Macrophomate Synthase: The First Structure of a Natural Diels–Alderase

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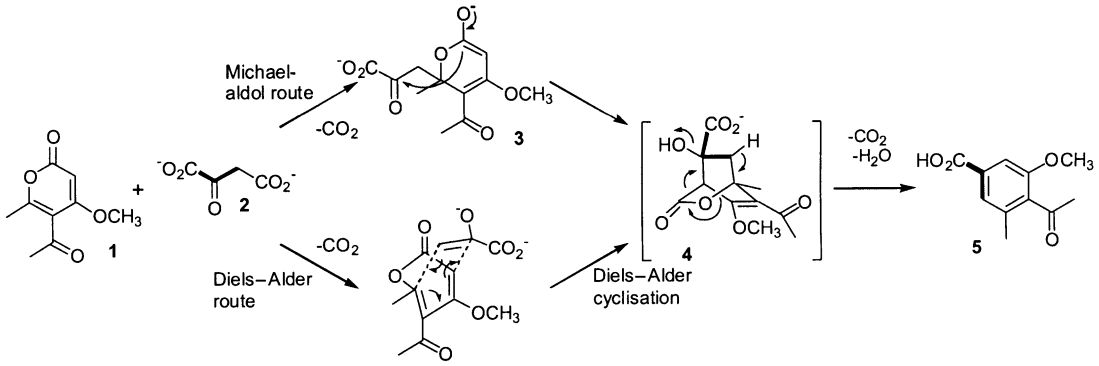
The involvement of enzymes in the catalysis of Diels–Alder reactions to form six-membered rings from an alkene and a 1,3-diene has been postulated for over a hundred natural products.[1] However, to date direct evidence from biosynthetic studies with purified or partially purified enzymes is provided for only three distinct naturally occurring Diels–Alder reactions.[2] Now the quest for natural Diels–Alderases has resulted in the elucidation of the first crystal structure of such an enzyme, which gives mechanistic insight into the formation of macrophomic acid (**5**).[3] In the presence of the 2-pyrone **1** and oxalacetate **2**, macrophomate synthase from the phytopathogenic fungus *Macrophoma commelinae* catalyses the formation of the aromatic metabolite macrophomic acid (**5**; Scheme 1). The complex transformation involves two carbon–carbon bond formations, two decarboxylations and a dehydration step.

This formation of a benzoic acid derivative is exceptional since it does not follow common routes like the shikimate or the polyketide pathway. A series of biosynthetic studies have revealed how macrophomate synthase, an enzyme with a molecular weight of 36 kDa,[4] catalyses this reaction. Two different pathways towards macrophomic acid (**5**) have been discussed, namely a route involving a stepwise carbon–carbon bond formation by a Michael-aldol sequence[5, 6] and a transformation by a Diels–Alder reaction[7] with concerted formation of the two carbon–carbon bonds. In a series of studies using substrate analogues[6, 8, 9] and bicyclic inhibitors[10] the evidence for the involvement of such a pericyclic reaction has been accumulated but only recently have the structural details of the Diels–Alderase been unravelled by Ose et al.[3] These researchers reported the 1.7-Å crystal structure of macrophomate synthase in complex with pyruvate. The provided structural model consists of 299 residues; the missing 40 residues do not contribute to catalytic activity, as was shown with deletion mutants. Macrophomate synthase was crystallised in the presence of pyruvate and Mg^{2+} ions, which are essential for catalytic activity, and the protomer structure is shown in Figure 1a. The hexameric protein is associated by intense hydrophobic interactions.[3]

The active site is located at the C terminus of an eight-stranded β barrel, which is covered by a long loop from the three-fold-related chain. In this site, a Mg^{2+} ion is octahedrally coordinated by two carboxy



Scheme 1. *Possible pathways for the formation of macrophomic acid (**5**) from oxalacetate (**2**) and the pyrone **1**. Upper path: a stepwise formation of the carbon–carbon bonds by a Michael-aldol sequence; lower path: concerted bond formation through [4+2] cycloaddition.*

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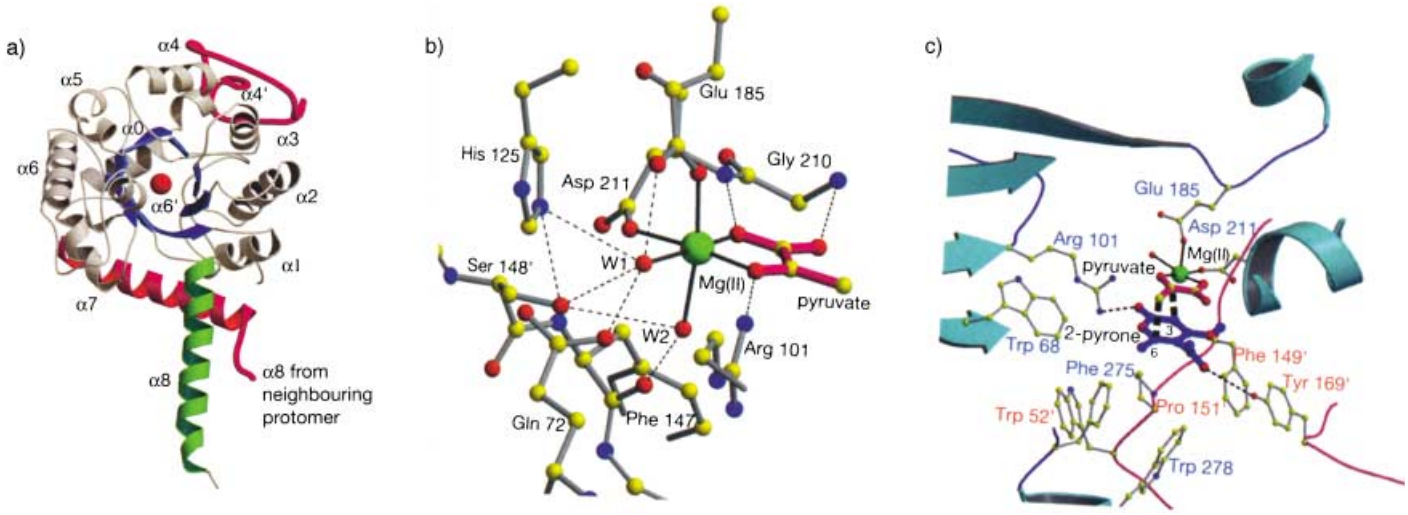
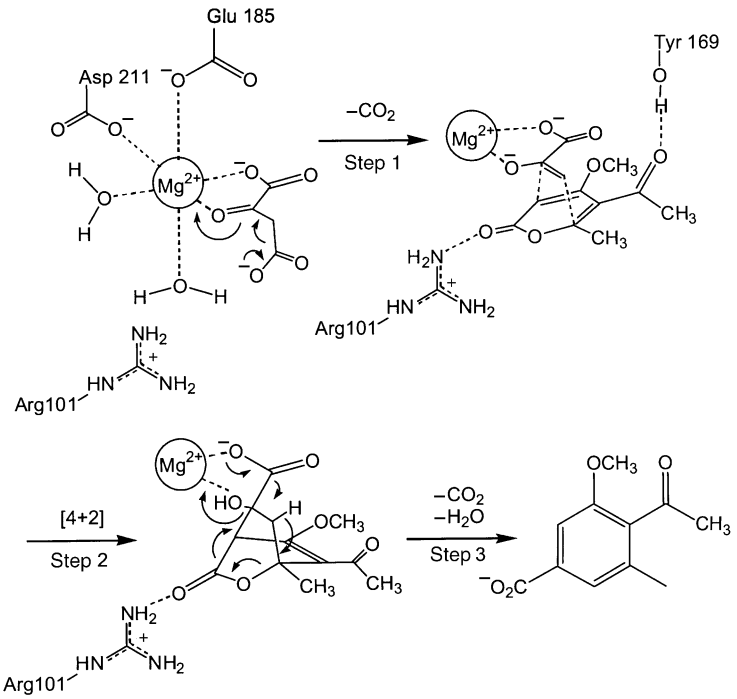


Figure 1. *a) Crystal structure of the macrophomate synthase protomer showing an α-helix swapped $(\beta/\alpha)_8$ barrel fold. The red α8 helix belongs to the neighbouring protomer. b) Active site view showing Mg^{2+} ion coordination. c) Proposed model for the very early transition state of the Diels – Alder reaction after decarboxylation of oxalacetate. Reproduced from ref. [3] with permission from Nature Publishing Group.*

be deducted from this arrangement. Initial decarboxylation of oxalacetate **2** is promoted by the Lewis acidity of the magnesium ion which also stabilises the formed enolate (Step 1, Scheme 2). This complex undergoes reaction with the 2-pyrone **1**, which is fixed in place by two hydrogen bonds and shielded from water by a flexible hydrophobic loop (Step 2, Scheme 2).

These two hydrogen-bonding groups not only act as residues that provide the appropriate orientation for the [4+2] cycloaddition, but also act as electron-withdrawing groups and increase the reactivity of the pyrone **1** in the inverse electron demand Diels – Alder reaction. The modelled position of the pyrone **1** is also confirmed by previous transformations with modified pyrone substrates of different spatial requirements.[8] The bicyclic product **4** can still be hosted by the large cavity of the active site and the final steps towards macrophomic acid (**5**) that prevent product inhibition after the cycloaddition happen without reorientation. *Anti* elimination of water and decarboxylation thus occur in association with Mg^{2+} (Step 3, Scheme 2). These two transformations are rate-limiting in the formation of macrophomic acid **5**.[6] This process might be attributed to the unfavourable intramolecular deprotonation of the pro-*R* hydrogen with the Mg^{2+}-complexed carboxy group (Scheme 2). Two mutants with Arg101 and Tyr169, which are supposed to bind the pyrone **1**, replaced by serine and phenylalanine, respectively, confirm this mechanistic proposal. In both cases the decarboxylase activity towards oxalacetate **2** is still present, while the Diels – Alder product formation is strongly inhibited.



Scheme 2. *Involvement of the identified catalytic residues in the acceleration of macrophomic acid (**5**) formation.*

Macrophomate synthase thus does not exclusively catalyse the Diels – Alder carbon–carbon bond formation, but also a preceding reaction that forces the educts into a reactive conformation. The enzyme therefore acts both as a producer of a reactive substrate and as an entropy trap for the [4+2] cycloaddition. Interestingly, the two other investigated Diels – Alderases also catalyse a transformation preceding the actual pericyclic reaction.[2]

The promotion of pericylic reactions by enzymes has been studied in most detail

for the Cope rearrangement of chorismate to prephenate, which is catalysed by chorismate mutases. In this case, detailed studies of the active sites have led to the mechanistic proposal that the enzymes stabilise a chairlike transition state by electrostatic and hydrogen bonding and thus reduce the influence of the activation entropy.[11] This seems also to be the case for the enzymatically promoted Diels – Alder reaction.

Calculations as well as work with mutants and inhibitors will have to clarify to what extent the Diels – Alder reaction in the enzyme active site of macrophomate synthase does indeed follow a concerted [4+2] cycloaddition pathway. The crystal structure now provides the basis for these investigations on how nature promotes the unusual transformation and adds to our sparse knowledge of enzymatically catalysed pericyclic reactions.

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