**Plasmodium falciparum** possesses organelle-specific α-keto acid dehydrogenase complexes and lipoylation pathways


Division of Infection and Immunity, Institute of Biomedical and Life Sciences, Joseph Black Building, University of Glasgow, Glasgow G12 8QQ, U.K.

**Abstract**

The human malaria parasite *Plasmodium falciparum* possesses a single mitochondrion and a plastid-like organelle called the apicoplast. Both organelles contain members of the KADH (α-keto acid dehydrogenase) complexes – multienzyme complexes that are involved in intermediate metabolism. In the asexual blood stage forms of the parasites, the α-ketoglutarate dehydrogenase and branched chain KADH complexes are both located in the mitochondrion, whereas the pyruvate dehydrogenase is exclusively found in the apicoplast. In agreement with this distribution, *Plasmodium* parasites have two separate and organelle-specific pathways that guarantee lipoylation of the KADH complexes in both organelles. A biosynthetic pathway comprised of lipoic acid synthase and lipoyl (octanoyl)-ACP:protein

**Introduction**

α-Lipoic acid is an essential cofactor of KADH (α-keto acid dehydrogenase) complexes such as PDH (pyruvate dehydrogenase), α-ketoglutarate dehydrogenase and BCKDH (branched chain α-keto acid dehydrogenase). These enzyme complexes catalyse the oxidative decarboxylation of α-keto acids generating acyl-CoA, NADH and CO₂. In most eukaryotes, KADH complexes are located in mitochondria where they are integral to energy metabolism and amino acid degradation [1,2]. In plants and algae, a second, distinct PDH is present in the plastid, where it provides metabolites for fatty acid biosynthesis [1]. KADH complexes are composed of three independent multimeric proteins, a deacetylase (or E1), a transacetylase (or E2) and a dihydrolipoamide dehydrogenase (or E3); the E2 subunits form the catalytic core of the protein complexes [2,3]. Lipoic acid is covalently attached to specific lysine residues of the lipoyl-domain of the E2 proteins and is crucially involved in substrate channelling through the enzyme complexes [2]. α-Lipoic acid, necessary for this post-translational modification, is either synthesized de novo or salvaged from the environment. The biosynthetic pathway uses octanoyl-ACP (where ACP stands for acyl-carrier protein), an intermediate of fatty acid biosynthesis and introduces two sulphurs into the 6 and 8 position of the substrate. The enzyme catalysing this reaction is LipA (lipoic acid synthase), an S-adenosylmethionine-dependent, [Fe-S] cluster-containing protein [4–6]. This reaction can occur either before or after LipB [lipoyl (octanoyl)-ACP:protein N^δ-lipoyltransferase B] has ligated the cofactor/precursor to the E2 subunit of KADH complexes [7]. Plants, bacteria and fungi synthesize α-lipoic acid de novo via this LipA/LipB pathway [8–10]. However, this pathway appears not to operate in mammals. Genes encoding LipA and LipB have been identified in mammalian genomes, but their significance is not fully understood because it is generally believed that mammals primarily rely on salvaged α-lipoic acid [11]. The salvaged α-lipoic acid is ligated to the E2 protein of mammalian KADH complexes by a two-step mechanism involving lipoate-activating enzyme and lipoyl-AMP:N^ε-l-lysine lipoyltransferase (Scheme 1) [12,13]. The ligation of salvaged lipoic acid in plants, bacteria and fungi is different from that found in mammals. One enzyme, LplA (lipoate protein ligase A), catalyses both activation and ligation of lipoic acid to their E2 subunits [14–16] (Scheme 1).

**KADH complexes in the malaria parasite Plasmodium falciparum**

*P. falciparum* and related apicomplexan parasites possess a single mitochondrion and a plastid-related organelle, the apicoplast [17]. Both contain key metabolic pathways essential for parasite survival [18,19]. The erythrocytic stages of *P. falciparum* generate energy primarily via glycolysis and the role of the mitochondrion for energy supply is not yet fully understood. However, it has been established that it contains a functional electron-transport chain that is essential for the biosynthesis of pyrimidine precursors [18]. In addition, components of the haem biosynthesis pathway...
are present in the mitochondrion [20]. The apicoplast is responsible for the biosynthesis of fatty acids and isoprenoids (reviewed in [19]). Both pathways differ significantly from those of the mammalian host and have been proposed as promising targets for the development of new antimalarials [21,22].

Genome analyses have revealed that *P. falciparum* and other apicomplexan parasites possess genes encoding the different subunits of all three KADH complexes [23,24] and their expression in the erythrocytic stages of the parasites has been established by microarray analyses, reverse transcriptase–PCRs and Western blotting [23–26]. Conspicuously, the parasites possess only a single, apicoplast-located PDH and seem to lack a mitochondrial counterpart [23,24]. Bioinformatics analyses and localization studies suggest that the other two multienzyme complexes are mitochondrial [24] (see Figure 1).

The apicoplast location of PDH represents an unusual metabolic situation, especially as genes encoding proteins that are required for a fully functional tricarboxylic acid cycle have been identified in the *Plasmodium* genome. This situation poses questions regarding how the tricarboxylic acid cycle is fuelled if acetyl-CoA is not provided by a mitochondrial PDH. One possibility is that the metabolite is obtained from the apicoplast via an acetyl-CoA transporter. A gene encoding a potential peptide-acetyl-CoA transporter has been identified in the parasite genome, but its location and functional role are yet to be elucidated [27]. Moreover, other metabolic mechanisms that supply acetyl-CoA within the mitochondrion may well exist, e.g. the degradation of the amino acids valine, leucine and isoleucine via the mitochondrial BCKDH. Another possibility is that pyruvate is not the primary source of fuel for the tricarboxylic acid cycle in these organisms and alternative metabolic pathways may compensate for the absence of mitochondrial PDH. α-Ketoglutarate could, for instance, act as an entry into the tricarboxylic acid cycle. This metabolite can be generated by glutamate dehydrogenase or alternatively by aspartate aminotransferase. The latter converts glutamate into α-ketoglutarate and simultaneously produces aspartate from oxaloacetate. The required oxaloacetate might be provided by phosphoenolpyruvate carboxylase, which is encoded in the parasite genome. This hypothesis is based on the idea that one of the prime functions of the tricarboxylic acid cycle in *P. falciparum* is to provide the metabolic precursor succinyl-CoA for haem biosynthesis [23,24]. Additional evidence supporting the hypothesis that the role of the tricarboxylic acid cycle differs from that in mammals is provided by the unusual localization of some components of the tricarboxylic acid cycle in *P. falciparum*. For instance, an aconitate-like
Figure 1 | Localization of components of the mitochondrial branched chain KADH complexes

The localization of the E1β-subunit of *P. falciparum* BCKDH complex and the mitochondrial lipoamide dehydrogenase were analysed by expressing the first 390 and 653 nucleotides respectively tagged with GFP in erythrocytic stages of the parasite. (A) E1β-subunit of *P. falciparum* BCKDH. Phase, phase contrast image; GFP-E1β, image obtained with GFP channel; Mito, image obtained with rhodamine channel representing the localization of MitoTracker CMred; Merge, merged image. (B) Mitochondrial lipoamide dehydrogenase. Phase, phase-contrast image; GFP-mE3, image obtained with GFP channel; Mito, image obtained with rhodamine channel representing the localization of MitoTracker CMred; Merge, merged image.

Protein was reported to be cytosolic and enzymatically inactive [28]. Further, an NAD+—dependent isocitrate dehydrogenase appears to be absent from *P. falciparum* and the mitochondrial NADP+—dependent enzyme seems to be involved in redox control rather than energy metabolism [29].

The presence of the PDH in the apicoplast is necessary because type II fatty acid biosynthesis occurring in the organelle requires both acetyl-CoA and NADH. This fatty acid biosynthesis is essential for the development of *P. falciparum* [22]; thus it is highly likely that apicoplast PDH is also essential and so may represent a potential target for the design of new antimalarials.

**Lipoic acid metabolism in *P. falciparum***

The presence and distribution of the KADH complexes in *Plasmodium* implies that the parasites need lipoic acid and the ability to ligate it to their E2 subunits in the mitochondrion and apicoplast (reminiscent of the situation found in plants and algae). Genome analyses revealed a single gene encoding a protein with similarity to LipA. Sequence analyses and localization studies using GFP (green fluorescence protein) fusion proteins unambiguously showed that LipA is an apicoplast protein in *Plasmodium* and the related apicomplexan parasite *Toxoplasma gondii* [30,31]. Furthermore, three distinct lipoate protein ligase genes have been identified in *Plasmodium*. One gene similar to lipB encodes a protein with a typical bipartite apicoplast-targeting sequence, whereas the second gene encodes a bacterial-like LplA that is mitochondrial [31]. The third gene is more similar to predicted plant lplA and its functionality and localization are under investigation.

Current evidence suggests that apicomplexan parasites supply their plastid PDH with lipoic acid via the biosynthetic pathway (LipA and LipB). This is in agreement with the presence of the fatty acid biosynthetic pathway in the organelle providing the precursor octanoyl-ACP for lipoic acid biosynthesis (Scheme 1). Interestingly, the parasite’s mitochondrion is devoid of lipoic acid biosynthesis and *Plasmodium* seems to rely entirely on salvage of the metabolite to supply it to the organelle. This is in agreement with the absence of fatty acid biosynthesis from the mitochondrion, which would be a necessary prerequisite for the supply of the precursor.
needed for lipoic acid biosynthesis. The organelle-specific distribution of lipoic acid biosynthesis and salvage pathways in *P. falciparum* (and related apicomplexan parasites) suggests that either both act independently or that lipoic acid generated in the apicoplast is also provided to the mitochondrion. There is some evidence that in *T. gondii* no direct transfer of newly synthesized lipoic acid from the apicoplast to the mitochondrion occurs [32]. These results strongly support the suggestion that the two lipoylation pathways act independently and that lipoic acid is not exchanged between apicoplast and mitochondrion in apicomplexan parasites as has been previously discussed [30,31].

### Other roles of lipoic acid

Lipoic acid is not only an essential cofactor for the KADH complexes, but also is renowned for its antioxidant capacity. Dietary supplementation of lipoic acid is beneficial for the treatment of Type II (non-insulin-dependent) diabetes, by reducing the oxidative stress, which is often increased in diabetic patients [33]. It is therefore possible that the metabolite is not only an essential cofactor of the parasite's KADH complexes but may also act as an antioxidant protecting the parasite’s organelles from oxidative damage. Indeed, in *Mycobacterium*, it was shown that protein-bound lipoic acid acts as a reducing agent for a thioredoxin-like protein, which in turn reduced a peroxiredoxin, leading to the detoxification of hydroperoxides [34,35]. This antioxidant role of lipoic acid could also be important in *Plasmodium* and this hypothesis is currently being investigated [36]. We have shown that the free dihydrolipoamide dehydrogenase/lipoamide redox pair reduces *P. falciparum* and *T. gondii* thioredoxin with appreciable second-order rates of 10^{-4} M^{-1} s^{-1} [37].

### Conclusions

In apicomplexan parasites, KADH complexes occur in both the mitochondrion and the apicoplast, but the single PDH is only present in the plastid. This unusual distribution raises intriguing questions about the supply of metabolites for the mitochondrial tricarboxylic acid cycle. This situation also dictates that both apicoplast and mitochondrion must possess functional lipoylation machineries. It is now known that the apicoplast contains a biosynthetic pathway comprising LipA and LipB similar to that in other plastid-containing organisms, whereas the mitochondrial relies entirely on the supply of exogenous lipoic acid as it is devoid of a biosynthetic pathway in contrast with some other eukaryotes. These unusual circumstances suggest that both lipoylation pathways are essential for parasite survival and are potentially excellent targets for the design of new antimalarials.

---

This research was funded by the Wellcome Trust. S.G. is a recipient of a Boehringer Ingelheim PhD Scholarship. S.M. is supported by a Wellcome Trust Senior Fellowship.

### References


Received 21 June 2005

©2005 Biochemical Society