similar systems with optical methods as a function of external magnetic fields. Our results provide independent evidence for the validity of this[14] explanation for the fast occurrence of ^3A, which has been a puzzle and the subject of controversy for some time.[15,16]

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Joachim Bargon

IBM Research Laboratory, San Jose, California 95193

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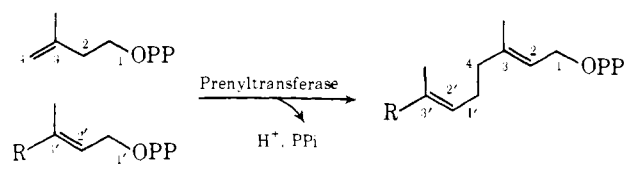
On the Mechanism of the Prenyltransferase Reaction. Metal Ion Dependent Solvolysis of an Allylic Pyrophosphate[1]

Sir:

The basic polymerization reaction of polyterpenoid biosynthesis is the condensation of C_4 of isopentenyl PP with the C'_1 of an allylic pyrophosphate with the concomitant elimination of pyrophosphate and the generation of the five-carbon homologue of the allylic substrate. This reaction is catalyzed



by the enzyme prenyltransferase. Recently strong evidence has been presented that this reaction proceeds by an ionization-condensation-elimination mechanism.[2] The enzyme requires a divalent cation, Mg^{2+} or Mn^{2+}, for activity, and, since we have shown the substrates for prenyltransferase bind to the enzyme in the absence of these cations, we have concluded that these ions are required for catalysis rather than binding.[3] Thus, it is possible that the role of metal ions is to assist in ionization of the allylic substrate. This consideration, along with the indication that divalent cations enhance the solvolysis of allylic pyrophosphates,[4] has led us to undertake a more thorough examination of the solvolysis of allylic pyrophosphates in the presence of Mg^{2+} and Mn^{2+}. We have also measured metal ion dependent rates of solvolysis of chrysanthemyl PP which is an analogue of the immediate precursors of both squalene and phytoene.

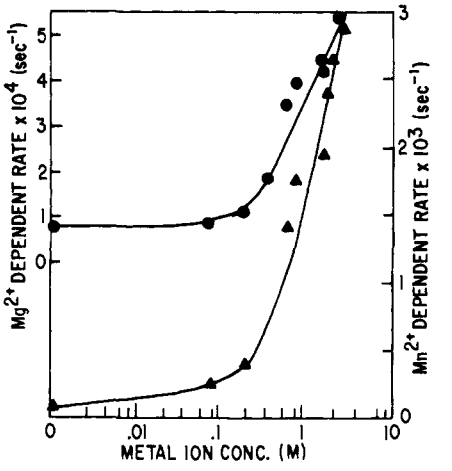


Figure 1. The effect of Mg^{2+} (●) and Mn^{2+} (▲) on the rate of solvolysis of geranyl PP. A constant ionic strength of 4.8 was maintained.[10]

Table I. Effect of Mg^{2+} on the Solvolysis of Chrysanthemyl Pyrophosphate

Mg^{2+}, M[a]	Rate × 10^4, s^{-1}
0	0.28
0.07	2.3
0.29	6.9
0.68	8.3
1.16	8.5
1.53	7.7

[a] A constant ionic strength of 4.8 was maintained.

For measuring rates of solvolysis, the pyrophosphates both labeled on the carbinol carbon with ^3H were sealed in ampules with 1 mL of buffered water containing variable concentrations of either $MgCl_2$ or $MnCl_2$ and NaCl to constant ionic strength.[5] After heating at 55 °C, the ampules were quenched at 0 °C and crushed, and the products of solvolysis extracted into hexane for radioisotope determination. First-order rates of solvolysis were obtained, each rate constant being determined by a set of six to nine time intervals. Rates were calculated from the slope of a least-squares plot of the data with the point of infinite solvolysis being set by acid hydrolysis of a control tube.

Alumina chromatography of the products indicated that 95% were alcohols and the remainder hydrocarbons. Analysis by GLC of the alcohols generated during solvolysis showed that geraniol and linalool were generated in a ratio of 1:5.[9] The proportions of the various products of solvolysis of geranyl PP were independent of the cation used. The products of solvolysis of chrysanthemyl PP were not analyzed.

The rates of solvolysis of geranyl PP at 55 °C as a function of Mn^{2+} and Mg^{2+} concentration are shown in Figure 1. The Mg^{2+}-dependent solvolysis rates of chrysanthemyl PP are in Table I. At metal ion concentration lower than 1 mM, the rates of solvolysis of geranyl PP were not significantly greater than that found in the absence of metal.[12] As the concentration of either divalent cation was increased from 1 to 10 mM, there was a gradual increase in the solvolysis of both substrates. Further increases in cation concentration from 0.07 M to ~1.5 M led to a large increase in the solvolytic rate. The limited solubility of NaCl prevented us from testing all divalent metal ion concentrations at the same ionic strength. The highest concentrations of Mg^{2+} were tested at an ionic strength of 12, and the rates observed were 1.8, 1.8, and 2.0×10^{-3} s^{-1} for 2.0, 2.3, and 2.63 M Mg^{2+}, respectively.[13] Thus, at these high

concentrations of metal ion the rates of solvolysis are leveling off.

The enhanced rate of solvolysis as a function of cation concentration strongly suggests that the solvolysis results from the formation of a dissociable complex between geranyl PP or chrysanthemyl PP and a divalent cation. From the dissociation constants of Mg geranyl PP and Mn geranyl PP, one can calculate that at 1 mM metal ion concentration essentially all of the geranyl PP exists as the monometal salt.[15] Yet, at this concentration the rate of solvolysis of the allylic pyrophosphate is not significantly above control values. Consequently, the formation and decomposition of another, most likely M_2-geranyl PP rather than M_1-geranyl PP, must be responsible for the enhanced solvolysis we have observed. The shape and position of the two curves are very similar. Thus the fivefold greater rate observed with Mn^{2+} could be attributed to the stability of the metal ion allylic pyrophosphate complex rather than to a difference is dissociation constants. The dissociation constants for the formation of M_2-geranyl PP can be estimated from the curves graphed in Figure 1 and are ~0.7 M.

The requirement for a second divalent cation for promotion of the solvolysis of an allylic PP is of particular interest since we have shown that two metal ions are bound per catalytic site of prenyltransferase when substrate is present.[3] Since the divalent cations are required for catalysis by prenyltransferase, we would like to postulate that the two metals bound with the allylic substrate serve to ionize this substrate and in so doing initiate the catalytic sequence of prenyltransferase and that one of the functions of the enzyme is to properly orient the metals and allylic pyrophosphate in the catalytic site.

The demonstration that the rate of chrysanthemyl PP solvolysis is enhanced by a divalent cation strengthens the suggestion that enzymes that use cyclopropylcarbinyl PP systems as substrates (phytoene and squalene synthetases) also proceed by a reaction mechanism that is initiated by ionization.[17,18]

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David N. Brems, Hans C. Rilling*

Department of Biochemistry
University of Utah College of Medicine
Salt Lake City, Utah 84112

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Selenium-77 Relaxation Time Studies. Considerations Regarding Direct Observation of Selenium Resonances in Biological Systems

Sir:

Selenium has for a number of years attracted biological interest, most notably for the selenosis that adversely affects livestock and its implication as a carcinogen. More recently it has been linked to the metabolism of vitamin E and the prevention of liver necrosis.[1] Its importance has prompted us to consider selenium NMR as a probe for investigating its role in these biological systems. Appealing features of ^{77}Se NMR are the pronounced sensitivity of the nuclear shielding to the chemical environment (the established shift range is over 1500 ppm)[2] and the stereospecificity of the coupling constants.[3] The technique therefore has great potential to afford valuable new information to supplement the present knowledge of this essential nutrient.

The low natural abundance (7.5%) and relative NMR sensitivity (6.97×10^{-3} that of the proton) of the ^{77}Se isotope combined with the concentration problems which one generally must be concerned with in biological applications pose significant experimental difficulties. These problems can be minimized by the use of Fourier transform (FT) NMR through which substantial gains in the signal-to-noise ratio over conventional NMR may be realized.[4] To take full advantage of the FT method a knowledge of the inherent spin-lattice relaxation time, T_1, of the selenium nucleus is desirable since it influences the time duration of the experiments (via the recycle time between pulses).[5] The T_1s also provide valuable information concerning molecular dynamics. In general, several mechanisms are known to contribute to the relaxation of spin $\frac{1}{2}$ nuclei and the possible variation in the magnitude of T_1 is considerable,[5b,6] but there is no information to date relative to selenium-77. Accordingly, we have initiated a study of ^{77}Se spin-lattice relaxation in some model compounds.[7]

All samples were degassed by a series of freeze-pump-thaw cycles before sealing under dynamic high vacuum. The T_1 and nuclear Overhauser effect (NOE) enhancements were determined in a standard manner.[6a,8,9] The select group of compounds investigated ($(CH_3)_2Se$, CH_3SeH, $(CH_3)_2Se_2$, $(C_6H_5)_2Se_2$, and $(C_6H_5CH_2)_2Se_2$) are representative of the three most common chemical environments in which selenium is expected to be found in biological systems.

The NMR parameters for the series of compounds are shown in Table I. For all compounds an Arrhenius plot of the observed relaxation rate, R_1*, vs. the reciprocal temperature exhibited a negative slope. The linearity of these plots (Figure 1) along with the lack of a Se-{^1H} NOE enhancement points