\textbf{De novo synthesis of a sunscreen compound in vertebrates}

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\textbf{Introduction}

Current paradigms suggest that gadusol, a sunscreen and antioxidant found in fish, is derived from 4-deoxygadusol, a precursor of mycosporine-like amino acids (MAA) produced by cyanobacteria, fungi, algae, and marine invertebrates. The accumulation of these compounds in marine animals has been proposed to be of dietary or symbiotic origin. Here, we report that gadusol is synthesized de novo in zebrafish (Danio rerio) from a pentose phosphate pathway intermediate, sedoheptulose 7-phosphate, by the sugar phosphate cyclase (SPC) 2-epi-5-epi-valiolone synthase (EEVS) and a methytransferase/oxidoreductase (MT-Ox). This is the first example of a SPC in vertebrates.

\textbf{Sugar phosphate cyclases and their enzymatic reactions}

\textbf{Experiments}

- A phylogenetic analysis of SPCs.
- Zebrafish genes were codon optimized and expressed in Escherichia coli and enzyme assays were performed.
- Transcription levels of each gene were measured in zebrafish embryos.
- Gadusol was isolated from zebrafish embryos at 72 hpf and the compound was analyzed by HPLC, MS, and NMR (NMR not shown).
- Heterologous expression of EEVS and MT-Ox genes in yeast was carried out in S. cerevisiae BY4742 pan1Δ::KanMX4 trp1Δ::URA3 a strain and gadusol production was analyzed by UV, HPLC, MS, and NMR (NMR not shown).
- The UV exposure experiments were performed on wild-type and rad11a yeast mutant strains using a 302 nm lamp.

\textbf{Results}

<table>
<thead>
<tr>
<th>Protein phylogeny of the vertebrate EEVS and sugar phosphate cyclases</th>
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<tbody>
<tr>
<td>Bacterial EEVS</td>
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<tr>
<td>Stramenopile EEVS</td>
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<tr>
<td>Ascomycota DDGS</td>
</tr>
<tr>
<td>Basidiomycota DDGS</td>
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<tr>
<td>Archaeal DHQS</td>
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<tr>
<td>DHQS like</td>
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</tbody>
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\textbf{Conserved animal genome organization}

- Both genes were codon optimized for overexpression in Escherichia coli.
- Enzymatic products were analyzed via MS and NMR (NMR not shown).

\textbf{Gadusol was identified by HPLC and MS from Zebrafish embryos and Yeast}

- Gadusol was identified in:
  1. 72 hpf zebrafish embryos
  2. Gadusol-producing S. cerevisiae

- The S. cerevisiae rad11a mutant is UV sensitive. This yeast strain was treated with gadusol through suspension in a supernatant from a gadusol-producing S. cerevisiae. After the gadusol treatment is applied, the mutant can survive exposure to UV at 302 nm.
- The same effect is seen when gadusol is applied to S. cerevisiae that has a functional rad11 gene.

\textbf{Conclusions}

Gadusol, previously thought to be accumulated through the diet of marine fish, is actually produced de novo in zebrafish. A sugar phosphate cyclase, 2-epi-5-epi-valiolone synthase, and a methytransferase/oxidoreductase are responsible for producing gadusol. Genetic data suggests that non-marine mammalian vertebrates (birds, amphibians, reptiles) also produce gadusol. The function of gadusol in vertebrates remains unknown. The genes responsible for gadusol production were inserted into S. cerevisiae and gadusol was produced. Gadusol acted as a sunscreen for the UV-sensitive S. cerevisiae rad11a.

\textbf{Acknowledgements}

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\textbf{References}