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β-Oxidation of fatty acids

A century of discovery

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Regulatory aspects, genetic defects, structure and mechanisms of acyl-CoA dehydrogenases and oxidases

The β-oxidation of fatty acids is a central metabolic process providing electrons to the respiratory chain and thus energy for a multitude of needs in aerobic organisms. It is a complex process occurring inside mitochondria, involves more than a dozen enzymes and must be carefully regulated, in particular in its relationship to the other sources of energy such as carbohydrate and amino acid catabolism. The consequences of dysfunctions in β-oxidation can be many-fold and severe with regard to human health, and thus an understanding of the basic mechanisms is of great relevance. The scope of the present series of minireviews is to highlight aspects of β-oxidation from different, complementary points of view. Thus, in the first review, Bartlett and Eaton address the interplay and regulation of the various enzymes involved in the β-oxidation cycle itself, and of those involved in the transport and transformation of fatty acids and conjugates. They also deal with the effects of malfunction of these enzymes, e.g. as a consequence of genetic defects. The second review is by Gregersen, Bross and Andresen and addresses a topic that has increased in importance in the recent past: the consequences on human health of genetic defects affecting β-oxidation. Since the discovery of the first defects in the 1970s, this field has gained steadily in relevance. One reason is undoubtedly the central role of β-oxidation in 'energy metabolism'. The second is associated with the finding that one specific mutation affecting medium-chain acyl-CoA dehydrogenase is among the most common genetic defects in humans of Caucasian descent, its frequency being higher than that of phenylketonuria. This has spurred great advances in the methodologies for the detection of specific metabolites in clinical chemistry, in genetic analysis and by this in the identification of specific defects. The review also draws attention to the importance of the interplay of medical studies with basic biochemical investigations that aim to elucidate the molecular basis of genetic defects. The third (Kim and Miura) and fourth contributions (Ghisla and Thorpe) deal with the family of enzymes involved in β-oxidation that has received most attention recently: the enzymes involved in the first step of the cycle, the α,β-dehydrogenation of fatty acid acyl-CoA conjugates. These enzymes constitute a family of flavoproteins whose chemistries regarding the catalytic event are similar. However, they differ significantly in their specificity, tissue distribution and quantitative occurrence. The members of this family have grown steadily and reach nine at present. The last two and most recent additions have emerged from the identification of corresponding genes in the human genome. The characteristics of this family raise the question of why nature evolved to use such a large number of related enzymes instead of a small number to do the same job. The answer is still uncertain though it can be speculated that it resides in control and in the fact that α,β-dehydrogenation is the slowest step in the β-oxidation cycle and has fostered a corresponding evolutionary pressure. Medium-chain acyl-CoA dehydrogenase, one of the 'oldest members' of the family, has received much attention from a biochemical/mechanistic point of view, and has advanced to become a model enzyme for the study of the chemistry underlying α,β-dehydrogenation. Also, with its cousin, the acyl-CoA oxidase(s), it shares the mechanism of substrate dehydrogenation but has completely different tastes for electron acceptor. It transfers electrons to a specific acceptor (electron transferring flavoprotein) and protects its redox equivalent from reaction with oxygen. This contrasts with the oxidase that specifically uses O_2 as an acceptor. The two enzymes have thus recently become models for investigating the factors that govern oxygen reactivity of flavoproteins. In their review, Kim and Miura highlight the salient features of the 3D structure of medium-chain acyl-CoA dehydrogenase in comparison with that of related members of the family and, importantly, with that of the specified acyl-CoA oxidase. Perhaps the most surprising aspect emerging from this comparison is the fact that the two enzymes do not differ in their functional groups at the active site. They differ, however, in subtle aspects such as the presence of a 'more open' active site in the case of the oxidase that might facilitate physical access of dioxygen to the active site, whereas access of solvent and dioxygen to that of the dehydrogenase appears to be repressed. The fourth contribution by Ghisla and Thorpe discusses primarily mechanistic aspects of acyl-CoA dehydrogenases from a (bio) chemical point of view. Although a number of important details still elude our understanding, it is fair to say that we have advanced to the point where there is a consensus on the basic mechanism shared by this class of enzyme and that of the oxidases. Importantly, there is good agreement between the deductions drawn from 3D data and biochemical studies.

Dedication: This minireview series is dedicated to H. Beinert*, one of the pioneers in the field of fatty acid β-oxidation, on the occasion of his ninetieth birthday.

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Finally, this digression into biochemical aspects brings us to the appreciation of the prominent role of H. Beinert, who has been a pioneer in the study of enzymes involved in β-oxidation, and to whom this series is dedicated on the occasion of his 90th birthday. The following section is his retrospective view of the progress that has occurred in the last century in this field.

Sandro Ghisla is in the Department of Biology at the University of Konstanz. He studied organic chemistry in Basel and then, in 1968, moved to the laboratory of the late Prof. P. Hemmerich at the newly founded University of Konstanz. There he worked on the chemistry of flavins, and on the structural elucidation of the covalent flavin of succinate dehydrogenase. This was followed by a longer stay (1971–75) in the laboratory of Prof. V. Massey where he got acquainted with the biochemistry of flavin-dependent enzymes. He then returned to the University of Konstanz and has since worked on various mechanistic topics related mainly to flavoproteins. Examples are bacterial luminescence, the activation of dioxygen, acyl-CoA dehydrogenases, amino acid oxidases and the biosynthesis of biopterins. Prof. Ghisla is a member of the Editorial Advisory Board of EJB.

β-Oxidation: from analysis of dog urine to crystal-structures and enzyme mechanisms within a century

Helmut Beinert

The mechanism by which saturated fatty acids, i.e. essentially saturated hydrocarbons, are degraded and utilized in living organisms has been a matter of keen interest to physiologists and biochemists for many years. It was a long and arduous road until the basic processes, and the catalytic proteins and necessary cofactors were recognized and obtained as pure substances. There were a number of crucial observations and developments in adjacent fields, scattered through the years for almost half a century, which eventually had to come together to lead to the final clarification of the processes and ingredients involved. In the following I intend to point out the various crucial stations along the way to success.

In 1904 Franz Knoop did his famous experiments [1], probably the first 'tracer' experiments, by feeding dogs phenylated fatty acids of various chain lengths, from which it became clear that fatty acids are degraded by successively chopping off two-carbon units at a time through introduction of a double bond between the β- and γ-carbon of the fatty acid, hence the name β-oxidation. This was supported in experiments with unlabeled fatty acids in 1908 by Dakin [2]. It was established that special attention should be given to the role of a two-carbon fragment, presumably some form of acetate. However, acetate is known to be a fairly unreactive substance. In experiments on yeast, Lynen [3] observed in Wieland's laboratory in the early 1940s that, when respiring yeast was oxygenated until its endogenous substrates had been exhausted, it was only able to resume oxidation of acetate after a lag period, as though acetate had to be prepared for oxidation by some activation process. This process could be facilitated by adding small quantities of a readily oxidizable substrate such as ethanol, indicating that some energy had to be provided by the cosubstrate, which was therefore called the 'sparker'. Out of such observations arose the idea of an 'activated' form of acetate.

In the same period, there happened to be interest in an 'active form' of acetate among pharmacologists and neurologists, because such a compound was obviously required for the enzymatic acetylation of choline [4,5] and of sulfanilamide [6], through which the latter lost its antibacterial activity. These acetylation reactions then furnished simple and practical assays for 'active acetate'. It was also noticed that, in addition to the respective acetylase proteins, a water-soluble substance of low molecular mass was required, which was called the coenzyme of acetylation, CoA [7]. Lipmann and his group were able, following the sulfanilamide assay, to obtain active concentrates of the new coenzyme, which they then subjected to various analyses for its constituents [8,9]. In this endeavour, the availability of qualitative and quantitative microbial assays for various growth factors, which had been or were developed just in those years, became of critical importance.

The CoA concentrates contained β-alanine, which pointed to the presence of pantothenic acid [10], and it was shown that the activity of the preparations in the acetylation assay were parallel to the pantothenic acid content. Pantothenic acid, an amide of pantoic acid and β-alanine, had been established as a vitamin in 1938 [11–13]. However, these ingredients could not account for the function of CoA. The crucial observation came via yet another growth factor, namely Snell's *Lactobacillus bulgaricus* factor, LBF [14]. LBF is the acid amide of pantothenic acid and cysteamin, called pantethein. CoA was shown to be pantethein-4′ phosphate [15]. With a crude liver extract it was possible to synthesize CoA from LBF [16,17]. The substance obtained was able to catalyze the formation of citrate from acetate and oxaloacetic acid with Ochoa's 'condensing enzyme' [18]. Work on LBF was considered important as it established the presence of cysteamine and, now for the first time, it gave a hint as to a plausible reactive site in the CoA molecule, which had up to this point not been obvious.

We must digress here for a moment and mention work that had a considerable bearing on the search for the identity of active acetate. In the late 1930s and early 1940s, Lipmann found with *Lactobacillus delbrueckii* that oxidation of pyruvate led to the formation of acetylphosphate [19]. Similarly, Barker and Stadtman, in their work with *Clostridium kluyveri*, had identified acetylphosphate [20], which was involved in performing acetyl- and phosphate-transfer reactions. This was taken as a first hint as to what kind of compound 'active acetate' could be. However, as an anhydride of a strong acid with a weaker acid, acetylphosphate was far less stable than the 'active acetate' being sought and was unable to act as an acetyl donor for citrate formation or for acetylation of sulfanilamide [7]. However, pantetheine, as an acetylmer-

captan, would be more prone to acetylate a suitable substrate, rather than undergo hydrolysis. Thus, the SH-function of CoA, as it had been revealed by the work on LBF, furnished the critical clue to the behaviour observed with the so far hypothetical active acetate.

At this point Lynen resumed the studies with starved yeast and obtained concentrates of the substance that was produced in the sparking process, which he expected to be 'active acetate'. Indeed, all the reactions observed with acetyl-CoA were produced [21]. All these events occurred within a span of at most two years in a breathtaking and often highly competitive race.

With acetyl-CoA in hand it was now possible to understand the process of β-oxidation, namely that hydration of the carbon–carbon double bond would follow the dehydrogenation step, with a second oxidation to the keto-form, acetoacetate, and a thiolase to separate the two acetyl CoA residues. The primary oxidizing enzymes, specific for certain carbon chain lengths, or branched chains, all turned out to be flavoproteins without transition-metal constituents [22] and the second oxidizing enzyme, a hydroxy-acid dehydrogenase, was dependent on NAD. Many of these and other enzymes of the pathway have now been crystallized and their structures and mechanisms of action have been determined.

The connection of this oxidation system to the respiratory chain, i.e. the cytochrome system, was not clear. It was found in 1954 that, for the oxidation of the flavoproteins of the β-oxidation system, yet another flavoprotein was required, ETF: the 'electron transfer flavoprotein'. However, this protein would still not communicate with the terminal electron transport system; for this an Fe-S-flavoprotein, ETF-ubiquinone oxidoreductase, was needed, which was only identified in 1975–77 [23].

Helmut Beinert was born in 1913 in Lahr in southern Germany. He pursued his studies mainly in Heidelberg and he graduated in chemistry in 1943 (at the University of Leipzig) with R. Kuhn, at the time one of the most eminent authorities in the field. In the aftermath of the postwar turmoil, he moved to the US and started his scientific career as a postdoctoral fellow at the Institute for Enzyme Research in Madison Wisconsin. Today, at the age of 90, he is still in Madison, and scientifically most active. On the other hand, over the years he has traveled and established collaborations all over the world, this being the basis of his achievements and testifying to his scientific versatility. Beinert is famous for two main reasons: one is the discovery, starting from the early 1960s, of several of the key enzymes involved in β-oxidation and electron transfer in mitochondria. The second, and probably the most recognized contribution, is the discovery of Fe-S proteins based on the use of EPR spectroscopy. He is thus one of the founders of what is now called 'Bioinorganic Chemistry'.

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