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MINI-REVIEW

Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential

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Abstract Amongst the diverse and potent biological activities of free fatty acids (FFAs) is the ability to kill or inhibit the growth of bacteria. The antibacterial properties of FFAs are used by many organisms to defend against parasitic or pathogenic bacteria. Whilst their antibacterial mode of action is still poorly understood, the prime target of FFA action is the cell membrane, where FFAs disrupt the electron transport chain and oxidative phosphorylation. Besides interfering with cellular energy production, FFA action may also result from the inhibition of enzyme activity, impairment of nutrient uptake, generation of peroxidation and auto-oxidation degradation products or direct lysis of bacterial cells. Their broad spectrum of activity, non-specific mode of action and safety makes them attractive as antibacterial agents for various applications in medicine, agriculture and food preservation, especially where the use of conventional antibiotics is undesirable or prohibited. Moreover, the evolution of inducible FFA-resistant phenotypes is less problematic than with conventional antibiotics. The potential for commercial or biomedical exploitation of antibacterial FFAs, especially for those from natural sources, is discussed.

Keywords Antibiotic · Antimicrobial · Drug resistance · Lipid · Natural products

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Introduction

Fatty acids (FAs) are ubiquitous molecules typically found bound to other compounds such as glycerol, sugars or phosphate headgroups to form lipids. Lipids are integral components of cell structures, e.g. membranes, which are made up of phospholipids, and energy stores that are often composed of triglycerides. FAs can be released from lipids, typically by enzyme action, to become free fatty acids (FFAs), which have diverse and potent biological activities (Table 1).

FFAs consist of a chain of carbon atoms attached to hydrogen atoms (Fig. 1). The number of carbon atoms varies, but those in biological systems usually have an even number between 10 and 28, and this review mainly concentrates on these. At one end of the carbon chain is a carboxyl group (–COOH) and, at the other end, is a methyl group ($–CH_3$; Fig. 1). The carboxyl group is hydrophilic and ionised when solubilised in water, whereas the carbon chain is hydrophobic, making the entire molecule amphipathic. FAs with <8 carbon atoms are considered short chain, whereas those with >16 carbon atoms are regarded as long chain. Unsaturated FAs have one or more C=C double bonds in the carbon chain, while the carbon atoms in saturated FAs are all joined by C–C single bonds (Fig. 1). Lipases, the group of lipolytic enzymes that cleave FAs from lipid headgroups by hydrolysis to give FFAs, can be specific for certain types of lipid, but sometimes, only certain FAs can be cleaved according to their position on the headgroup and the length and unsaturation of the carbon chain.

The biological activities of FFAs have roles in host defences against potential pathogenic or opportunistic microorganisms. An important aspect of this is growth inhibition or the direct killing of bacteria. There is now an

Table 1 Selected bioactivites of various saturated and unsaturated FFAs

Activity	Fatty acid(s)	Key reference
Antimicrobial		
Anti-algal	C8:0, C10:0, C12:0	McGrattan et al. (1976)
	C18:4n-3	Kakisawa et al. (1988)
	C18:1, C18:2, C18:4, C18:5, C20:4, C20:5, C22:6	Arzul et al. (1995)
	C18:2n-6, C18:3n-3	Ikawa et al. (1997)
	C16:0, C18:0, C18:1n-9, C18:2, C18:3n-3, C20:5n-3, C22:6n-3	Wu et al. (2006)
	C16:0, C16:1n-7, C16:1n-7*t*, C16:4n-3, C18:0, C18:1n-9, C18:2n-6, C18:3n-3, C18:4n-3, C20:0, C20:1n-9, C20:4n-6, C20:5n-3, C22:0, C22:1n-9, C22:6n-3	Alamsjah et al. (2008)
Antibacterial (Gram-negative)	C20:4n-6	Knapp and Melly (1986)
	C10:0, C12:0	Bergsson et al. (1998)
	C10:0, C12:0, C14:0, C16:1	Bergsson et al. (1999)
	C15:0, C16:0, C17:0, C18:0, C18:1, C18:4, C20:4, C20:5, C22:0, C22:4, C22:5	Benkendorff et al. (2005)
Antibacterial (Gram-positive)	C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, C18:1, C18:2, C18:3	Galbraith et al. (1971)
	C10:0, C12:0, C14:0, C14:1, C16:0, C16:1, C18:1, C18:2, C18:3	Kabara et al. (1972)
	C8:0, C9:0, C10:0, C11:0, C12:0, C13:0, C14:0, C14:1n-5, C16:1n-7, C16:1n-7*t*, C18:2n-6, C18:3n-3, C18:3n-6, C20:1n-9, C20:3n-6, C20:3n-3, C20:4n-6, C22:2n-6, C22:3n-3, C20:4n-6, C22:6n-3	Feldlaufer et al. (1993)
	C16:1n-10	Wille and Kydonieus (2003)
	C15:0, C18:1, C18:4, C20:4, C20:5, C22:0, C22:4, C22:5	Benkendorff et al. (2005)
Anti-fungal	C10:0, C12:0	Bergsson et al. (2001)
	C10:0, C12:0, C14:0, C14:1, C16:1, C18:2	Kabara et al. (1972)
Anti-protozoan	C18:0, C18:1, C18:2, C18:3	Rohrer et al. (1986)
	C8:0, C10:0, C12:0	Dohme et al. (2001)
Antiviral	C8:0, C10:0, C12:0, C14:0, C16:1, C18:1, C18:2, C18:3, C20:4	Thormar et al. (1987)
	C10:0, C12:0, C14:0, C16:1, C18:1	Hilmarsson et al. (2006)
Cytotoxic		
Haemolytic (sheep erythrocytes)	C18:0, C18:1, C18:2, C18:3, C18:4, C18:5, C20:4, C20:5, C22:6	Arzul et al. (1995)
Haemolytic (human erythrocytes)	C20:4n-6, C20:5n-3	Fu et al. (2004)
Inhibits cell division (mammalian leukemic HL-60 cells)	C20:4n-6, C20:5n-3, C22:6n-3	Finstad et al. (1994)
Inhibits cell division (sea urchin eggs)	C18:4, C18:5, C20:5, C22:6	Sellem et al. (2000)
Inhibits development of fertilised echinoderm eggs	C16:4n-3	Murakami et al. (1989)
Inhibits photosynthesis	C16:1n-7	Peters and Chin (2003)
Reduces viability of rat Leydig cells	C16:0, C18:0	Lu et al. (2003)
Toxic to whole organisms		
Brine shrimp larvae	C8:0, C10:0, C12:0, C18:1, C18:2, C18:3, C20:4	Curtis et al. (1974)
Daphnia (Crustacean)	C18:3n-6	Reinikainen et al. (2001)
Fairy shrimp (Crustacean)	C20:5n-3	Jüttner (2001)
Fish	C20:5n-3	Marshall et al. (2003)
Mosquito larvae	C18:1, C18:2, C18:3n-6	Harada et al. (2000)
Tube worm (marine)	C20:4, C20:5	Pawlik (1986)

Table 1 (continued)

Activity	Fatty acid(s)	Key reference
Signalling		
Increases expression of bacterial proteins for energy metabolism, cell wall and protein synthesis	C16:1n-6, C18:2n-6	Kenny et al. (2009)
Induces larval settlement and metamorphosis	C16:1, C18:2, C20:4, C20:5	Pawlik (1986); Jensen et al. (1990)
Inhibits bacterial attachment	C18:1n-9	Stenz et al. (2008)
Reduces expression of bacterial virulence factors: β-lactamase and Toxic Shock Syndrome Toxin (TSST)	C12:0	Ruzin and Novick (2000)
Reduces expression of bacterial virulence factors: β-lactamase and haemolysin	C16:1n-6	Clarke et al. (2007)
Reduces expression of bacterial virulence factors: haemolysin	C12:0, C14:0, C16:0, C18:0	Liaw et al. (2004)
Regulates bacterial swarming	C12:0, C14:0, C16:0, C18:0, C18:1	Liaw et al. (2004)
Regulates protein kinase C activation	C20:4n-6	Khan et al. (1995)

Bond positions, where reported, are all in *cis* orientation unless marked *t* for *trans*

extensive literature concerning the antibacterial effects of various FFAs from a wide range of biological sources, including algae, animals and plants (McGaw et al. 2002; Wille and Kydonieus 2003; Desbois et al. 2008, 2009). Indeed, FFAs are often identified as the active ingredients in ethnic and herbal medicines (Yff et al. 2002; McGaw et al. 2002). This review aims to summarise some of this work and to discuss the various mechanisms and structural features of FFAs that causes them to prevent bacterial growth or survival. Furthermore, the potential for commercial or biomedical exploitation of antibacterial FFAs is discussed.

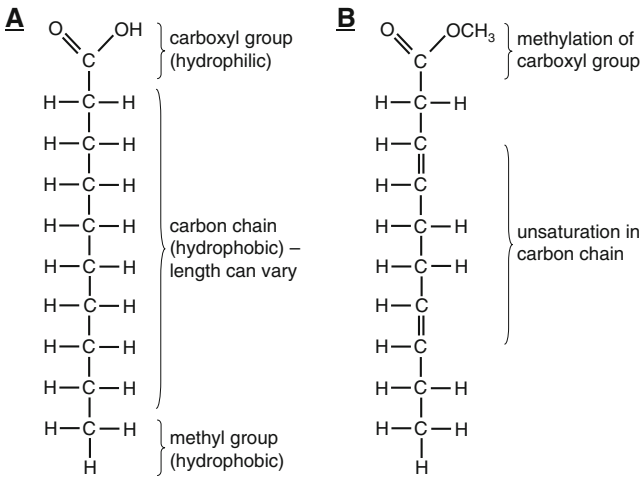

Fig. 1 Structure of free fatty acids (FFAs). **a** The saturated FFA, capric acid (C10:0), which has 10 carbon atoms in the carbon chain. The carbon chain length can vary but at one end is the carboxyl group (–COOH) while at the other end is a methyl group ($–CH_3$). The carboxyl group is hydrophilic and ionised when solubilised in water, whereas the carbon chain and terminal methyl group are hydrophobic, making the entire molecule amphipathic. **b** Unsaturated FFAs have one or more C=C double bonds in the carbon chain and, here, the fatty acid is methylated, as the carboxyl group has an additional $–CH_2$. This fatty acid is C10:2n-3, as there are 10 carbon atoms in the carbon chain, there are 2 C=C bonds of which the first of these is located 3 carbon–carbon bonds from the methyl end

Free fatty acids in antibacterial defence

The antibacterial effects of FFAs are frequently observed during bioassay-guided fractionation of extracts from a variety of organisms (Hemsworth and Kochan 1978; McGaw et al. 2002; Wille and Kydonieus 2003; Desbois et al. 2009). The antibacterial actions of FFAs are typically broad spectrum and of potencies comparable to natural antimicrobial peptides (AMPs) in vitro (Georgel et al. 2005). FFAs function in the antimicrobial defences of many multicellular organisms, including mammals (Hemsworth and Kochan 1978; Georgel et al. 2005), plants (Weber 2002), molluscs (Benkendorff et al. 2005), seaweeds (Küpper et al. 2006) and amphibians (Rickrode 1986). Whilst FFAs are not as structurally diverse as the more widely studied AMPs, their importance in the human innate immune system is well-established, particularly in the defence of skin and mucosal surfaces (Thormar and Hilmarsson 2007; Drake et al. 2008). Indeed, FFAs are the most active antimicrobial agents present in human skin lipid samples (Wille and Kydonieus 2003). There is 10–15 μg of FFAs per square centimetre on human skin, of which lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), sapienic acid (C16:1n-10) and *cis*-8-octadecenoic acid (C18:1n-10) are the most abundant (Wille and Kydonieus 2003; Takigawa et al. 2005). FFAs are produced on the skin by lipolytic cleavage of lipids secreted from the sebaceous glands (Shalita 1974; Fluhr et al. 2001; Drake et al. 2008), and their presence on the skin is sufficient to

control the bacterial microbiota (Takigawa et al. 2005; Georgel et al. 2005; Kenny et al. 2009). The most important antibacterial FFA in human skin exudate is C16:1n-10, a long-chain monounsaturated FFA, and skin deficient in this and other FFAs tends to be more susceptible to colonisation by the opportunistic pathogen, *Staphylococcus aureus* (Takigawa et al. 2005; Georgel et al. 2005). However, if the skin is treated with C16:1n-10, protection against colonisation is bolstered (Takigawa et al. 2005; Georgel et al. 2005). Besides inhibiting or killing bacteria directly, FFAs also make conditions unfavourable for the growth of certain bacteria on the skin surface by maintaining an acidic pH (Fluhr et al. 2001; Takigawa et al. 2005). FFAs may further affect the expression of bacterial virulence factors (Table 1) that are important or essential for the establishment of infection, probably by disrupting cell-to-cell signalling. Thus, saturated and unsaturated FFAs can prevent initial bacterial adhesion and subsequent biofilm formation (Kurihara et al. 1999; Osawa et al. 2001; Kankaanpää et al. 2004; Won et al. 2007; Stenz et al. 2008; Davies and Marques 2009). Moreover, the swarming behaviour of the urinary tract pathogen, *Proteus mirabilis*, is inhibited by medium- and long-chain saturated FFAs (Liaw et al. 2004). The expression of certain toxins, haemolysins, or enzymes that confer drug resistance are all down-regulated in the presence of various saturated and unsaturated FFAs (Ruzin and Novick 2000; Liaw et al. 2004; Clarke et al. 2007), while genes responsible for iron uptake and extracellular proteases may be similarly reduced (Kenny et al. 2009). The ability of various species of bacteria to resist the action of FFAs and subvert these epithelial defences certainly explains, at least in part, the success of certain skin and mucosal pathogens (Clarke et al. 2007; Drake et al. 2008).

Perhaps, less well-known is the role that FFAs play in the defence of single-celled eukaryotic organisms against bacterial threats. In microbial eukaryotes, such as microalgae, FAs are found primarily in the lipids that constitute the cell membranes and energy storage structures, but during cellular disintegration, large quantities of FFAs are released from cellular lipids by host lipolytic enzymes (Jüttner 2001; Wichard et al. 2007). A high proportion of the FFAs that are freed from the cell membranes, including those around the photosynthetic plastid, are mono- and polyunsaturated varieties (Cutignano et al. 2006). These FFAs are toxic to invertebrate grazers, which may have caused the microalgal cell to lose its integrity in the first instance (Jüttner 2001; Wichard et al. 2007). Therefore, the toxic FFAs act to reduce grazer numbers and ultimately grazing pressure (Jüttner 2001). At first, it might seem counterintuitive that this defence strategy requires the host cell to undergo mechanical damage and death, but in evolutionary terms, it has benefits because neighbouring microalgal cells, particularly in biofilms, would be expected to be clones or very closely related. That these same FFAs are potently antimicrobial means similar protection may be afforded to microalgae under threat from pathogenic bacteria or viruses. Whilst the initial host will not survive, FFAs released from a microalgal cell that has been damaged by a pathogen will act on pathogens in the local vicinity reducing their numbers, therefore conferring some protection of its neighbouring relatives from onward transmission. This 'population level' defence may be considered metabolically inexpensive, as the FFAs form essential cellular components with the lipases already synthesised and present within the cell to carry out vital processes.

Antibacterial activity and FFA structure

The antibacterial activity of each FFA is influenced by its structure and shape. This, in turn, is a function of the length of the carbon chain and the presence, number, position and orientation of double bonds (Fig. 2). The literature contains contrasting reports concerning the relationship between a FFA's structure and its antibacterial activity, but some general trends do emerge. The –OH group of the carboxyl group seems to be important for the antibacterial activity of FFAs, as methylated FFAs (Fig. 1) often have reduced or no activity (Kodicek and Worden 1945; Zheng et al. 2005).

Medium- and long-chain unsaturated FFAs tend to be more active against Gram-positive bacteria than Gram-negatives (Kodicek and Worden 1945; Galbraith et al. 1971). In general, unsaturated FFAs tend to have greater potency than saturated FFAs with the same length carbon chain (Kabara et al. 1972; Greenway and Dyke 1979; Feldlaufer et al. 1993; Zheng et al. 2005; Desbois et al. 2008). Within series of monounsaturated FFAs, the most potent usually have 14 or 16 carbon atoms (Kabara et al. 1972; Feldlaufer et al. 1993). Often, a direct correlation exists between the number of double bonds in an unsaturated FFA's carbon chain and its antibacterial efficacy (Saito et al. 1984; Knapp and Melly 1986; Feldlaufer et al. 1993). The double bonds in naturally occurring FFAs typically have *cis* orientation and these tend to have greater antibacterial activity than FFAs with double bonds in *trans* orientation (Galbraith et al. 1971; Kabara et al. 1972; Feldlaufer et al. 1993), probably because the structures of *trans*-bonded unsaturated FFAs resemble saturated FFAs (Fig. 2). Whilst only a few studies have investigated the effect of bond position in the carbon chain of FFAs, there is some evidence that the position of double bonds can affect potency and spectrum of antibacterial action (Kabara et al. 1977; Feldlaufer et al. 1993; Wille and Kydonieus 2003).

For saturated FFAs, the most active have 10 or 12 carbons in the chain and antibacterial efficacy tends to

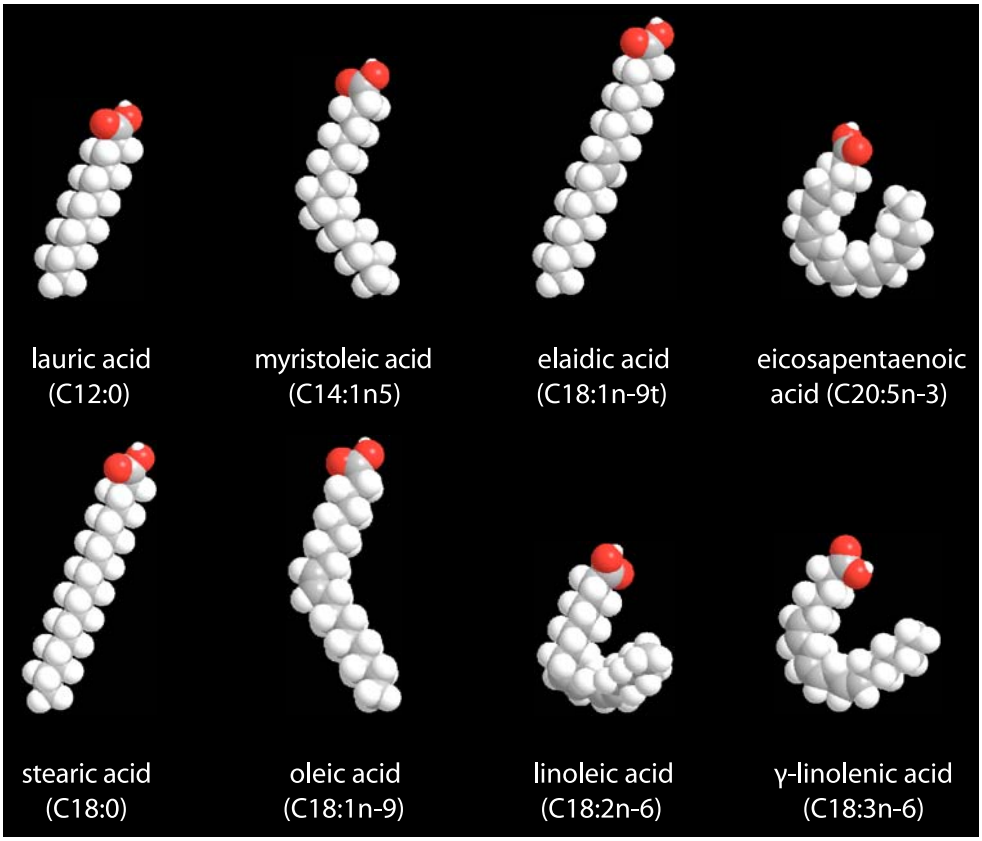

Fig. 2 Space-filled representations of eight free fatty acids (FFAs). Saturated FFAs (e.g. lauric and stearic acids) have a simple 'straight-line' structure. A *cis*-double bond causes a kink in the carbon chain (e.g. myristic and oleic acids). Additional *cis*-double bonds in the carbon chain cause further kinks (e.g. linoleic, γ-linolenic and eicosapentaenoic acids). However, *trans*-double bonds have little effect on the shape (e.g. elaidic acid), and these FFAs structurally tend to resemble saturated FFAs (e.g. stearic acid)

decrease as the chain length gets longer or shorter (Galbraith et al. 1971; Kabara et al. 1972; Bergsson et al. 2001; Sun et al. 2003; Wille and Kydonieus 2003). However, other workers have reported that FFAs with 14, 16 or 18 carbon atoms can be more potent than FFAs with 10 or 12 carbons against certain species of bacteria (Willett and Morse 1966; Galbraith and Miller 1973a; Miller et al. 1977). Comparisons are complicated because different authors have used a variety of methodological approaches to determine and measure potency with considerable variation between reports in the size of the inoculum and the incubation conditions. Moreover, the relative activity of FFAs may depend on whether a complete growth inhibition assay or an IC_{50} determination is used (Willett and Morse 1966). To enable simple comparison, ideally, all determinations of minimum inhibitory concentration and minimum bactericidal concentration for FFAs need to adhere to standardised definitions and protocols, such as those published by the Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute 2000).

Mechanisms of antibacterial activity

It remains unclear exactly how FFAs exert their antibacterial activities, but the prime target seems to be the bacterial cell membrane and the various essential processes that occur within and at the membrane (Fig. 3). Some of the detrimental effects on bacterial cells can be attributed to the detergent properties of FFAs on account of their amphipathic structure. This allows them to interact with the cell membrane to create transient or permanent pores of variable size. At higher concentrations, detergents, such as FFAs, can solubilise the membrane to such an extent that various membrane proteins or larger sections of the lipid bilayer are released. The key membrane-located process affected by FFAs is the production of energy caused by interference with the electron transport chain and the disruption of oxidative phosphorylation (Sheu and Freese 1972; Galbraith and Miller 1973b; Miller et al. 1977; Boyaval et al. 1995; Wojtczak and Więckowski 1999). Other processes that may contribute to bacterial growth inhibition or death include cell lysis, inhibition of enzyme activity, impairment of nutrient uptake and the generation of toxic peroxidation and autoxidation products (Fig. 3). FFAs can kill a bacterium outright (bactericidal action) or inhibit its growth (bacteriostatic action), which is reversible and means that the bacterium remains viable but cannot undergo cell division in the presence of the FFA (Kodicek and Worden 1945; Sheu and Freese 1972). Assays used to investigate the antibacterial activities of FFAs do not always discriminate between bactericidal and bacteriostatic actions, but it is reasonable to assume that growth inhibition cannot continue indefinitely, and eventually, a growth-inhibited bacteri-

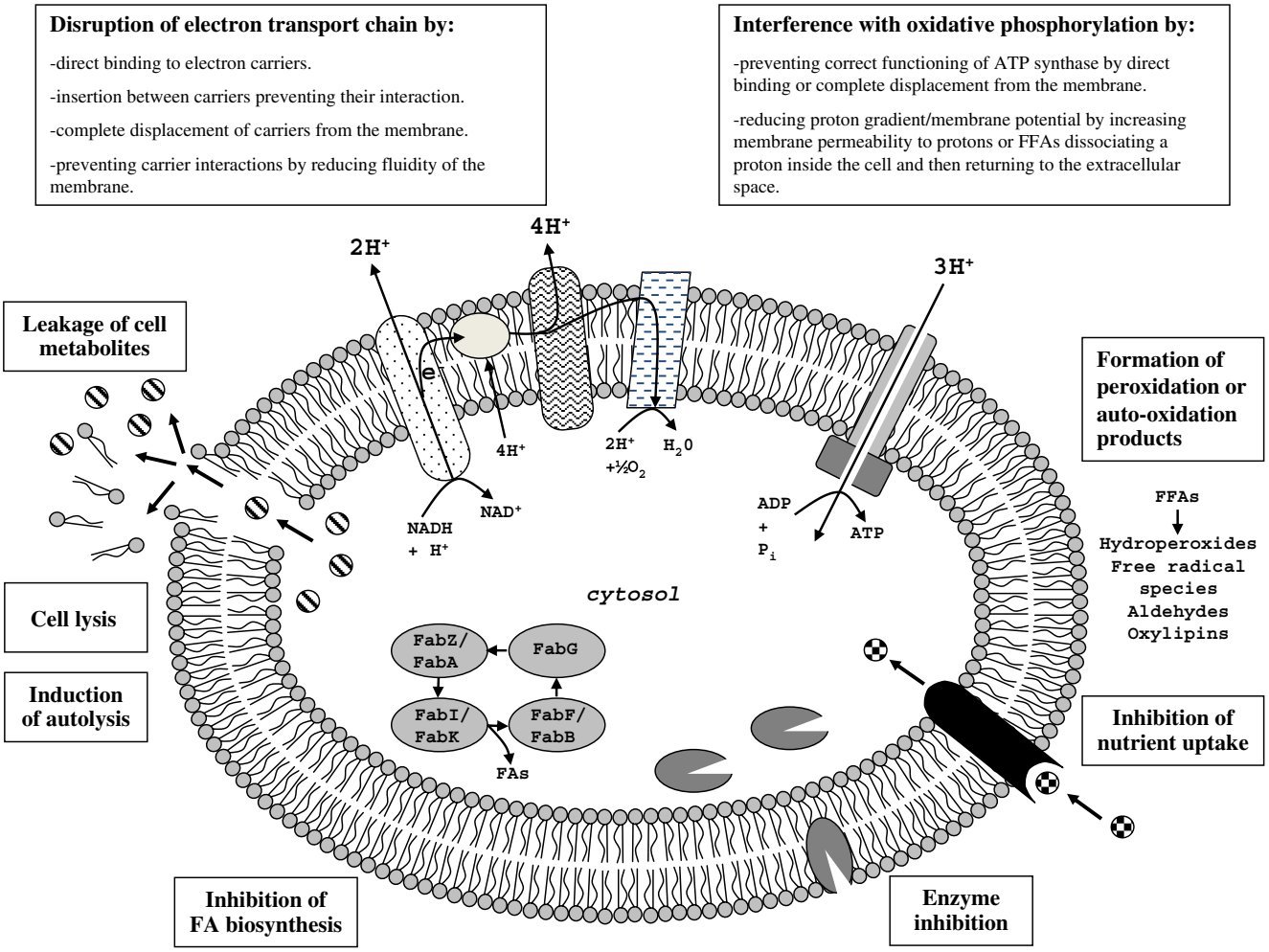

Fig. 3 Schematic representation of possible cell targets and mechanisms of antibacterial action of free fatty acids (FFAs). They may affect bacterial energy production by disrupting the electron transport chain and/or interfering with oxidative phosphorylation. FFAs can cause leakage of cell metabolites from the cell, complete cell lysis and autolysis. Membrane and cytosolic enzymes, including those required for fatty acid biosynthesis, can be inhibited by FFAs. They can impair active nutrient uptake by acting directly on the transport protein or as an indirect result of the cell's inability to produce ATP. Peroxidation and auto-oxidation products of FFAs may also have deleterious effects on the bacterial cell and play a role in cell killing. For clarity, only the bacterial inner cell membrane is shown

um will die. In describing the processes of antibacterial activity below, little distinction is made as to whether the outcome is bactericidal or bacteriostatic.

Disruption of electron transport chain

The inner membrane of Gram-positive and Gram-negative bacteria is an important site for energy production, and it is where the electron transport chain is located. The various carriers in the electron transport chain, which are embedded within the membrane, pass electrons from one carrier to another until two electrons combine with the final acceptor, usually oxygen, and two protons to form water (Mitchell 1961). During this process, protons are exported from the inside of the cell, whilst the concentration of electrons in the cytosol increases. This generates a proton gradient and membrane potential, which are crucial for the production of ATP by the enzyme, ATP synthase (Mitchell 1961). Medium- and long-chain saturated and unsaturated FFAs that gain access through the cell wall or outer membrane of a bacterium can perhaps bind to the carriers of the electron transport chain directly or insert into the inner membrane causing the electron carriers to move apart or be displaced from the membrane entirely (Galbraith and Miller 1973b; Peters and Chin 2003). In each case, the ability of the electron transport chain to transfer electrons is impaired so that the proton gradient and membrane potential are reduced. This results in a reduction in ATP production, and the bacterium becomes deprived of an essential source of energy. Both unsaturated and saturated FFAs could

translocate or bind the electron carriers directly, but complete displacement from the membrane is likely achieved by unsaturated FFAs only, probably because they increase membrane fluidity (Greenway and Dyke 1979; Chamberlain et al. 1991; Stulnig et al. 2001). This is because the *cis*-bonds in unsaturated FAs cause a kink in the carbon chain (Fig. 2) that prevents these FAs from packing tightly in the membrane. Thus, when medium- and long-chain unsaturated FFAs are incorporated into the membrane, there is an increase in fluidity that can develop into membrane instability (Chamberlain et al. 1991; Stulnig et al. 2001). Conversely, medium- and long-chain saturated FFAs (and *trans*-bonded unsaturated FFAs) that lack a kinked structure can be packed more tightly (Fig. 2). Hence, medium- and long-chain saturated FFAs can reduce membrane fluidity and disrupt electron transport, perhaps by restricting the movement of carriers within the membrane (Sheu and Freese 1972). Moreover, as explained above, solubilisation of the membrane by the detergent effect of FFAs could also account for the loss of vital components of the electron transport chain from the membrane.

Uncoupling of oxidative phosphorylation

FFAs may further prevent energy production by uncoupling oxidative phosphorylation. Thus, the potential energy created by the electron transport chain dissipates as heat rather than being used for ATP synthesis (Sheu and Freese 1972; Greenway and Dyke 1979; Beck et al. 2007). ATP synthase (also located on the bacterial inner membrane) uses the energy from the proton motive force, which results from the proton gradient and membrane potential, created by the electron transport chain, to convert ADP to ATP. Interaction of FFAs with the bacterial inner membrane can affect this process and reduce or prevent the production of ATP (Sheu and Freese 1972; Galbraith and Miller 1973b). A simple way that this could happen is for a saturated or unsaturated FFA to bind directly to ATP synthase itself, which could prevent the enzyme functioning correctly. Alternatively, FFAs can interfere with the proton gradient and membrane potential. This weakens the proton motive force upon which ATP synthesis relies. FFAs, particularly unsaturated ones, can reduce ATP synthesis by increasing the permeability of the membrane to protons (Borst et al. 1962; Boyaval et al. 1995). This could happen anywhere on the inner membrane or at specific proton pores, such as those already identified in mitochondria (Więckowski and Wojtczak 1998; Wojtczak and Więckowski 1999; Beck et al. 2007). Thus, protons enter the cytosol causing a reduction in the proton gradient and membrane potential. Moreover, the enzyme's ability to synthesise ATP is further diminished because the protons bypass ATP synthase (Boyaval et al. 1995; Więckowski and Wojtczak 1998). The proton gradient and membrane potential are also thought to be reduced by FFAs entering the cytosol, dissociating the proton from its carboxyl group and then returning across the membrane to the exterior, thus increasing the cytosolic concentration of protons (Wojtczak and Więckowski 1999; Beck et al. 2007; Schönfeld and Wojtczak 2008).

Cell lysis

Due to their structure, the insertion of unsaturated FFAs into the bacterial inner membrane causes it to become more fluid and permeable (Greenway and Dyke 1979; Chamberlain et al. 1991). The increased permeability of the membrane by the insertion of unsaturated medium- and long-chain FFAs can allow internal contents to leak from the cell, which can cause growth inhibition or even death (Galbraith and Miller 1973a; Greenway and Dyke 1979; Speert et al. 1979; Wang and Johnson 1992; Boyaval et al. 1995; Shin et al. 2007). If membrane fluidity increases excessively, the membrane can become unstable and the cell will ultimately lyse (Carson and Daneo-Moore 1980). Indeed, unsaturated FFAs have been shown to lyse single-celled algae (Wu et al. 2006), bacteria (Carson and Daneo-Moore 1980; Wang and Johnson 1992; Thompson et al. 1994; Shin et al. 2007), erythrocytes (Fu et al. 2004), mammalian cells such as sheep fibroblasts (Thormar et al. 1987) and vero cells (Thormar et al. 1987), or even enveloped viruses (Thormar et al. 1987). Aside from increased membrane fluidity, the detergent effect of FFAs, which, at high concentrations, may solubilise large sections of the cell membrane, could further account for complete cell lysis. In addition, saturated FFAs can induce autolysis of bacterial cell walls in some species, perhaps triggered by a reduction in membrane fluidity (Tsuchido et al. 1985; Cybulski et al. 2002; Kenny et al. 2009).

Inhibition of enzyme activity

FFAs are potent inhibitors of diverse enzymes, and unsaturated FFAs usually have greater inhibitory activity than saturated ones (Kurihara et al. 1999; Zheng et al. 2005; Won et al. 2007; Hamel 2009; Sado-Kamdem et al. 2009). Inhibition of enzymes in the membrane or cytosol that are crucial for bacterial survival and growth could account for some of the antibacterial effects of FFAs. Interestingly, Zheng et al. (2005) showed that unsaturated FFAs can inhibit bacterial FA biosynthesis in vivo, which will, in turn, affect the composition of the bacterial cell membrane. This could cause altered and inappropriate cell membrane fluidity and permeability, leading to the membrane-related problems described above.

Impairment of nutrient uptake

Saturated and unsaturated FFAs can inhibit the ability of bacteria to take up nutrients, such as amino acids, thereby effectively starving the bacterium of the nutrients it requires to remain viable (Galbraith and Miller 1973b; Shibasaki and Kato 1978). It is not clear whether FFAs reduce nutrient uptake by directly disrupting the membrane-located transporter proteins (by direct binding or complete displacement) or whether it is a consequence of the reduced proton motive force required for the energy-requiring process of active transport.

Peroxidation and auto-oxidation

Other workers have suggested that it is the action of secondary degradation products of FFAs that are responsible for their antibacterial activities. These could be produced by peroxidation that yields H_2O_2 and reactive oxygen species (Knapp and Melly 1986; Hazell and Graham 1990; Wang and Johnson 1992; Schönfeld and Wojtczak 2008) or auto-oxidation of unsaturated FFAs that creates oxylipins and short-chain aldehydes, which are antibacterial in their own right (Gutteridge et al. 1974; Adolph et al. 2004).

Of course, the specific mechanisms by which individual FFAs cause bacterial growth inhibition and/or death will depend on FA structure, the target bacterium and the sites that the FFA can access. Effective control of bacterial growth and survival might involve multiple mechanisms, each of which might, directly or indirectly, be affected by factors such as pH and temperature (Galbraith and Miller 1973c; Kabara et al. 1977; Miller et al. 1977; Shibasaki and Kato 1978; Greenway and Dyke 1979; Wang and Johnson 1992; Sun et al. 2003). Often it is not clear whether changes in antibacterial activity caused by different pH and temperature conditions is due to alterations in the solubility of the FFA or whether these conditions have greater influence on the physiology, and therefore the susceptibility, of the target bacterium.

Bacterial resistance to killing by FFAs

Some bacterial species are naturally resistant to the antibacterial action of FFAs. The cell walls of Gram-positive bacteria and the outer cell membranes of Gram-negative species protect against FFAs, as once these structures are removed, the cells are more susceptible (Galbraith and Miller 1973a; Miller et al. 1977). Differential susceptibility of bacterial species to the action of FFAs is likely to be due to the FFA's ability to permeate the outer membrane or cell wall, which will enable access to the sites of action on the inner membrane. Interestingly, *S. aureus* appears to upregulate the expression of genes encoding proteins involved in the synthesis of the cell wall upon exposure to unsaturated FFAs, a strategy that no doubt serves as a protective measure because a thicker cell wall makes it more difficult for FFAs to penetrate and exert their antibacterial effects at the cell membrane (Kenny et al. 2009). Furthermore, additional wall material makes the cell surface more highly charged and thus less hydrophobic. Therefore, FFAs are less attracted to the cell and are less likely to insert into the inner membrane (Clarke et al. 2007; Kenny et al. 2009). The ability of some bacteria to change their cell surface hydrophobicity (Clarke et al. 2007; Kenny et al. 2009) may explain why certain strains of the same species differ with respect to their susceptibility to the antibacterial effects of FFAs (e.g. Heczko et al. 1979; Ko et al. 1978; Lacey and Lord 1981; Kenny et al. 2009).

Another factor that might contribute to the resistance of some bacterial strains to disruption by FFA is the presence of membrane-located carotenoids. Carotenoids are antioxidants that also stabilise the cell membrane by decreasing its fluidity. Thus, their presence may counteract the effects of reactive FFA degradation products or FFA-induced increases in membrane fluidity (Chamberlain et al. 1991). Indeed, strains of *S. aureus* containing high levels of carotenoids are less susceptible to the antibacterial effects of unsaturated FFAs than strains with lower quantities of carotenoids in their membranes (Chamberlain et al. 1991; Xiong and Kapral 1992). Further work is necessary to ascertain whether similar differences in the presence of carotenoids, or other membrane-stabilising sterols, account for the variation in FFA susceptibility between different strains of other bacterial species. Certainly, there is a need to better elucidate the precise mechanism(s) of antibacterial action by FFAs in order to understand how certain bacteria evade or abrogate their bactericidal effects.

Uses and applications of antibacterial free fatty acids

The broad spectrum of activity and non-specific mode of action of at least some FFAs make them attractive as antibacterial agents for various applications in medicine, agriculture, food preservation and the formulation of cosmetics or nutraceuticals, especially where the use of conventional antibiotics is undesirable or forbidden. Many FFAs are plentiful in natural sources, non-toxic (Kabara 1979) and 'generally regarded as safe' (US Food and Drug Administration 1997). By and large, the evolution of inducible FFA-resistant phenotypes is less problematic than with conventional antibiotics (Lacey and Lord 1981; Petschow et al. 1996; Sun et al. 2003). Kenny et al. (2009) screened 5,000 transposon mutants of *S. aureus* for

FFA resistance but found none, and, in fact, most mutants were even more susceptible to FFA action. Yet, despite their obvious potential, the antibacterial properties of FFAs have still to be fully exploited. One reason may be because some FFAs, particularly long-chain polyunsaturated ones, can be unstable (Kodicek and Worden 1945; Gutteridge et al. 1974; Guil-Guerrero et al. 2001) and tend to bind non-specifically to proteins or other compounds (Kodicek and Worden 1945; Galbraith et al. 1971; Lacey and Lord 1981; Boyaval et al. 1995; Petschow et al. 1996). A further problem may be the perceived lack of patentable intellectual property concerning these ubiquitous antibacterial compounds. However, these problems can be overcome, and the usefulness of FFAs in antibacterial applications should not be dismissed.

Biomedical therapeutics

FFAs are defence molecules in the innate immune systems of multicellular organisms that could be manipulated for the prevention and treatment of bacterial diseases. The increasing prevalence of drug-resistant bacteria as well as an enhanced appreciation for the mechanisms of drug-resistance acquisition is necessitating the discovery and development of alternative anti-infectives to conventional antibiotics (Thormar and Hilmarsson 2007). The future exploitation of particular FFAs for systemic treatment may be limited by their toxicity at high doses to certain eukaryotic cells (Table 1), although Clarke et al. (2007) successfully used C16:1n-10 to treat systemic *S. aureus* infections in mice. At present, the best prospects for exploitation in medicine are for therapies aimed at enhancing the concentrations of natural FFAs on the skin.

Topical antibacterial decolonising agents are given to patients intranasally before surgery to disinfect the nose and reduce the chances of contracting a postsurgical infection (van Rijen et al. 2008). Presently, the antibiotic of choice for this is mupirocin, but resistance to this agent is becoming increasingly prevalent, and treatment failure is now more common (Simor et al. 2007). Linolenic acid (C18:3) can reduce *S. aureus* numbers on human skin and therefore could be exploited as an alternative to mupirocin (Lacey and Lord 1981). Furthermore, Lukowski et al. (2008) have shown that emulsions of FA-rich extracts from microalgae can reduce MRSA attachment to pre-treated skin. Thus, there is potential for the development of a gel containing one or more FFAs with potent activity for Gram-positive pathogens, such as MRSA, to prevent and reduce bacterial colonisation of the skin and nose.

As far as sexual health is concerned there are also possibilities for a FFA-containing product to reduce the transmission of sexually transmitted infections (STIs), especially those caused by *Neisseria gonorrhoeae* (Bergsson et al. 1999; Thormar et al. 1999), *Chlamydia trachomatis* (Bergsson et al. 1998; Thormar et al. 1999) or herpes simplex virus (Kristmundsdóttir et al. 1999). Indeed, formulations containing monoglycerides (single FAs bound to glycerol) have demonstrable efficacy against STIs in vivo (Neyts et al. 2000). Other potential therapeutic applications suggested for FFAs in humans might be in the prevention of dental caries (Kurihara et al. 1999; Osawa et al. 2001; Won et al. 2007), in reducing the incidence of infant gastrointestinal infections by adding to formula milk (Thormar et al. 1987; Isaacs et al. 1995), in the treatment of acne (Nakatsuji et al. 2009; Yang et al. 2009) or in the treatment of stomach ulcers caused by *Helicobacter pylori* (Hazell and Graham 1990; Thompson et al. 1994; Petschow et al. 1996).

Agriculture and aquaculture

Antibiotics are used as animal feed supplements to increase the production of meat or cultured fish, as they reduce bacterial abundance in the digestive system resulting in more energy being diverted to weight accumulation (food conversion) (Dibner and Richards 2005). However, concerns about antibiotic resistance transferring to human pathogens and anxiety about antibiotic residues and environmental contamination (Smith et al. 2002) has led to the ban on the use of conventional antibiotics in livestock foodstuffs in the European Union (European Union 2005), and similar bans are being considered elsewhere (Dibner and Richards 2005). Therefore, opportunities exist to replace these conventional antimicrobial agents, and FFAs may be a realistic alternative. Moreover, as FFAs are also active against methane-producing Archaea (methanogens) in the guts of ruminants, they could reduce emissions of this important greenhouse gas (Ungerfeld et al. 2005).

Piglets treated with a source of lipids and a lipolytic enzyme to release antibacterial FFAs in the animals' guts show a reduction in the abundance of gut microbiota and improved weight gain and feed conversion (Dierick et al. 2002). However, at present, the high cost of the lipid component remains the stumbling block to implementation (Dierick et al. 2002). Here, we suggest that single-celled algae could be an inexpensive source of FFAs. These microorganisms are autotrophic, negating the need for costly heterotrophic sources of carbon, can be cultured on non-arable land in salt water and can achieve growth rates similar to bacteria (de la Noue and De Pauw 1988). Moreover, technologies in the culture, harvest and manipulation of single-celled algae for their lipids are established but continue to improve, particularly due to recent interest in biofuels (Chisti 2008). Single-celled algal species can be selected and their lipid composition further manipulated to enrich for the particular mixture of FFAs required

(Borowitzka 1988). The algae, which are a nutrient source in themselves, typically also contain various health-promoting vitamins and antioxidants (de la Noue and De Pauw 1988), could be incorporated into animal feed. The addition of exogenous lipolytic enzymes may be unnecessary for certain algal species, as the cells contain their own enzymes activated on cell disintegration (Jüttner 2001; Wichard et al. 2007).

FFAs added to feed may also increase survival in commercial rabbit farms where losses to enteric diseases can be high. Rabbits experimentally infected with pathogenic *Escherichia coli* have a better chance of survival if the feed is supplemented with caprylic acid in its free form or as triglycerides (Skřivanová et al. 2008). These rabbits also have significantly fewer *E. coli* in their faeces and stomachs compared to rabbits fed a non-supplemented diet (Skřivanová et al. 2008). Emulsions of monoglycerides can reduce the burden of pathogenic bacteria, such as *Campylobacter jejuni*, in chicken feed (Thormar et al. 2006). A similar approach could be used for the prevention of disease in aquaculture and mariculture, as FFAs are active against industry-relevant bacterial pathogens (Benkendorff et al. 2005; Desbois et al. 2009) and pose virtually no environmental harm from leaching into the water. Finally, antibacterial FFAs have also been considered in the treatment of bovine mastitis (Hogan et al. 1987; Nair et al. 2005) and in the control of honeybee infections (Feldlaufer et al. 1993; Hornitzky 2003).

Discussion and concluding remarks

As discussed above, the antibacterial properties of FFAs are well-recognised, and because they act through different mechanisms to most conventional antibiotics, they offer potential for commercial exploitation. However, there are a few problems that have hindered progress thus far. First, some FFAs have an unpleasant taste (Stephan and Steinhart 2000; Refsgaard et al. 2000). Second, certain FFAs can be unstable and they also have a tendency to bind non-specifically to proteins (Kodicek and Worden 1945; Galbraith et al. 1971; Lacey and Lord 1981; Boyaval et al. 1995; Petschow et al. 1996; Guil-Guerrero et al. 2001). Finally, there may be a perceived lack of patentable intellectual property (IP) because FFAs are found so ubiquitously. As regards taste, a possible solution is to deliver the FFAs in the form of lipids together with a lipolytic enzyme. Such a combination, where the enzyme cleaves antibacterial FFAs from the lipid source, can be used to increase the in situ abundance of FFAs, such as inside an animal's gut (Dierick et al. 2002). This form of administration also subverts the problem of FFA instability because the FFAs will be delivered as stable lipids (e.g. triglycerides). If the problem of taste can be solved, one of the most lucrative areas for development could be in controlling the growth of pathogens or spoilage bacteria in food (Wang and Johnson 1992; Ouattara et al. 1997; Shin et al. 2007; Desbois et al. 2008, 2009). Monoglycerides, which tend not to have an unsavoury taste, are already used in food preservation (Shibasaki and Kato 1978). One of the most exciting potential applications for antibacterial FFAs is their use in topical medicine for the prevention and treatment of bacterial diseases. With respect to IP opportunities, new IP could be generated by exploring interactions between FFAs and conventional antibiotics or other agents with the aim of identifying synergistic combinations, as investigations to this end have been reported only sparsely (Shibasaki and Kato 1978; Wille and Kydonieus 2003; Drake et al. 2008). Combination therapies, where multiple antibacterial agents are given together, are desirable, as they can reduce the opportunity for bacterial resistance to emerge (Zhao and Drlica 2001). Treatments containing a FFA component will further reduce the opportunity for resistance to emerge due to the FFA's non-specific mode of action. Additionally, studies of chemically altered FFAs engineered for more desirable 'drug-like' characteristics may prove to be another fruitful avenue to success.

Ultimately, the choice of FFAs is application-dependent and will differ according to the requirements of the process and the bacteria to be targeted. Specific mixtures could be produced that are optimised for each application and finely tuned for potency and spectrum. These could be mixed with solvents, stabilisers, or other compounds to further enhance activity.

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