It is worthwhile to note that these enoyl-CoA reductases typically incorporate solvent at the α-carbon in a syn fashion,[4,7,8] with the only exceptions (i.e., anti addition) being found in yeast[9] and *Escherichia coli*.[10] The enoyl-CoA reductase which catalyzes the final step in the formation of the cyclohexanecarboxylic acid now represents a third exception although clearly it has a very different metabolic role to these enzymes.

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Chaotropic Salt Effects in a Hydrophobically Accelerated Diels-Alder Reaction

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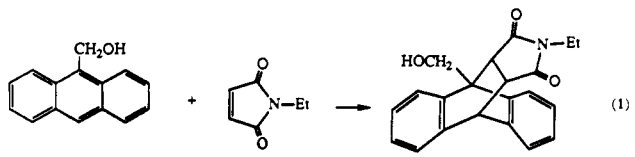
We have described the remarkable acceleration of some Diels-Alder reactions seen in water solution[1,2] and, as well, the changes in product selectivity.[2,3] Grieco has applied these water effects on selectivities to a number of cases of synthetic interest.[4] Although acids,[5] including hydrogen bonders,[6] can accelerate some Diels-Alder reactions, we offered several arguments that in our cases the hydrophobic effect is of principal importance. First of all, in reaction 1 the rate is slower in methanol than in less polar solvents, because an intracomplex hydrogen bond between the reactants is broken in the polar methanol.[1] However, despite this



(1)

the reaction is greatly accelerated in water, as the data in Table I show. Furthermore, recently Schneider has shown[7] that a Diels-Alder reaction rate as a function of solvent follows a "solvophobicity" parameter related to hydrocarbon solubility, not a polarity parameter. Hydrophobic packing of the two reactants in reaction 1, for instance, is the explanation that is consistent with both of these findings.

Some special salt effects on the reaction rates[1,2] and selectivities[2,3] of reaction 1 and other Diels-Alder reactions also argued for a hydrophobic packing effect in water solvent (similar salt effects are consistent with hydrophobic packing in the transition state for the benzoin condensation in water[8]). Reaction 1 in water is faster when LiCl is added, but slower when guanidinium chloride is added (Table I). LiCl is a "salting out" salt that increases the hydrophobic effect, by electrostriction of water that decreases the solubility of hydrocarbons and thus promotes their association.[9] Guanidinium chloride is a common denaturant of proteins and nucleic acids; such substances are sometimes called "chaotropic" agents.[9] They decrease the association of hydrocarbon residues in water and act as "salting in" materials that increase the water solubility of hydrocarbons such as butane or benzene.[9,10] Although it is usually thought that this occurs because materials like guanidinium ion or urea break up water structure, overcoming the electrostrictive effect of the chloride ion, we have recently shown that a different mechanism is responsible for the effects of such "salting in" denaturants.[11]

Table I. Second-Order Rate Constants for the Addition of *N*-Ethylmaleimide to Anthracene-9-carbinol (Reaction 1) in Various Media at 45 °C

solvent	$k_2 \times 10^3$, M^{-1} s^{-1}	k_{rel}
2,2,4-trimethylpentane[a]	8.0 ± 0.7	0.035
methanol[a]	3.4 ± 0.3	0.015
water[a]	226 ± 7	1.000
water[b]	230 ± 2	1.000
water + LiCl (4.86 M)[c]	560 ± 54	2.5
water + LiCl (4.0 M)[b]	498 ± 28	2.2
water + GnCl (4.86 M)[c]	72 ± 10	0.32
water + GnCl (2.0 M)[b]	129 ± 6	0.56
water + $LiClO_4$ (4.0 M)[b]	157 ± 3	0.68
water + $GnClO_4$ (2.0 M)[b]	86 ± 4	0.37

[a] Reference 1. [b] This work. All data are the average of at least three runs, in most cases of five runs. Reactions were carried to at least 7 half-lives. [c] Reference 2 and Ph.D. Thesis, D. Rideout, Columbia University, 1982.

The correlation between rate effects in the Diels-Alder reaction and salting out/in properties of the materials added might be a coincidence. Symons has pointed out the changes in water properties that occur when different types of ions are added.[12] In particular, he proposes that small coordinating cations bind the unshared electron pairs of water and lead to an excess of hydrogens available to hydrogen bond, while small coordinating anions bind to water protons and decrease its ability to hydrogen bond other substances. Our data on LiCl vs guanidinium chloride could be explained if Li^+ speeds reaction 1 and Cl^- slows it (but not as much) by the Symons effect and if hydrogen bonding by solvent is the principal modifier of the reaction rates.[13]

We have now tested this hypothesis. The hydrogen-bonding explanation would require that $LiClO_4$ speed the reaction even more than LiCl does; the salting-in explanation goes in the other direction,[8] contrasting the electrostrictive Cl^- with the charge-dispersed ClO_4^-. We find that $LiClO_4$ indeed slows reaction 1 (Table I), consistent only with the hydrophobic explanation. As an additional check, guanidinium perchlorate has an even larger salting-in effect than does guanidinium chloride;[14] we find that it slows reaction 1 even more (Table I). If the hydrogen-bonding explanation were operative, the order between these two salts should have been the reverse of that we observe.

These data confirm the idea that hydrophobic packing effects contribute to the Diels-Alder reaction in water, just as they do in the benzoin condensation. It seems likely that many organic

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reactions will show such effects. Furthermore, the data confirm the utility of chaotropic salts in diagnosing the presence of the hydrophobic effect in such reactions. As in the present case, it is clearly desirable to have such data for a variety of salts to exclude other explanations.

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Mechanism-Based Inactivation of Pyruvate Formate-Lyase by Acetylphosphinate: Evidence for Carbon-Phosphorus Bond Cleavage

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Pyruvate formate-lyase (EC 2.3.1.54; PFL) is an oxygen-sensitive enzyme (MW 170 kDa; dimer) that replaces pyruvate dehydrogenase during anaerobic glucose metabolism in *Escherichia coli* and a number of other prokaryotes.[1] The enzyme converts pyruvate and coenzyme A (CoA) to formate and acetyl coenzyme A via a ping-pong kinetic mechanism with covalent acetyl-PFL as an isolable intermediate.[2] PFL has no known organic or metal cofactor but does contain a protein-based organic free radical which is essential for the catalysis of this remarkable transformation.[3,4] A mechanism styled after a Minisci-type homolysis of pyruvate esters[5] and initiated by the enzyme radical (X•) has been proposed (Scheme I; path A).[6,7] We report here that acetylphosphinate (**1**), a pyruvate analogue, is a mechanism-based inactivator of PFL and present evidence for a novel carbon-phosphorus bond cleavage during the inactivation process.

Hypophosphite ($H_2PO_2^-$) has been the most thoroughly studied inactivator of PFL.[3,6-8] It inactivates both free PFL and acetyl-PFL,[9] quenches the enzyme radical with kinetics similar to the kinetics of inactivation, and covalently labels the enzyme. Both the reaction of acetyl-PFL with formate and the inactivation by hypophosphite are subject to primary kinetic isotope effects consistent with homolytic cleavage of the carbon-hydrogen and phosphorus-hydrogen bonds generating formate radical anion and hypophosphoryl radical anion, respectively.[6] Recent work has suggested that inactivation of acetyl-PFL by hypophosphite may involve carbon-phosphorus bond formation via the hypophosphoryl radical anion.[7,8]

On the basis of the above findings and the structural similarities of **1** to pyruvate, the possible inactivation of PFL by **1**[10] was examined by standard methods (Figure 1).[6] Incubation of PFL with **1** (45 μM) alone afforded inactivation kinetics which were biphasic in nature, comprising an initial rapid phase followed by a slow phase. In contrast to the result with hypophosphite,[9] preincubation of PFL with saturating levels of pyruvate (10 mM) afforded essentially complete protection from inactivation by **1**. Addition of formate (5 mM) or CoA (55 μM) resulted in a dramatic enhancement of inactivation by **1** (25 μM) with clean first-order kinetics ($t_{1/2} \sim 2.2$ min). Inactivated PFL did not regain activity after a 10 000-fold dilution in anaerobic buffer containing 10 mM pyruvate. A K_I of 303 ± 65 μM and k_{inact} of 5.8 ± 0.8 min^{-1} were also determined[15] (Figure 1, inset). All but

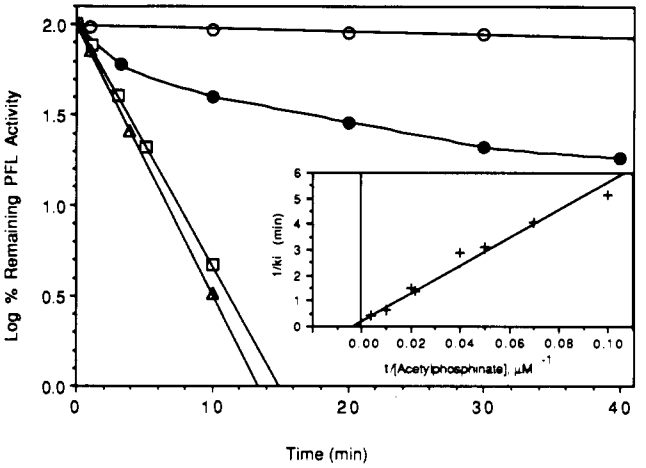
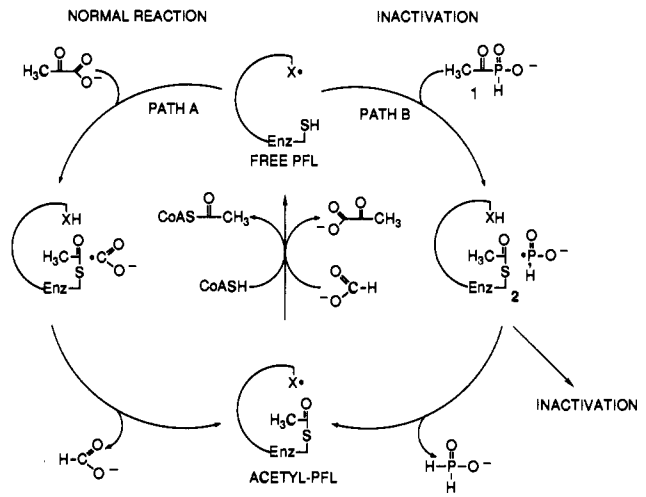


Figure 1. Time course for inactivation of PFL by acetylphosphinate (**1**). Active PFL (4 units) in anaerobic, 100 mM Tris buffer pH 8.1 (500 μL) at 25 °C was incubated with **1** under the following conditions: (●) 45 μM **1** alone; (○) preincubation with 10 mM pyruvate before addition of 45 μM **1**; (□) 55 μM CoA or (△) 0.5 mM formate and 25 μM **1**. Aliquots (20 μL) were removed at indicated times and quantitated for residual PFL activity.[6] Inset: Determination of K_I and k_{inact} for **1**. Rates of inactivation were determined in the presence of 55 μM CoA and 10-250 μM **1**. The line plot was determined by nonlinear regression.[15]

Scheme I



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