Mechanism of the Isomerization of Isopentenyl Pyrophosphate in *Rhodotorula rubra*¹

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Stereospecifically labeled mevalonic acid was incorporated into the carotenoids of *Rhodotorula*. The randomized results are discussed in relation to mechanisms proposed for the conversion of isopentenyl pyrophosphate to dimethylallyl pyrophosphate and the prenyl transferase enzyme system to *Rhodotorula rubra*.

Several mechanisms have been proposed for the isomerization of isopentenyl pyrophosphate (IPP) to dimethylallyl pyrophosphate (DMAPP). A concerted mechanism involving the attachment of a thiol group of the isomerase to C3 of IPP has been proposed by Lynen et al. (4). Others (6) have proposed, instead, a nonconcerted reaction involving the protonation of the methylene group of IPP following by the formation of a carbonium ion.

Tefft et al. (7) studied the stereochemistry of torularhodin biosynthesis by the use of (2-¹⁴C, 2⁻H₂)-labeled mevalonic acid (MVA) incorporated into torulene and torularhodin by *Rhodotorula rubra* and *R. glutinis*. It was found by this technique and by a decarboxylation reaction that the gem dimethyl groups of the acyclic part of torulene were not randomized.

The lack of randomization in the formation of torularhodin, and also necessarily in the formation of DMAPP, led these authors to propose a concerted rather than a multi-step mechanism for the formation of DMAPP from IPP. Through thorough study of the stereochemistry of the IPP-DMAPP isomerization, Cornforth et al. (1) favored a concerted reaction. It can be argued, however, that the results of Tefft et al. (7) would be consistent with the nonconcerted mechanism if the effect of the proposed carbonium ion intermediate were minimized by the lack of a significant back reaction from DMAPP to IPP.

A number of authors have studied the incorporation of (2-¹⁴C, (2R)-2⁻H₂]MVA and (2-¹⁴C, (2S)-2⁻H₂]MVA into terpenoids. The plant sterols and carotenoids showed a lack of specificity, whereas stereospecific labeling was observed in animal systems (2, 5). The cause of this lack of stereospecificity was explained by the differentially slower effective rate of the prenyltransferase enzyme mediating the head-to-tail condensation of molecules of IPP and DMAPP in plants (3).

Experiments were designed in which [2-¹⁴C, (2R)-2⁻H₂]MVA and [2-¹⁴C, (2S)-2⁻H₂]MVA were incorporated into the medium of *R. rubra* and the pigments were extracted and purified as described previously (7). These experiments were conducted to characterize the yeast IPP isomerase system. The results from these experiments showed nearly the same atomic ratio for each of the isolated carotenoids regardless of substrate utilized (Table 1). If one considers all the possible ratios that might be expected from this experiment, the results would range for both R and S from 8/8 to 3/8 for torulene and 7/8 to 2/8 for torularhodin. One would expect a completely randomized system to show ratios between the expected range, and this result was obtained. Randomization should not be a factor once the carotenoids are formed, and stepwise changes should be observed. The atomic ratio for the conversion of torulene to torularhodin shows the stepwise change for R, but it is slightly higher for S, probably due to adventitious radioactive compounds included in the crystalline S torularhodin. These results indicated that the prenyltransferase system of *Rhodotorula* resembles that of plants, and therefore a significant equilibrium between DMAPP and IPP exists.

Consequently, the lack of randomization of carbon label in torularhodin (7) must be due to a concerted reaction for this isomerization (IPP → DMAPP) rather than a nonconcerted one involving no back reaction. Thus, the conclusions of Tefft et al. (7) (based on experiments with this experimental system) and Cornforth et al. (1) are substantiated.

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<table>
<thead>
<tr>
<th>Compound</th>
<th>Radioactivity (dpm)</th>
<th>Radioactive ratio 3H/14C</th>
<th>Atomic ratio (3H/14C)</th>
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<tr>
<td></td>
<td>R</td>
<td>S</td>
<td>R</td>
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<tr>
<td>Substrate</td>
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<td>Torulene</td>
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<td>Torularhodin</td>
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<td>7,644</td>
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