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Correlation between sequence, structure and function for trisporoid processing proteins in the model zygomycete *Mucor mucedo*

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HIGHLIGHTS

- TSP1 and TSP2 are two NADP-dependent, trisporoid-binding dehydrogenases.
- TSP1 of *Mucor mucedo* is an aldo–keto reductase with a TIM-barrel structure.
- TSP2 of *Mucor mucedo* is a short-chain dehydrogenase with a Rossmann fold motif.
- Binding pockets are defined by protein families not by substrate structure.
- Active sites of both dehydrogenases contain a lysine and a tyrosine.

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ABSTRACT

Terpenoids, steroids, carotenoids, phytoenes and other chemically related substance groups fulfill multiple functions in all realms of the organismic world. This analysis focuses on trisporoids that operate as pheromones in the phylogenetically ancient fungal group of mucoralean zygomycetes. Trisporoids serve as pheromones for recognizing complementary mating partners and for inducing the differentiation program towards sexual spore formation. Trisporoids are synthesized by oxidative degradation of β-carotene. Structurally, they are related to retinoids in mammals and abscisic acid in vascular plants. In order to evaluate evolutionary relationships between proteins involved in trisporoid binding and also for checking possibilities to recognize functionally related proteins by sequence and structure comparisons, we compared representative proteins of different origins. Towards this goal, we calculated three-dimensional structures for 4-dihydromethyltrisporate dehydrogenase (TSP1) and 4-dihydrotrisporin dehydrogenase (TSP2), the two proteins involved in trisporic acid synthesis that have unequivocally been correlated with their catalytic function for the model zygomycete *Mucor mucedo*. TSP1 is an aldo–keto reductase with a TIM-barrel structure, TSP2 belongs to short-chain dehydrogenases, characterized by a Rossmann fold. Evidently, functional conservation, even implying very similar substrates and identical cosubstrates of enzymes in a single organism, turns out to be essentially independent of basic protein structure. The binding sites for NADP and trisporoid ligands in the proteins were determined by docking studies, revealing those regions affecting substrate specificity. Despite the pronounced differences in amino acid sequence and tertiary structure, the surfaces around the active sites are comparable between TSP1 and TSP2. Two binding regions were identified, one sterically open and a second closed one. In contrast to TSP1, all docking models for TSP2 place the trisporoid into the second, channel-like region.

1. Introduction

1.1. Scientific background

Terpenoids, here the fungal trisporoids, which are sometimes referred to as apocarotenoids according to botanical language usage, fulfill many different functions as communication mediators in all organismic groups. Often, they are hormones like steroids or modulate directly the activity of transcription factors like many retinoids in mammals or abscisic acid in plants, or, as in *Mucor*-like fungi (Schimek and Wöstemeyer, 2006, 2009) and other zygomycetes (Schimek et al., 2003), they act at several developmental levels as pheromones, the so-called trisporoids, mediating the recognition of mating partners. In this organismic group, these molecules represent the general principle of recognition between (+) and (−) mating types and are also believed to fulfill regulatory functions at later stages of sexual differentiation (Schimek and Wöstemeyer, 2006). They also play an important role in parasitic

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interactions in the mycoparasite *Parasitella parasitica* (Schultze et al., 2005), where they form the chemical basis for communication between *Parasitella parasitica* and its various mucoralean hosts (Rosewich and Kistler, 2000; Wöstemeyer et al., 2002). Although the chemical building blocks are identical for all terpenoid molecules – all of them are the result of condensation reactions between isopentenyl pyrophosphate moieties – the corresponding enzymes are often completely different. Thus, it is impossible to identify and clone genes involved in terpenoid processing by making predictions based on sequence similarity. Even within the phylogenetically clearly delimited group of *Mucor*-related fungi and regarding exclusively trisporoid-processing enzymes, predictions for hitherto unknown genes based on sequences of known ones have failed. We therefore analyzed a range of proteins involved in terpenoid metabolism or recognition, in order to understand ligand binding properties in more detail and to define similarities at levels other than primary amino acid sequence between TSP1, TSP2 and well-known terpenoid binding proteins, in order to create alternative search patterns for additional trisporoid converting enzymes.

Towards this aim we used retinoid-binding proteins as well as a plant abscisic acid receptor to point out structural similarities with the trisporoid converting dehydrogenases of zygomycetous origin. The pronounced chemical similarity between all these oxidative carotene degradation products prompted us in a first approach to treat the proteins involved as analogous, if not phylogenetically homologous.

This analysis reveals that the proteins, which we considered in our studies, showed higher sequence and/or structure similarity when they had similar function, independently if they bound the same substrates or not. We found that the two trisporoid converting dehydrogenases from *Mucor mucedo* TSP1 and TSP2 are two proteins with enzymatically identical function and very similar ligands, but with completely different structures.

1.2. Ligands: structures and functions

Retinoids, trisporoids and abscisic acid are structurally similar. Retinoids are monocyclic diterpenes (Fig. 1A). Cell growth, differentiation and apoptosis are just some of the important functions controlled by retinoic acid signaling (Theodosiou et al., 2010). The traditional functions of retinoid-binding proteins are transport and storage of retinoids. In addition, retinoids have the ability to regulate their own availability, metabolism and activity (Noy, 2000; Gudas and Wagner, 2011). Some of them modulate the activity of transcription factors and affect gene regulation by binding to nuclear receptors (Chambon, 1996; Pfahl and Chytil, 1996).

In addition to their role as mediators of sexual development in zygomycetes, trisporic acid (Fig. 1B) and some of its precursors positively regulate their own synthesis, too. Moreover, trisporic acid and possibly other trisporoids are secreted by the mucoralean biotrophic mycoparasite *Parasitella parasitica*, thus mimicking mating partners and consequently attracting host hyphae followed by cytoplasmic fusion (Schultze et al., 2005). The general trisporoid structure in Fig. 1B (Schimek and Wöstemeyer, 2009) shows that the compounds are distinguished by the substituents pattern at C1 and C4. The substituents pattern at the ring (C2, C3, C13) characterizes the derivatives. The TSP1 substrate is 4-dihydromethyltrisporate (R_1=CH_3, R_2=$COOCH_3$, R_3=OH). The TSP2 substrate is 4-dihydrotrisporin (R_1=CH_3, R_2=CH_3, R_3=OH). We concentrated predominantly on the B-series of trisporoids, because *Mucor mucedo* has a pronounced substrate preference for B-trisporoids with a keto-group (=O) at the end of the side chain (C13).

Abscisic acid is a monocyclic sesquiterpene (Fig. 1C), a phytohormone, regulating seed dormancy, plant development, drought tolerance and adaptive responses to environmental stresses (Nishimura et al., 2009; Raghavendra et al., 2010).

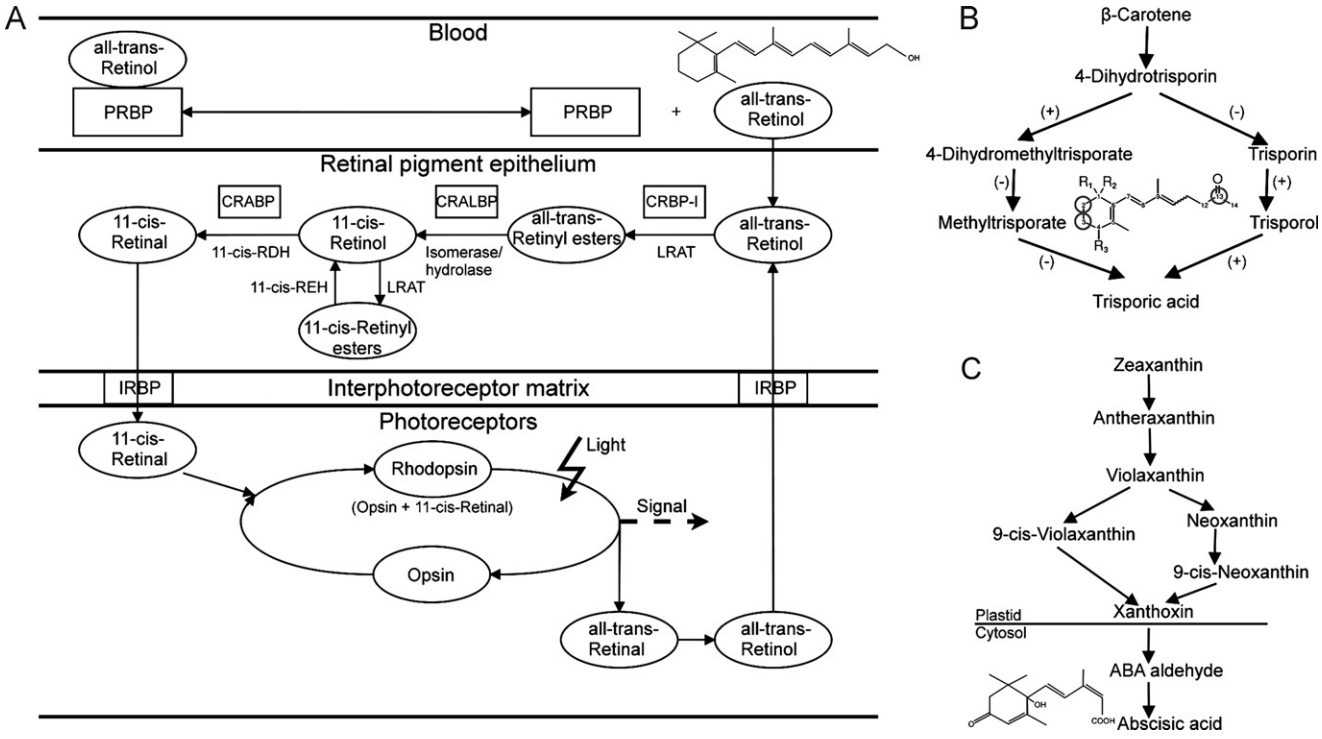

Fig. 1. Structure and biosynthesis of the ligands: (A) retinoids and retinoid-binding proteins in the visual cycle (Noy, 2000) (11-*cis*-RDH, 11-*cis*-retinol dehydrogenase; 11-*cis*-REH, 11-*cis*-retinyl ester hydrolase; LRAT, lecithin:retinolacyltransferase). (B) Trisporoids (Werkman, 1976, modified) 4-dihydrotrisporin: R_1=CH_3, R_2=CH_3, R_3=OH; trisporin: R_1=CH_3, R_2=CH_3, R_3=O; trisporol: R_1=CH_3, R_2=CH_2OH, R_3=O; trisporic acid: R_1=CH_3, R_2=COOH, R_3=O; methyltrisporate: R_1=CH_3, R_2=$COOCH_3$, R_3=O; 4-dihydromethyltrisporate: R_1=CH_3, R_2=$COOCH_3$, R_3=OH. (C) Abscisic acid (Seiler et al., 2011).