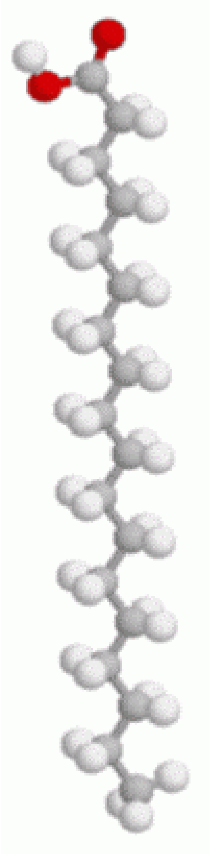
FREE (UNESTERIFIED) FATTY ACIDS

1. Occurrence and Biochemistry

Free or unesterified fatty acids are ubiquitous if minor components of all living tissues. In animals, much of the dietary lipid is hydrolysed to free acids before it is absorbed and utilized for lipid synthesis. Intact lipids in tissues can be hydrolysed to free acids by a variety of enzymes (*e.g.* lipoprotein lipase, hormone-sensitive lipase, phospholipase A), before being metabolized in various ways including oxidation, desaturation, elongation or re-esterification. As free acids can interact with a wide range of enzyme systems in both specific and non-specific ways, they must be rapidly sequestered in tissues by various means to ensure that their activities are closely regulated.



Monomeric fatty acids in the free state have very low solubilities in aqueous media. In serum, they are transported between tissues bound to the protein albumin, which has up to six strong binding sites and a large number of weak binding sites where non-polar interactions are possible between the fatty acid hydrocarbon chains and uncharged amino acid side chains. In this way, the concentration of a long-chain fatty acid in serum can be increased by as much as 500 times above its normal maximum. However, the bound fatty acids can diffuse into the aqueous phase, where they are rapidly taken up into the outer leaflet of the plasma membrane by non-enzymatic mechanisms. It is then possible that fatty acids can then cross the membrane simply and rapidly by a biophysical process, i.e. by ‘flip-flop’. On the other hand, there is also evidence that specific transporter proteins may be involved in part to activate by formation of **acyl-coA** prior to further esterification, but also to ensure vectorial transport so that specific fatty acids are directed towards particular purposes. Certainly within the cell, a family of fatty acid binding or transport proteins has essential functions in fatty acid trafficking pathways and in fatty acid activation. It appears that cells have several overlapping mechanisms that ensure sufficient uptake and directed intracellular movement of the fatty acids required for their physiological functions.

Apart from their obvious role as a source of energy (see our web page on **acylcarnitines**, for example), unesterified fatty acids can act as second messengers required for the translation of external signals, as they can be produced rapidly as a consequence of the binding of specific agonists to plasma membrane receptors. In this way, they can substitute for the second messengers of the inositide pathways. Fatty acids are effective also in operating at specific intracellular locations reversibly to amplify or otherwise modify signals. For example, they influence the activities of protein kinases, phospholipases, G-proteins, adenylate and guanylate cyclases, and many other metabolic processes. Part of the action of fatty acids may occur indirectly via metabolism of arachidonic acid to eicosanoids. On the other hand, there is much evidence that fatty acids *per se* are messengers that mediate the responses of the cell to extracellular signals. Many of these reactions are specific to particular fatty acids. For example, polyunsaturated fatty acids, including docosahexaenoic and arachidonic acids, bind to the retinoid X receptor and induce activation. Some related processes appear to occur in plants.

In addition in animal tissues, long-chain polyunsaturated fatty acids are involved in regulating gene expression, mainly targeting genes that encode proteins with roles in fatty acid transport or metabolism. In this respect, (n-3) fatty acids are more potent than (n-6) fatty acids. Straight-chain saturated and monoenoic fatty acids do not appear to be involved in the process, but surprisingly poly-methyl-branched fatty acids, such as phytanic and pristanic, may have a function. In some circumstances, both the free acids *per se* and their **coenzyme A** esters may be involved.

The mechanisms by which modulation of gene transcription occurs are only partially resolved, and this is the subject of considerable research effort, especially with respect to the family of transcription factors, i.e. peroxisome proliferator-activated receptors (PPARs) in the nuclei of cells. However, it is believed that fatty acid-binding-proteins are intimately involved and in effect act as nutrient sensors. They bind long-chain fatty acids with high affinity in the cytoplasm and transport them to nuclei, which they enter via the nuclear pores, where they are able to form complexes with nuclear receptors enabling them to regulate receptor activation.

The effects can be highly specific, different fatty acids binding to or activating different types of PPAR, although the PPARα and hepatocyte nuclear factor 4α (HNF4α) are especially important. In particular, polyunsaturated fatty acids may exert beneficial effects by up-regulating the expression of genes encoding enzymes involved in oxidation of fatty acids, while at the same time down-regulating genes for enzymes involved in lipid synthesis. They also influence glucose metabolism. As a result, unesterified fatty acids may mitigate the undesirable symptoms of the metabolic syndrome and may even reduce the risk of heart disease. In contrast, abnormal PPAR activation can be a factor in the lipotoxicity observed with obesity, insulin resistance, type 2 diabetes and hyperlipidemia.

Similarly, in bacteria, it has been demonstrated that the bacterial fatty acid transport and trafficking system leads to fatty acid-responsive regulation of gene expression.

Free fatty acids have potent antimicrobial, antiviral and antifungal properties, and they exert such effects in some living systems, especially the skin and mucosa of the lung. As they are powerful detergents and will inhibit very many enzymes systems in a non-specific manner, it is not clear whether the biocidal properties are also non-specific. Unsaturated fatty acids seem to have the greatest effects, but this may be because they can insert more readily into membranes.

2. Analysis

Accurate measurement of free fatty acids concentrations in plasma and tissues can be a useful measure of metabolic status. Unfortunately, it is very easy to generate free acids artefactually by faulty storage or extraction. Lipases can continue to function slowly in some tissues, even at –20°C, and the process will accelerate if tissues are allowed to thaw prior to extraction. In one important series of experiments [Kramer, J.K.G. and Hulan, H.W., *J. Lipid Res*., **19**, 103-106 (1978)], it was shown that if animal tissues were pulverized and extracted at –70°C, very low levels only of free fatty acids were detected in comparison to more conventional procedures. High concentrations reported occasionally in the literature are patently impossible in living tissues and are due to inappropriate sample handling.

Following extraction of lipids from tissues by a suitable procedure, a free fatty acid fraction can be isolated by thin-layer chromatography or by solid-phase extraction chromatography on a bonded amine phase. This can be methylated and analysed by gas chromatography with an internal standard.

Recommended Reading

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