Chapter 1
The Origin and Evolution of Antibiotics

Julian Davies

Abstract Microbes are the most prevalent living organisms in the biosphere; they constitute about 50 % of the Earth's weight. They are also the most prolific in terms of the production of antibiotics and other bioactive small molecules. This rich store of chemical diversity (termed the Parvome) provides an inexhaustible source of therapeutic agents that has barely been investigated. Devising new ways of harvesting these compounds is a major challenge that requires developing new insights into their origin and evolution and also predictions of their roles in chemical and biological evolution. Only with this information will it be possible to exploit their pharmaceutical potential to the full.

1.1 Introduction

The biosphere is populated with an enormous collection of low-molecular weight organic compounds with an extraordinary diversity of molecular structures produced by living organisms (the Parvome) (Davies and Ryan 2012). Although a significant proportion of these compounds may be products of the normal processes of biodegradation, the majority are made by defined, regulated biosynthetic pathways and are involved in many of the functions and interactions of cells, tissues, organs, and organisms (both positive and negative). These molecules have highly specific interactions with cellular targets (although very few have been identified), and may act both extra- and intracellularly. It is likely that all living beings make bioactive small molecules for these purposes. It has been suggested that the "central dogma of biology" is more than just the triumvirate of DNA, RNA, and protein and should include the wealth of bioactive small molecules

J. Davies (✉)
Department of Microbiology and Immunology, Life Sciences Institute, University of British Columbia, 2350 Health Sciences Mall, Vancouver BC V6T 1Z3, Canada
e-mail: jed@mail.ubc.ca

F. Marinelli and O. Genilloud (eds.), *Antimicrobials*,
DOI: 10.1007/978-3-642-39968-8_1,

(Schreiber 2005). They play essential, but as yet, largely unidentified roles in the maintenance of the biosphere.

One of the best recognized groups of bioactive small molecules are those that have antibiotic activity; they are produced principally by bacteria, fungi, plants, and sponges, but in all probability most living organisms, including humans and elephants, etc., make such molecules. They are critically important, not because of their number or distribution, but because of their demonstrated therapeutic properties. However, it is likely that, they represent only a small fraction of the Parvome.

The word "antibiotic" was coined by Selman Waksman (Strohl 1997) soon after the discovery of streptomycin in the early 1940s. This compound, together with penicillin (historically and therapeutically), presaged the most successful medical epoch ever, the age of antibiotics. Infectious diseases became curable and billions of humans have been saved from the historical and modern plagues.

Although Waksman's definition was convenient, it ignored the vast number of bioactive compounds without detectable therapeutic potential that are produced by living organisms. In reality, the word antibiotic defines a property or an activity and not a compound.

1.2 What's in a Name?

The word antibiotic is often incorrectly used. At the present time, almost any small molecule made by a microbe is termed an antibiotic: this is loose-thinking! What can these products be called when their functions are so broad and when they have such a wide range of structures, activities, and biochemical origins?

The name "secondary metabolite" has frequently been used for bioactive compounds; this term was employed originally with reference to plant products. The simplest definition comes from "Wikipedia:" "Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism."

It is true that antibiotic activity is most frequently detected when the logarithmic phase of growth begins to slow down, when a microorganism is no longer dividing exponentially (Campbell 1984). However, although antibiotic activity cannot be detected earlier, bioactive compounds could well be produced, at concentrations not detected by inhibition. This definition also seems somewhat derogatory, since the compounds produced play many critical roles and can in no sense be referred to as "secondary"! The fact is, secondary metabolites are mostly synthesized using primary metabolites as precursors. The timing of their production with respect to growth is of no real consequence.

The word "idiolyte" has also been used as a substitute for secondary metabolite; this refers to an association with a production phase late in microbial growth. This word has not really caught on, perhaps because of its use in immunology.

1.3 Many Sources, Uses, and Functions

Bioactive small molecules have many properties and applications in medicine, industry, agriculture, and other uses (Demain and Sanchez 2009); these go far beyond Waksman's definition which states that "an antibiotic is a chemical substance produced by a microorganism which has the capacity, in dilute solutions, to inhibit the growth of, or to kill other microorganisms." It was not realized at the time that, at even lower dilutions, antibiotics might actually stimulate growth or influence other biochemical functions in microorganisms. For example, the formation of biofilms.

In order to discuss the evolution of microbially produced bioactive compounds in the context of the evolution of the cell, it is essential to realize that bioactive small molecules can, and do have many sources and probably played numerous roles in the origins of living organisms.

Thus, the Parvome components have evolved to serve numerous ecological functions in different organisms and under many circumstances. The biosynthetic pathways for small molecules may share many commonalities and probably evolved in similar ways, but their roles in the organism that produces them are often intimately related to the lifestyle of the producer. Bioactive compounds may play different roles depending on their hosts. For example, the adrenergic hormones: these are relatively simple organic molecules that have specific functions depending on the circumstances. They have been characterized as hormones produced by and playing important roles in humans and animals, but recently they have been shown to affect the properties of bacterial populations.

Norepinephrine acts as a stress hormone and a neurotransmitter in animals, but also stimulates bacterial growth and enhances bacterial virulence functions. This catecholamine was one of the first compounds identified in studies of the growing discipline of microbial endocrinology (Freestone et al. 2008). Similar activities have been reported for other congeners of this family of compounds. Interestingly, the mammalian protein hormone insulin has been shown to enhance the growth of certain bacterial pathogens (Plotkin and Viselli 2000). These are good examples of the extensive biological plurality of functions exhibited by bioactive small molecules. This is especially true for bacterial products that were first characterized for their antibacterial activity and used therapeutically. When tested employing a range of assays and concentrations for their biological activity, most so-called antibiotics prove to have a surprising range of concentration-dependent biochemical activities (Davies et al. 2006). In addition, most antibiotics have multiple toxic side effects due to their ability to bind to specific human receptors of one kind or another. Yet, for our convenience, they remain labeled as antibiotics.

Biologically active compounds with a diversity of biological activities (including antibiosis) have been present in the biosphere for eons. For example, the lichens, which are ancient mutualistic associations of fungi/algae/bacteria produce a large number of bioactive low molecular weight compounds that may originate from any of the symbionts. Evidence suggests that they play roles in the

maintenance of the lichen structure, but many have antibacterial activity (and are called antibiotics) (Muller 2001). Plants are also prolific sources of bioactive small molecules with a huge range of functions and applications (Firn and Jones 2003).

The Parvome refers to the enormous diversity of organic compounds in the biosphere: these chemical entities must be essential but what roles do they play in their natural habitats? It is becoming increasingly obvious that microbes exist in all living organisms as communities or microbiomes (Banfield and Young 2009): Bioactive small molecules are likely involved in the establishment and maintenance of microbial communities through inter-species signaling activities. These broad ecological functions are not well understood and studies of their activities in situ are still in their infancy. It is unfortunate that the pervasive notion of small molecules as weapons of attack and defence has suppressed their recognition as ubiquitous agents of communication in biology.

1.4 How Old Are "Antibiotics"?

It does not make sense to discuss the evolution of antibiotics without some consideration of their origins. How old are these compounds and in what way and when, did their biosynthetic pathways evolve? This is distinct from the commercial evolution of antibiotics taking place at this time, driven by the competition between the pharmaceutical industry and resistant pathogens.

Calculations show that a biosynthetic pathway responsible for making a complex non-ribosomal peptide antibiotic (NRP) is at least one billion years old (Baltz 2010). The biosynthetic gene cluster for daptomycin is 128 kB in size (see Baltz, this volume). However, the precursor amino acids for their synthesis must have been present in the biosphere from earlier times. The NRPs include both protein-associated amino acids and other amino acids that have only been found in NRP structures. Thus, both protein and non-protein amino acids are very old and are thought to have been delivered to the Earth as organic components of meteorites. Meteorites have been shown to transport a number of different amino acids, both protein and NRP-associated (Pizzarello and Shock 2010) into the biosphere, and they likely played roles in prebiotic chemistry (van der Gulik et al. 2009). As an example, the components of the pharmaceutically important NRP antibiotic daptomycin with 10 different amino acids, including the rare 3-methylglutamic acid, have been detected in meteorites.

1.5 Antibiotic Myths

The notion that the antibiotic activities of small molecules are used as competitive weapons is mentioned frequently, but is largely unproven. After all, there are many natural products, chemically related to the compounds used in the clinic that have no antibiotic activity and could not have been identified in conventional screens.

Similarly, there is a long-held belief that Streptomycetes and related spore-forming Actinomycetes produce the majority of useful antibiotics. This also, is not correct: the large family of *Actinobacteria* are possibly the most fruitful microbes in terms of small molecule production (Miao and Davies 2010). Even the Pseudomonads and Firmicutes produce many bioactive small molecules; not all have demonstrated antibacterial or antiviral (phage) properties but many of the compounds play roles in pathogenesis and in various signaling processes. The eukaryotes also have their champions: the Fungi are rich in small molecule production and chemical diversity and have been exploited extensively by the pharmaceutical industry.

If the truth be known it is probable that all microbes, prokaryotic and eukaryotic, produce bioactive small molecules that may exhibit antibiotic activity under certain conditions. One defining feature is that all of these products are made by large and often complex, tightly regulated biosynthetic pathways. The gene clusters vary considerably: That for tetracycline (see Genilloud and Vincente, this volume) is around 30 kB and for pristinamycin (see Kirst, this volume), more than 200 kB.

Is chromosomal DNA of high G+C composition a prerequisite for small molecule production? A number of microbes with low G+C content (Firmicutes such as Staphylococci) are known to make non-ribosomal peptides but, in general, genomes with higher G+C content appear to have the greatest potential for small molecule production. There could be a reason for this: GC-rich genomes might be considered more "ancient."

Considering the evolution of bioactive molecules without having a clear idea of their true biological roles is difficult: in most cases their small molecule productivity appears to endow no specific selective advantage to the producing host. What roles might they have played in biochemical evolution? Until exhaustive small molecule screening and genome mining have been employed to investigate the microbial world, such questions will remain unanswered.

1.6 Mode of Action and Evolution of Targets

Assuming that the majority of bioactive small molecules are ancient (possibly as old as amino acids), what types of selection pressure determined their evolution? And what can be said about the development of their complex biosynthetic pathways? The evolution of the biosynthetic pathways for molecules such as daptomycin, tetracycline, and other well-known antibiotics is of great interest (Fischbach et al. 2008; Ridley et al. 2008). It is easy to say that they are old, but how did these complex genetic systems evolve and over what period of time? There has been much speculation over the evolution of "simple" biosynthetic pathways such as those for the protein amino acids (Teichman et al. 2001).

What are the benefits of small molecules to the producing organism? There are countless microbial natural products that cannot be detected using conventional

screening approaches. What are the evolved functions for this large number of fascinating molecules?

With respect to the process of chemical evolution, low molecular weight compounds (monomers) are likely to be ancient and were used as precursors to generate more complexity: peptides followed amino acids (van der Gulik et al. 2009). The same is true for the evolution of complex organelles found in cells: ribosomes and cell walls, for example. It is now generally accepted that "early" RNA was a ribozyme and this was the precursor of the protein-rich ribosome and other catalytic RNA structures (Noller 2012).

One can imagine that the primordial synthesis of simple polymers, such as peptides/proteins required that small molecule effectors bound to the catalytic RNA and so facilitated polymerization reactions. Under certain conditions, protein synthesis inhibitors can actually enhance peptide bond formation. Similar in vitro studies may mimic the primordial catalytic reactions of RNA. In an RNA world, activities and binding sites for effectors on the RNA could eventually become the binding sites for antibiotic inhibition in ribosomes (Davies et al. 1992). This suggests that structural relationships exist between small molecule binding sites on pro- and eukaryotic organelles such as ribosomes or nucleic acid synthesis complexes. Non-ribosomal peptides may have played roles as catalysts of primitive reactions by binding to nucleic acid fragments and enhancing the activity of ribozymes. They might have evolved into site-specific binding functions that led to their subsequent activity as inhibitors. Similar evolutionary transitions might have resulted in the formation (or conservation) of small molecule binding sites on human and animal hormone receptors (Catnach and Fairclough 1992).

Modern-day antibiotics have been shown to have a wide range of biological activities depending on the concentrations used. This phenomenon, referred to as hormesis (low concentration: positive effect, high concentration: negative effect) probably applied to all bioactive molecules throughout evolution (Kendig et al. 2010). Hormesis is the key to identifying true biological activity. It can be assumed that primordial bioactive molecules appeared in the environment at low concentrations and interacted with different target molecules/structures at concentrations well below inhibitory levels (before defined biosynthetic pathways had evolved). Many relics of these reactions remain: binding of low concentrations of antibiotics to the translation system can, under some circumstances, stimulate peptide bond formation. The peptidyl transferase reaction can be enhanced by some antibiotics. The same is true for nucleic acid processes, that required ribozymes: small molecules could have modulated their activity.

1.7 Parallel Chemical and Protein Evolution

Antibiotic "evolution" during the past 60 years has essentially been a synthetic chemical process. Almost all drugs have undergone successive rounds of chemical remodeling in efforts to overcome the appearance of pathogens with acquired

resistant to the current generation of antibiotics. This has been a typical "catch-22" situation. The mechanisms of resistance have been well characterized: inactivation/destruction of inhibitor, protection of target, secretion from cell, and others. The best-studied and most dramatic example, that of the β-lactam antibiotics, has seen the evolution by mutation and selection of over 1000 β-lactamase enzymes, each with subtle variations in active site (see Leemans et al. this volume). This has occurred in response to rounds of chemical improvements of the penicillin and cephalosporin antibiotics (Bush and Jacoby 2010).

1.8 Conclusions

Microbial small molecules are ancient, huge in number, and diverse in structure and function. This brief overview of the origins of bioactive small molecules leaves many unanswered questions, particularly with respect to evolutionary mechanisms. What are the evolved natural functions for these fascinating molecules? A discussion of the evolution of these compounds without having a clear sense of their true biological roles is difficult. How many different roles might they have? They did not evolve to challenge chemists, amuse biochemists or microbiologists, or to cure diseases that were absent on the Earth before the advent of man.

The following are recommendations for future studies:

(a) Low molecular weight organic molecules played important roles in the evolution of the biology of the cell. A better understanding of these processes will lead to the identification of new receptors and compounds that bind to them.
(b) Harvesting the Parvome, using genome mining and heterologous gene cluster expression, will revolutionize the pharmaceutical industry. There is no shortage of novel compounds!
(c) Studies of small molecule activity should focus on cell–cell signaling rather than on antagonistic activities.
(d) Creative studies on the activities of bioactive small molecules in microbiomes will aid in the understanding of all aspects of health and disease.

References

Baltz RH (2010) Genomics and the ancient origins of the daptomycin biosynthetic gene cluster. J. Antibiot (Tokyo) 63:506–511

Banfield JF, Young M (2009) Variety-the Splice of Life-in Microbial Communities. Science 326:1198–1199

Bush K, Jacoby GA (2010) Updated functional classification of β-lactamases. Antimicrob Agents Chemother 54:969–976

Campbell IM (1984) Secondary metabolism and microbial physiology. Adv Microb Physiol 25:1–60

Catnach SM, Fairclough PD (1992) Erythromycin and the gut. Gut 33:397–401

Davies J, von Ahsen U, Wank H, Schroeder R (1992) Evolution of secondary metabolite production: potential roles for antibiotics as prebiotic effectors of catalytic RNA reactions. In: Chadwick DJ and Whelan J (eds) Secondary metabolites: their function and evolution. Ciba Foundation symposium:171 Wiley, Chichester, pp 24–32

Davies J, Spiegelman GB, Yim G (2006) The world of subinhibitory antibiotic concentrations. Curr Opin Microbiol 9:1–9

Davies J, Ryan KS (2012) Introducing the Parvome: bioactive compounds in the microbial world. ACS Chem Biol; 7:252–9

Demain AL, Sanchez S (2009) Microbial drug discovery: 80 years of progress. J Antibiot (Tokyo) 62:5–16

Firn RD, Jones CG (2003) The origin of secondary metabolism-a unifying model. Mol Microbiol 37:989–994

Fischbach MA, Walsh CT, Clardy J (2008) The evolution of gene collectives: How natural selection drives chemical innovation. In: Proc Natl Acad Sci USA,105:4601–4608

Freestone PP, Sandrini SM, Haigh RD, Lyte M (2008) Microbial endocrinology: how stress influences susceptibility to infection. Trends Microbiol 16:55–64

Kendig EL, Le HH, Belcher SM (2010) Defining hormesis: evaluation of a complex concentration response phenomenon. International J of Toxicol 29:235–246

Miao V, Davies J (2010) *Actinobacteria:* the good, the bad, and the ugly. Antonie van Leeuwenhoek doi: 10.1007/s10482-010-9440-6

Muller K (2001) Pharmaceutically relevant metabolites from lichens. Appl Microbiol Biotechnol 56:9–16

Noller, HF (2012) Evolution of protein synthesis from an RNA world, Cold Spring Harb Perspect Biol, 4: a003681.

Pizzarello S and Shock E (2010) The organic composition of carbonaceous meteorites: the evolutionary story ahead of biochemistry. Cold Spring Harbor Perspect Biol, Mar 2010.

Plotkin BJ, Viselli SM (2000) Effect of insulin on microbial growth. Curr Microbiol 41:60–64

Ridley CP, Lee HY, Khosla C (2008) Evolution of polyketide synthases in bacteria. Proc Natl Acad Sci USA 105:4595–4600

Schreiber SL (2005) Small molecules: the missing link in the central dogma. Nat Chem Biol 1:64–66

Strohl WR (1997) Industrial antibiotics: today and the future. In: Strohl WR (ed) Biotechnology of Antibiotics, 1st edn. Marcel Dekker, New York

Teichman SA, Rison SCG, Thornton JM, Riley M, Gough J, Chothia C (2001) The evolution and structural anatomy of the small molecule metabolic pathways in *Escherichia coli*. J Mol Biol 311:693–708

van der Gulik P, Massar S, Gilis D, Buhrman H, Rooman M (2009) The first peptides: the evolutionary transition between prebiotic amino acids and early proteins. J Theor Biol 261:531–539