Protein prenylation involves the attachment of C15 (farnesyl) or C20 (geranylgeranyl) groups to proteins and is catalyzed by a class of enzymes known as prenyltransferases. The observation that inhibition of Ras farnesylation arrests the growth of tumor cells has been the motivating factor in developing inhibitors of prenyltransferases that can serve as anticancer drugs; currently, several candidates are in Phase 3 clinical trials. Mechanistic analysis of enzymatic reactions can provide insights that are potentially useful in drug design. Since enzymes must bind to the transition state (TS) with greater affinity than the ground state, molecules that mimic the structure of the TS will have the highest possible affinity for the enzyme. Enzyme inhibitors based on such principles can manifest extraordinary affinity and selectivity; accordingly, we are interested in determining the TS structure for the reaction catalyzed by protein farnesyltransferase. Moreover, the detailed knowledge gained in these experiments should increase our understanding of how enzymes activate isoprenoid diphosphates for subsequent reaction. Such knowledge could be particularly useful for manipulating the reactivity of prenyltransferases and the closely related terpene cyclases for biotechnology purposes.

At present, the most reliable method to determine TS structure is through the use of computational methods in conjunction with experimentally measured kinetic isotope effect (KIE) measurements. While a large body of literature exists for KIE measurements performed on benzyl systems, reports for related allylic systems are sparse. Thus, as a prelude to enzymatic measurements, it was decided to investigate several model reactions, shown in Figure 1, first. Results from such experiments would provide KIE values for limiting associative (SN2) and dissociative (SN1) mechanisms and allow us to validate the computational methods that would be used in the subsequent determination of the enzymatic TS. For a model substrate, dimethylallyl chloride (1) was chosen. Solvolysis of 1 in benzyl alcohol (2) was used as a dissociative model while displacement with triphenylphosphine (5) was employed as an associative model. Kinetic analysis of these reactions revealed that the solvolysis reaction was first order in 1 and zero order in 2, whereas the other reaction was first order in both 1 and 5. To measure the 13C KIEs for the reaction, an NMR method was employed based on the work of Singleton and others; that approach involves the integration of 13C NMR spectra obtained at natural abundance of reactant obtained prior to reaction and after substantial conversion. In the work reported here, the model reactions were performed, monitored by GC, and terminated by vacuum distillation to recover the remaining starting material which was then derivatized by reaction with dimethylmalonate (7) and the resulting product (8) purified by flash chromatography. 13C NMR spectra were obtained and integrated using C-4 as an internal standard. 13C KIEs were calculated from the ratios of peak areas determined from samples of the derivatized starting material (8) before and after reaction. A significant primary 13C KIE at C-1 was measured in the SN2 model reaction (1.040 ± 0.003), whereas a value near unity (0.997 ± 0.003) was observed in the SN1 model reaction.

Figure 1. Model reactions and derivatization chemistry for SN1 and SN2 prenylation reactions.
the TS structures between the yeast and mammalian enzymes that could be exploited for drug design.

In summary, we present here a TS structure for the reaction catalyzed by PFTase that complements X-ray crystallographic studies and provides a clear structural framework for understanding the results of previous mechanistic investigations of this enzyme, including stereochemical and kinetic analyses. The experiments reported here provide powerful insights into an important class of biological reactions through a combination of model chemistry, computation, and kinetic analysis. Finally, it should be noted that the KIE analysis described here was accomplished through experiments performed with stable isotopes and did not require radiolabeled compounds. It is likely that the role of ESI-MS and NMR in KIE analyses of biological processes will continue to grow in the future.

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Supporting Information Available: Details for the synthesis of [1-13C]-GPP, c1 determination, KIE experiments, TS calculations, and data analysis are included. This material is available free of charge via the Internet at http://pubs.acs.org.

References


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Figure 3. Proposed TS structure for PFTase-catalyzed reaction based on calculations using GPP and ethane thiolate. The second isoprene unit is omitted for clarity. Colors: S (orange), C (green), and O (red).