some such as MRSA are almost a death sentence. Currently Vancomycin is the only largely successful commercial drug to treat MRSA infections. Due to the constant evolving world of microbes, there is a constant need for new compounds to treat them. The standard approach has been to take a known working drug and slightly modify one of the side chains or groups on the main compound to hopefully make it different enough to again be able to kill the intended bacteria. In the early 90's, Dr. Ronald Zuckermann at Northwestern University decided to experiment with keeping the same side chains, but

rearranging their placement on the carbon skeleton. Beginning with simplistic molecules for a test of concept, he created a peptide with the side chains from the α-carbon switched to the nitrogen, and created what is now called “peptoids”. Research into peptoids has been very promising, showing they have an increased stability compared to their peptide counterparts, control of chirality, low immune responses, and are generally unrecognized and unaltered by bacteria and eukaryotes alike.

To first understand peptoids it is best to start with how they are created. Peptides are polymers of amino acids bonded together by peptide bonds. As detailed in Figure 1, peptide bonds are bonds created by the hydroxyl group of one molecule reacting with hydrogen from the other molecule’s amine group, which releases a water molecule and creates a covalent bond.

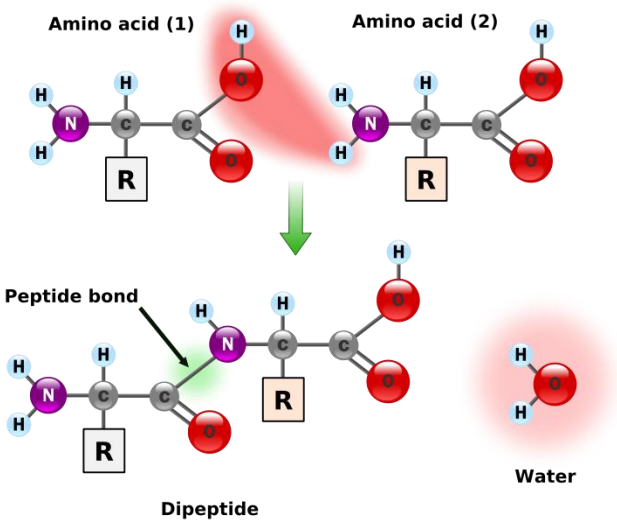

Figure 1. Peptide Bond Formation (Mrabet, 2007)

Peptoids have the same structure as peptides though the side chains are on the nitrogen in the backbone instead of the α-carbon. Peptides are naturally synthesized through the facilitation of an enzyme, Peptidyl Transferase, but because this and other enzymes do not recognize of the nature of the peptoid monomers (see Figure 2) they will not readily create peptide bonds between them, so a different method is required for peptoid synthesis. The first step in creating a peptoid

is to take the preassembled side chain attached to an amine and attach it to a haloacetic acid (Zuckermann, *et al.* 1992). The haloacetic acid is a place holder that is only on the first side chain in the desired sequence. It will not come off through the peptoid synthesis process, and thus prevents the peptoid from sequencing in the wrong direction. The haloacetic acid is not removed until after completion of the synthesis and it is cleaved by a solvent that will specifically target whichever acid is used. A haloacetic acid generally contains two oxygen molecules and a halide, making it a very electronegative region (Figure 2).

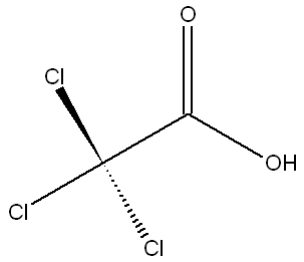

Figure 2. Trichloroacetic acid (a haloacetic acid)

Electronegativity is a way to measure relative electron affinities of molecules based on how strongly they can pull electrons away from other groups. The high electronegativity of the acid will pull electron density towards itself and away from the less electronegative parts, such as the nitrogen. This effect is transferred through the nitrogen, pulling the electrons in the nitrogen-hydrogen bond towards the nitrogen and away from the hydrogen, weakening the bond, and making it easier for the hydrogen to leave the compound, leaving a negatively charged nitrogen atom. In step one, the compound is then introduced into a solution containing dimethylformamide (DMF), Diisopropylcarbodiimide (DIC) and a bromated glycine (which is a glycine with bromine in place of the ammonia molecule). Both DIC and DMF are polar aprotic solvents, meaning the entire molecule has partially positive and partially negative regions created from differences in electronegativity. The solvents though do not have a hydrogen bonded to a

highly electronegative atom, and therefore cannot induce hydrogen bonding. Due to their polarity, they are able to induce the OH^- group from leaving the glycine and form with the H^+ from the nitrogen to yield a water molecule. The glycine is then left with a positively charged carbon atom which the negatively charged nitrogen atom readily bonds to. This then creates the intermediate shown in the center of Figure 1.

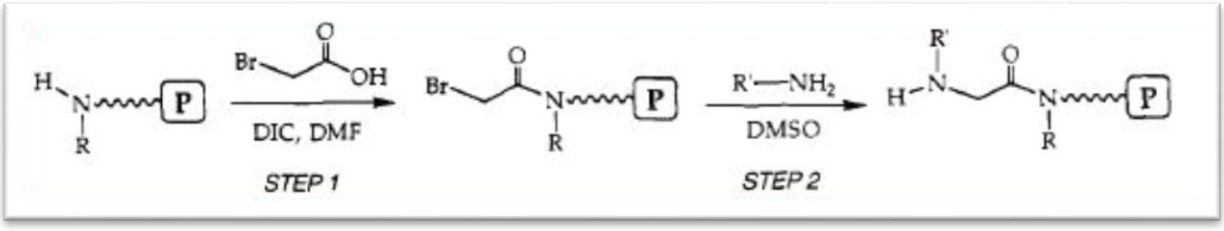

Figure 3. Synthesis of a Peptoid (Zuckermann, *et al.*, 1992)

The next desired side chain with an amine bonded to it is then added, in solution of Dimethyl Sulfoxide (DMSO), to the intermediate. The amine group on this side chain (and all amines added further in sequencing) contain two hydrogen as opposed to just one from the amine group in step one, because the amine in step two will bond to two separate carbon chains, and thus needs two sites that can readily change bonds. DMSO is also a polar aprotic solvent, and functions much like DIC and DMF. One of the hydrogen atoms in the amine group comes off due to the polarity of the DMSO, and thus the side chain has a negatively charged nitrogen atom. The bromine bonded to the glycine backbone readily leaves the glycine to form negative bromine ions in solution, leaving behind a partially charged carbon behind. Bromine is a halogen and thus only requires one electron to fill its 4p orbital giving it a very stable configuration, so it will readily leave the carbon, taking both electrons from the bond with it due to its high electronegativity. The positive carbon atom then bonds with the nitrogen attached to the side chain to form the final product of Figure 3, which is a peptoid consisting of two side chains. This process is then repeated with the desired side chains to form the peptoid of with the desired

length and structure. Due to DMSO, DIC, and DMF being unreactive throughout this process, the solvents can be readily added and extracted, and so the entire process can be automated on a peptide synthesizer.

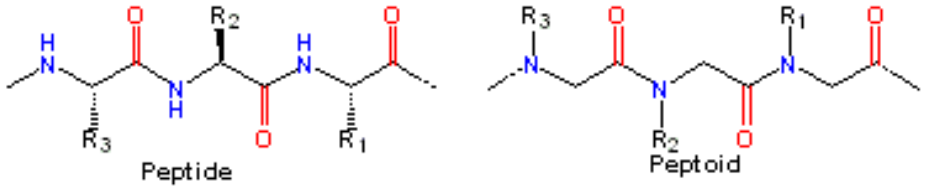

Figure 4. Structural Differences of a Peptoid vs. a Peptide (Wetzler, 2010)

The next step in understanding peptoids is to compare them to their counterpart, peptides, and see how they are different. Above in Figure 4 is a diagram of a peptoid and peptide, which could both contain the exact same side chains. From figure 4 it can be seen that the largest difference is the side chains (represented by R) are bonded to nitrogen on peptoids and carbons on peptides, thus the carbons in peptoids have an extra hydrogen, while in peptides, that hydrogen is on the nitrogen. This might sound inconsequential, but the sedative used for morning sickness released in the 1950's named Thalidomide would cause birth defects if the other enantiomer was also given (Moghe, Kulkarni, Parmar, 2008). The difference between enantiomers, as detailed in Figure 5 below, is a simple difference in the 3-D orientation of a bond between a nitrogen and carbon.

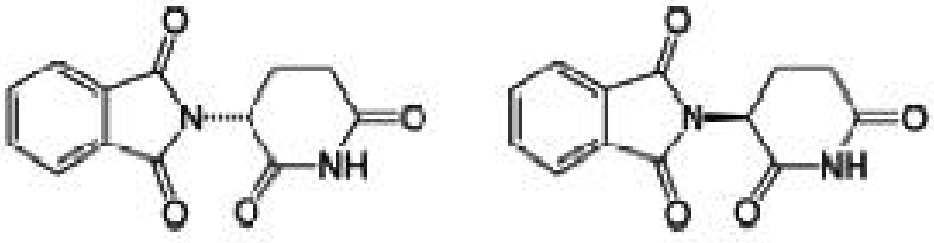

Figure 5. (R)-Thalidomide on the right (relieves morning sickness) and (S)-Thalidomide on the left (causes birth defects) (Moghe, Kulkarni, Parmar, 2008)

The side chain location differences are known as primary structure, which is simply the structure of what bonds are formed between which atoms. Aside from these, peptoids and peptides do have different secondary structure, which is the 3-D structure of segments in relation to others (Chongsiriwatana *et al.* 2008). In peptides, secondary structure is largely influenced by the hydrogen bonding of amine groups. The nitrogen is more electronegative than the hydrogen bonded to it, thus giving the hydrogen a partially positive charge which will attract to partially negative charges of other nitrogens. No actual bonds are made but the attraction is strong enough to keep the molecule stabilized. Because the nitrogen bond is based off polarity, when introduced into an aqueous solution (which is polar) it can change the arrangement of the bond. Certain peptides have a stable enough secondary structure to not denature in water, but not all do, and it is dependent on the sequence of the side chains.

Peptoids on the other hand do not have this hydrogen bond because the nitrogen does not contain hydrogen on it. However, due to the large bulky nature of the sidechains, they orient themselves to cause the least interference with each other (known as steric hindrance) (Wu *et al.* 2008). From figure 6 it is clear that the side chains are large complex groups. These groups create steric hindrance because around each atom is an electron cloud. These clouds push against each other because an electron repels another electron (due to similar charges repelling), thus the entire group will rotate and move to cause the least amount of strain, which will be the greatest distances between repelling clouds. This repulsion makes up the largest majority of stabilizing peptoid secondary structure. Since the structure of peptoids isn't based on hydrogen bonding, when introduced into an aqueous solution, peptoids won't easily change shape (Sanborn *et al.* 2001).

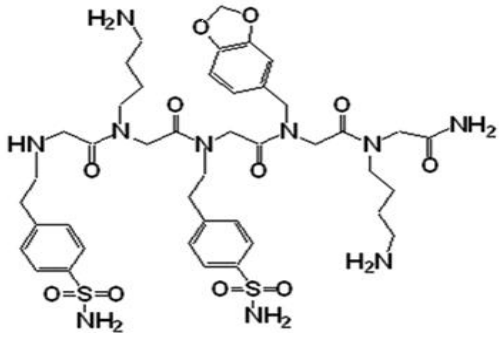

Figure 6. A Peptoid Designed for Use in a Cancer Research Project

In the biochemical world subtle differences can manifest large scale polar opposite effects, but fortunately for peptoids, this isn't true for every facet of the molecule. The high specificity between structure and function seems to work against bacteria here. If the function of a peptide is known, then a mimicking peptoid, which is a peptoid of the same sequence of side chains, can potentially perform the same function (Simon *et al.* 1992). Aside from functioning like peptides, peptoids are protease resistant. Protease is an enzyme that bonds to peptides and breaks peptide bonds by inserting a water molecule into the bond, reversing the process. Protease has been tested against peptoids and cannot break the peptide bonds in peptoids because the enzyme protease cannot recognize the peptoid as a molecule containing peptide bonds (Culf, Ouellette 2010).

With a much more complete comprehension of peptoids, it can now be understood why they are so promising, and preferred over peptides. Because evolution works towards finding a better solution, not the best solution, peptoids have never been found in nature and are thus unrecognized by cells which they have currently been tested with, which is a huge benefit for their use as antimicrobials. A peptoid can potentially perform the same function as a peptide with a certain antimicrobial affect, but the bacteria will not recognize the peptoid (Simon *et al.* 1992). This will prevent the cell from trying to fight off peptoid, such as removing it from its system or

degrading it or any of the other mechanisms it would use against a peptide. Due to this huge lack of resistance to peptoids, many drugs could potentially be resynthesized from peptoids instead of peptides and be “reused”.

All antimicrobials to be used therapeutically are designed to target something about the bacteria that is different from the host cell, so the antimicrobial isn’t destroying host cells as readily as it is infectious cells. But sometimes host cells can uptake the antimicrobial on accident and it can become poisonous through various side effects. With peptoids though, human cells do not recognize them much like bacterial cells don’t, and assume they are inert garbage and do not readily uptake them. Because of this, they have proven to be much less toxic to human cells that some of their peptide counterparts have proven to be. This also means that peptoid based drugs should be more effective in lower quantities since some isn’t being degraded or wasted before it gets to the target. The last added benefit of being a difficult to recognize molecule is that the immune system does not consider it as a threat and thus petoids are promising candidates for drugs because they have a low chance of triggering an immune response and causing an allergic reaction.

Aside from their ease of function and low toxicity, peptoids have one last benefit over conventional peptides. Nitrogen has the electron configuration of $1s^2\ 2s^2\ 2p^3$ which simply means the most outer orbital ($2p^3$) is only half filled, since the p orbitals hold a total of six electrons, and consequentially will only make three bonds under most circumstances. Carbon has the electron configuration of $1s^2\ 2s^2\ 2p^2$, which means it readily makes four bonds. The implications of this difference are extraordinary when one examines the differences of peptoids and peptides (Figure 3) and if one knows some basic principles of chirality. Chirality refers to the asymmetry of an entire molecule, or a single atom. If a plane of symmetry can be drawn through the center

of the molecule or the atom, then it is known as achiral, but if it cannot be drawn, it is chiral. The best general rule for chirality of a single atom is if all the groups bonded to it are different, it has to be chiral, but if two or more of the groups are the same, it is achiral. When reexamining Figure 3 and paying special attention to the groups the side chains are bonded to, it becomes clear that with peptoids, there is a control over chirality. In the peptide, the side chain is bonded to the carbon, which is bonded to two distinct carbon chains (treating each side not as a single carbon, but the whole chain as a group) and also bonded to hydrogen, giving it four distinct groups, making it chiral. But only the orientation of the two carbon chains is controlled, the side chain and the hydrogen can switch position to give products with different 3-D orientations. This gives rise to enantiomers, which as explained earlier, can cause huge problems. In peptoids however, the side chain is bonded to the nitrogen, which is then bonded to the two distinct carbon chains. Because nitrogen can only readily make three bonds, there is nowhere for the side chain to switch positions with, and thus can only be in one distinct orientation. Also, the carbons are no longer chiral in the peptoid because they both have two hydrogen groups, meaning even if they switched orientation, it's the same structure, and the same product. For this reason, the synthesis of peptoids allows for a complete control over the 3-D structure of the molecule without giving rise to unwanted enantiomers.

Over the past two decades peptoids have increasingly grown in the world of antimicrobials and therapeutic drugs. As more research is concluded they are proving to have more strengths instead of unforeseen flaws. Due to their novel structure never encountered before in nature there has yet to be an enzyme able to recognize and interact with them. Such a trait will severely reduce side effects from taking a drug made from peptoids, since host cells cannot accidently modify the drug and create something toxic. Their alien structure also is very

promising for antimicrobials since it opens up a whole new field of potential molecules to fight drug resistant strains of organisms. A combination of these two traits should potentially lower costs for medication since they will be relatively easy to synthesize compared to more complex peptides, and will require a smaller dosage to guarantee the same effect. Peptoid based drugs have also proven to be less toxic to mammalian cells then some of their peptide counterparts, which will reduce allergic reactions to medication, and further decrease potential harmful side effects. The structure of the peptoid alone drastically reduces the chance for impurities through recombinations and the sequencing in the wrong order. The structure also forces the peptoids to be synthesized in a process that has to be tailor made to create such a slightly modified compound. Though this has slowed the research and thus the production of peptoids, it decreases the possibility for more impurities and overall reducing the costs of synthesis in the long run. Last, due to their high stability, they don't need much modification to survive host cell environments to function in the required medium. Such a benefit makes peptoids a promising candidate for cancer research. A cancerous cell will produce certain waste, or biomarkers, specific to the processes that make it cancerous. If these biomarkers are known, then a blood sample can be screened for these biomarkers. A problem with this process though is that the molecule detecting the biomarkers has to be able to attach to the biomarker, without deforming to the point that a positive signal cannot be detected. The stability of peptoids and potential for high specificity has pushed peptoids ahead in consideration for biomarker validators. All of these attributes in sum make peptoids a revolutionary family of molecules that holds a bright future in medical research, and will expand the ability of modern medicine.

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