Chapter 13

Enzymatic Catalysis in Organic Media: Fundamentals and Selected Applications

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ABSTRACT

The use of enzymes in organic solvents significantly extends conventional aqueous-based biocatalysis. This field is reviewed with respect to the salient factors that govern enzymatic catalysis in nonaqueous media. Specific examples of peroxidase catalysis in organic media are discussed (e.g., phenolic polymerizations and enzymic temperature abuse sensors). Given that membranous parts of a cell are highly nonaqueous in character, the study of enzymes in poorly hydrated media may provide clues as to the function of membrane-bound enzymes and their response to different gravitational forces.

INTRODUCTION

Water is a poor solvent for nearly all applications in industrial chemistry. Most organic compounds of commercial interest are very sparingly soluble and are often unstable in aqueous solutions. Chemists have realized the limitations of aqueous-based catalysis and have long ago replaced water with more suitable organic solvents. Unlike chemical processes, conventional biocatalysis has been performed in aqueous solutions. This is due mainly to the preconceived notion that nature intended enzymes to be catalytically active in water and that organic solvents serve only to destroy the catalytic power of enzymes. This notion is simplistic and incorrect. Enzymes are used routinely in water containing a small proportion of a water-miscible organic cosolvent (for reviews see Butler, 1979). Enzymes are also used in the soluble aqueous components of both biphasic aqueous-organic systems (Lilly, 1982; Carrea, 1984; Halling, 1987) and inside reverse micelles dissolved in nonpolar solvent (Martinek et al., 1986; Luisi and Laane, 1986). Finally, enzymes are employed as insoluble catalysts in nearly anhydrous media (Dordick, 1989; Klibanov, 1986).

This chapter highlights fundamental studies carried out in our laboratory performed with peroxidases in nonaqueous media. These include free-energy correlations of substrate and solvent hydrophobicities with catalytic activity, and applied studies including peroxidase-catalyzed polymerization of phenols and the development of an enzyme-mediated temperature abuse sensor.

In these studies the organic solvent system can be defined as being "monophasic." Such a system lacks a distinct aqueous phase and hence involves insoluble enzyme preparations (enzymes are insoluble in organic solvents) in both nearly anhydrous media and water-miscible organic-aqueous cosolvents employing the organic solvent as the predominant system component. Unlike other organic solvent reaction systems, enzymes in monophasic systems are not in direct contact with a bulk water phase and exist in an environment vastly different from an aqueous milieu. Potential advantages of using enzymes in monophasic organic media are highlighted in Table I.

Table I. *Potential Advantages of Employing Enzymes in Organic as Opposed to Aqueous Media* (Dordick, 1989; Klibanov, 1986)

1. Increased solubility of non-polar substrates
2. Shifting of thermodynamic equilibria to favor synthesis over hydrolysis (e.g., syntheses of esters, peptides, lactones)
3. Reduction in water-dependent side reactions
4. Immobilization is often unnecessary; enzymes are insoluble in organic solvents. Recovery of enzymes is possible by simple filtration
5. Ease of product recovery from low boiling solvents
6. Enhanced thermal stability of enzymes; water is required to inactivate enzymes at high temperatures
7. Elimination of microbial contamination
8. Potential of enzymes to be used directly within a chemical process

PARAMETERS WHICH GOVERN ENZYMATIC CATALYSIS IN ORGANIC MEDIA

The application of enzymes in nonaqueous media is a new endeavor. This is especially the case in monophasic

organic systems where the enzyme does not exist in a predominantly aqueous solution. Thus conventional kinetic and mechanistic comparisons to aqueous-based enzymology are not straightforward. In particular, three variables exert profound control over enzymatic catalysis in organic media: the water content of the organic solvent system, the physical nature of the biocatalyst, and the nature of the organic solvent itself.

Role of Water for Enzymatic Catalysis in Organic Media

Water is vital for enzymatic activity. Nearly all the noncovalent interactions that are required for correct protein structure and function depend on the direct or indirect role of water. Replacing water with a completely anhydrous organic solvent would destroy catalytic activity by destroying the delicate balance of forces that holds proteins together. While the need for water is clear, the amount of water required in enzymic systems is less well understood. Recent evidence suggests that an enzyme molecule in aqueous solutions becomes fully hydrated when surrounded by a few layers of water (Zaks and Klibanov, 1988). Estimates of the thickness of the water layer are subject to speculation. One common hypothesis is that the enzyme molecule requires a small hydration layer that acts as the primary component of the enzymic microenvironment. This layer acts as a buffer between the enzyme surface and the bulk reaction medium. In this case, it is highly improbable that an enzyme molecule will be affected by the water phase beyond the hydration layer (e.g., the bulk aqueous phase) unless significant alteration of the enzymic microenvironment is caused by the bulk reaction medium (Zaks and Klibanov, 1985).

If one extends this argument to enzymatic catalysis in organic media, then it should be possible to keep an enzyme fully hydrated and active with a small amount of water, perhaps as little as that bound on selective sites of the protein. The bulk reaction medium, in this case, could be nearly anhydrous.

The actual amount of water required for catalytic activity does appear to be enzyme dependent. For example, chymotrypsin suspended in octane remains catalytically active even if only 50 molecules of water per enzyme molecule are present (Zaks and Klibanov, 1986). Horseradish peroxidase, however, often requires 0.25% (v/v) water or more for activity in hydrocarbon solvents (Kazandjian et al., 1986). Such a great variation in water requirements for enzymes in organic media may be a consequence of the method of correlating activity to the water content of the solvent. It has recently been shown that measurements of the amount of enzyme adsorbed to an enzyme in organic solvents, as opposed to the water content of the solvent itself, afford a better correlation to enzyme activity (Zaks and Klibanov, 1988). Clearly, each enzyme must be examined at various levels of hydration in organic solvents, and it is very likely that enzymes previously reported to be inactive in organic media were actually employed at non-optimal levels of hydration.

Biocatalyst Preparation

The insolubility of enzymes in monophasic organic systems has a controlling influence on the kinetics of enzymatic catalysis in organic media: insolubilized enzymes are subject to intraparticle and external diffusional limitations which can mask the true, intrinsic kinetics of catalysis. Methods used to eliminate intraparticle and external diffusional limitations in conventional heterogenous chemical systems have been effectively applied to enzymatic catalysis in organic media.

One such method is to spread the enzyme onto materials with large surface areas, such as glass beads (Kazandjian and Klibanov, 1985; Kazandjian et al., 1986). Because enzymes are insoluble in organic media, the bound enzyme will have no propensity to desorb from the glass surface, and facile immobilization is feasible without the cumbersome covalent attachment or entrapment procedures required in conventional aqueous-based systems. The enhancement of substrate diffusion by this method is illustrated in the horseradish peroxidase-catalyzed oxidation of *o*-phenylenediamine carried out in our laboratory. In benzene the oxidation rate is 160-fold faster following deposition of the enzyme onto 75-150 μm glass beads. Peroxidase is clearly highly active in organic media and is susceptible to internal diffusional resistances. Once the enzyme is deposited onto the glass beads, internal diffusion may still be rate limiting. Adsorption of the enzyme forms multiple layers on the support. This "overcrowding" can be relieved, theoretically, if monolayer enzyme coverage is obtained. Using peroxidase-catalyzed oxidation of *p*-cresol in dioxane, we have shown that the rate of catalysis greatly depends on the enzyme loading onto the glass beads. Once a monolayer of enzyme has been approximated, a reduction in the enzyme loading onto the beads has no further effect on reaction rates (Ryu et al., 1989). The value of optimal loading (0.1 mg peroxidase/g glass beads and a bead size of 75-150 μm) correlates well with the expected surface area coverage of peroxidase on this size particle as calculated from the hydration radius of the enzyme (Cantor and Schimmel, 1980).

External diffusion can be optimized by simply increasing agitation. Unlike aqueous or liquid-liquid biphasic systems (Lilly, 1982), high agitation in monophasic organic solvent reactions will not lead to shearing of the enzyme molecules, perhaps because the enzymes are insoluble and incapable of dissolving and denaturing at an interface. It should be stressed that external diffusion in monophasic solvent systems is defined by the static boundary layer around the solid enzyme particles. Because no separate aqueous phase exists in such systems, diffusional limitations that result from inadequate partitioning of hydrophobic substrates through a well-defined aqueous layer are uncommon.

Effect of Organic Solvent on Biocatalysis and Choice of Solvent

The nature of the organic solvent affects enzymatic catalysis in three distinct ways. First, the solvent can cause inhibition or inactivation by directly interacting with the enzyme. In this case, the solvent alters the native conformation of the protein by disrupting hydrogen bonding and hydrophobic interactions, thereby leading to reduced activity and stability (Cremonesi et al., 1974). This is a major problem with soluble enzymes in biphasic systems and in aqueous systems with low concentrations of water-miscible solvents (Williams et al., 1987). The insolubility of enzymes in monophasic organic solvents, however, appears to prevent the enzyme from undergoing severe inactivation in the presence of the solvent. This is similar to the phenomenon observed in stabilization of enzymes to thermoinactivation by immobilization (Klibanov, 1983). The inability of enzymes to dissolve in organic solvents keeps the enzyme locked into its proper orientation for catalytic activity. In fact, many enzymes in monophasic organic media are stable to vigorous vortexing (Kazandjian et al., 1986).

A second way solvents can affect enzymatic activity is by interacting with diffusible substrates or products of the reaction. For example, chloroform, a good phenoxy radical quencher, significantly diminishes the catalytic activity of peroxidase-catalyzed oxidation of phenols (Kazandjian et al., 1986), a reaction that is initiated by the enzymatic generation of phenoxy radicals in the presence of hydrogen peroxide.

Finally, the organic solvent may interact directly with the essential water in the vicinity of the enzyme. While this interaction may not directly affect the enzyme, it does have major consequences for catalysis. Specifically, highly polar solvents are capable of solubilizing large amounts of water and will tend to strip away the layers of essential water from the enzyme, causing inactivation. Conversely, hydrophobic solvents are less able to remove or distort the enzyme-associated water and are less likely to cause inactivation.

Several factors must be taken into account in determining which solvent is most appropriate for a given reaction. First and foremost is the compatibility of the solvent with the reaction of interest. For example, sugars are soluble only in hydrophilic, water-miscible solvents such as pyridine or dimethylformamide. The use of hydrophobic, water-immiscible solvents is impractical for enzyme-catalyzed sugar modifications, as there would be no interaction between the insoluble substrate and insoluble enzyme. Similarly, the compatibility of the reaction products with the solvent is important. Polar products tend to remain in the vicinity of the enzyme and can cause product inhibition or can undergo unwanted side reactions. This latter consequence has been observed with polyphenol oxidase catalysis in hexane. The polar quinone products are insoluble in hexane and undergo unwanted polymerization in the water layer around the enzyme (Kazandjian and Klibanov, 1985). In addition, this polymerized material fouls the enzyme particles and reduces their catalytic activity. In the more polar chloroform, the quinones partition into the bulk solvent and do not inactivate the enzyme.

Another factor is that the solvent selected must be inert to the reaction. Transesterification reactions involve a nucleophilic attack by an alcohol on an ester to produce a second ester. If the solvent is also an ester, then high conversions of an unwanted solvent-based ester will form at the expense of the desired product. Similar results will occur if the solvent is an alcohol. There are cases, however, where an ester or alcohol solvent is used for transesterification reactions in order to employ high concentrations of reactants and to push the equilibrium controlled reaction to completion (Cesti et al., 1985). Additional factors which may influence the choice of solvent include solvent density and viscosity, surface tension, toxicity, flammability, waste disposal, and cost.

Assuming that a large number of potential solvents does exist, the choice of the appropriate solvent(s) is not straightforward and, until now, is generally arbitrary. This situation is being addressed by a number of research groups. Specifically, models have been developed which correlate the physicochemical characteristics of individual solvents with observed enzymatic activity. One model advanced by Brink and Tramper (1985) for the epoxidation of propene is the use of the Hildebrand solubility parameter. Solvents with low polarities and high molecular weights (> 200 D) were found to support catalytic activity. Similarly, Laane and coworkers (1987a and b) found a quantitative correlation between the hydrophobicity of the solvent and the activity retention of the biocatalyst. They reasoned that solvents with high values of log P, where P is defined as the partitioning of a given solvent between water and 1-octanol in a two-phase system (Equation 1), are optimal for enzymatic activity.

$$P = [\text{Solvent}]_{\text{octanol}} / [\text{Solvent}]_{\text{water}} \qquad (1)$$

Values of log P can be easily calculated from hydrophobic fragmental constants (Rekker and de Kort, 1979). Correlations with solvent molecular weight are unnecessary. Values of log P in excess of 4 (e.g., decanol, hexadecane, diphthalate esters) supported high degrees of activity retention in the propene epoxidation reaction. Solvents with log P values below 2 (e.g., short chain alcohols, water-miscible solvents, short chain esters and ethers) were unsuitable for biocatalysis.

A great deal of confusion persists with respect to the definition of catalytic activity in the log P correlations. Strict distinctions must be made between enzyme stability and intrinsic catalytic activity. Much of the log P data has been obtained by measuring activity after the biocatalyst has been placed in the organic solvent for a few hours. This is more an indication of stability rather than activity. A far more accurate model would be one which accounts for the effects of solvents on the intrinsic kinetics, as determined by the

catalytic rate constants k_{cat} and K_m. In this manner, the effect of solvents on enzymes can be quantitatively related to enzymatic activity on the basis of initial, intrinsic rate analysis. Separate correlations to enzyme stability could also be developed. Our recent work with horseradish peroxidase in monophasic organic solvents highlights one specific kinetic approach to understanding and quantifying enzymatic catalysis and substrate specificity in nonaqueous media, namely, the use of linear free energy relationships on enzymatic catalysis in organic media (Ryu and Dordick, 1989, submitted).

FREE ENERGY CORRELATIONS OF SUBSTRATE AND SOLVENT HYDROPHOBICITIES WITH PEROXIDASE CATALYSIS IN MONOPHASIC ORGANIC MEDIA

The effects of organic solvents on the catalytic activity and substrate specificity of enzymatic catalysis are not well understood. Relatively few kinetic studies have been carried out to date (Ryu et al., 1989; Zaks and Klibanov, 1986; Sakurai et al., 1988). Such quantitative analyses are crucial for the development of kinetic models that can be used to predict optimal choice of solvent and substrate for enzymatic reactions in organic media. We have examined horseradish peroxidase-catalyzed oxidation of phenols in monophasic organic solvents as a model to elucidate solvent-induced kinetic alterations of enzymatic catalysis, particularly with respect to substrate and solvent hydrophobicities. In all experimental cases, internal diffusional limitations have been eliminated by adsorbing the enzyme onto non-porous glass beads (Ryu et al., 1989).

Our experimental strategy has been to determine the steady-state kinetic constants, V_{max}, K_m, and V_{max}/K_m in water and a variety of organic solvents with varying degrees of hydrophobicity. The solvents used range in hydrophobicity from water (log P = -2.17) to butyl acetate (log P = 1.70). Added water ranges from 0 - 30% (v/v) in dioxane, and 2%, 1.5%, and 1% in ethyl, propyl, and butyl acetates, respectively. Some water is required in organic solvents for peroxidase to express activity. In the water-immiscible ester solvents, this water is at a concentration just below saturation levels, an amount proven to be optimal for similar solvent systems (Zaks and Klibanov, 1988).

Phenols with *para* substitutions that differed in hydrophobicities have been employed and range from methoxy to *tert*-butyl. Such peroxidase substrates exert only minimal differences in steric hindrance around the phenolic moiety, and are similar in electronic factors (Gordon and Ford, 1972). Figures 1 and 2 depict the dependence of catalytic efficiency (V_{max}/K_m) on the substrate hydrophobicity, π, in aqueous buffer (10 mM phosphate, pH 7) and several nonaqueous solvents. The catalytic efficiency is profoundly lower (up to four orders of magnitude) in organic solvents as compared to aqueous buffer. Furthermore, in all solvents, catalytic efficiency decreases as substrate hydrophobicity increases. This substrate effect, however, becomes more

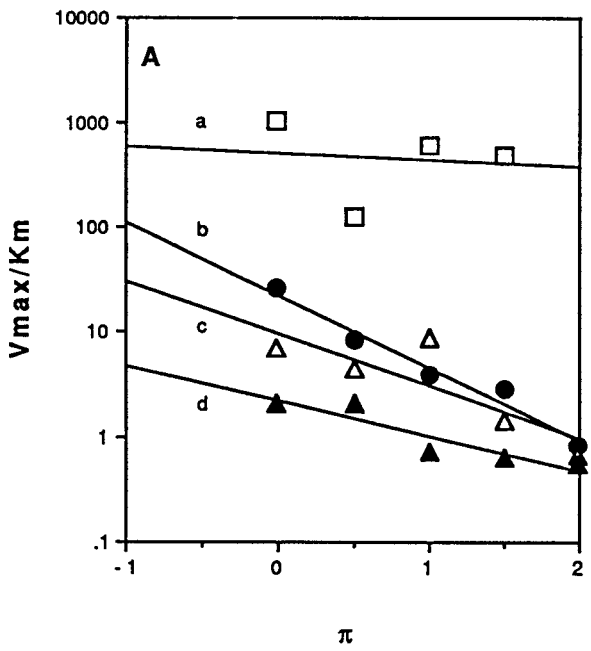

Figure 1. Role of substrate hydrophobicity, π, on catalytic efficiency of horseradish peroxidase in water-miscible organic solvents. a = water; b = 70% dioxane; c = 80% dioxane; d = 95% dioxane. Residual volumes consisted of aqueous buffer, pH 7 (10 mM phosphate).

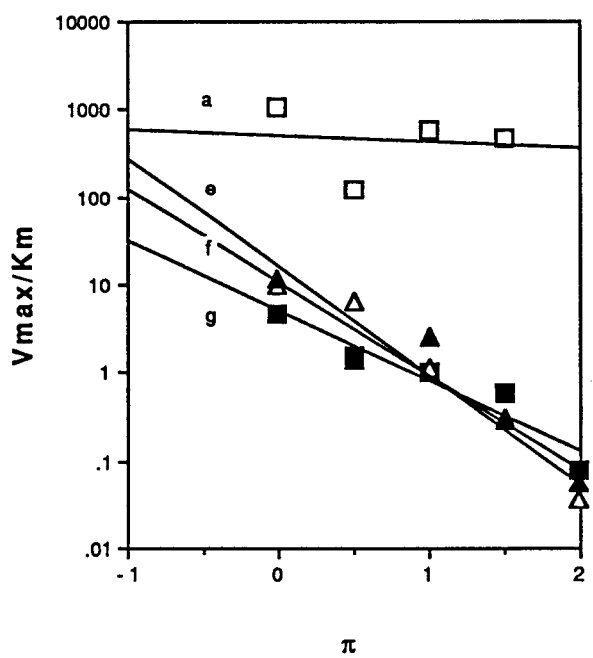

Figure 2. Role of substrate hydrophobicity, π, on catalytic activity of horseradish peroxidase in water-immiscible organic solvents. a = water; e = butyl acetate (1% aqueous buffer); f = propyl acetate (1.5% aqueous buffer); g = ethyl acetate (2% aqueous buffer).

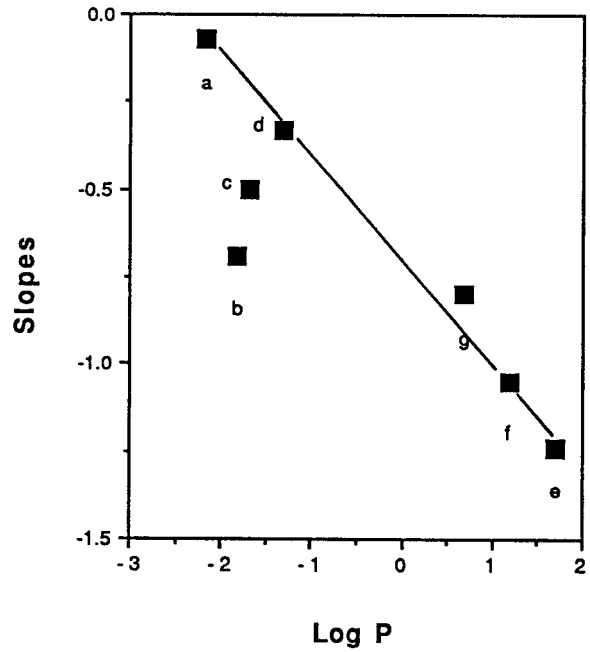

Figure 3. Linear free energy relationship between solvent hydrophobicity and substrate hydrophobicity and catalytic activity of horseradish peroxidase. Slopes indicate the slopes of V_{max}/K_m vs π. Log P represents a measure of solvent hydrophobicity as described in the text.

pronounced as solvent hydrophobicity increases; the slopes of catalytic efficiency vs. π become more negative as a function of log P (Figure 3) and with high linearity (correlation coefficient of 0.99), neglecting the dioxane-water mixtures. These results indicate that a linear free energy relationship exists between catalytic efficiency and both substrate and solvent hydrophobicities. Furthermore, substrate hydrophobicity becomes a significant reaction variable as solvent hydrophobicity increases.

The above findings may be explained purely by the partitioning behavior of phenols between the bulk reaction medium and the peroxidase active site. This partitioning is likely to diminish as substrate and solvent hydrophobicities increase, thereby necessitating a larger concentration of phenols to saturate the enzyme. This results in an increase in the apparent K_m of the phenols in organic versus aqueous media. Similar increases in apparent K_m have been observed for trypsin catalysis in dioxane-water mixtures (Douzou and Balny, 1977). The anomalous results we have obtained with dioxane-water mixtures are intriguing and worth further speculation. It is possible that a conformational change in the peroxidase occurs in such solvents. While enzymes in water-immiscible solvents appear to retain their native structural integrity and are rigid (Dordick, 1989; Clark et al., 1989; Zaks and Klibanov, 1985), the high water content used in the dioxane solvent systems (5 - 30%, (v/v)) may have enabled the peroxidase to become more mobile than in a less hydrated, water-immiscible solvent. Such a structural modification in dioxane-water mixtures may have resulted in the observed deviations from linearity. Along these lines, 95% dioxane solution deviates the least.

The effect on K_m has been verified by calculation of the values of apparent K_m. All phenols tested have significantly higher apparent K_m's in organic solvents than in water. In some cases, this increase is over three orders of magnitude. For example, in aqueous buffer, the apparent K_m's of *p*-methoxyphenol, *p*-cresol, *p*-ethylphenol, and *p*-propylphenol are 0.63, 0.70, 0.25, and 0.15 mM, respectively. In butyl acetate, however, the apparent K_m's were 28, 42, 110, and 250 mM for the four phenols, respectively; an increase from 44- to over 1660-fold in butyl acetate than in water. Furthermore, these differences become more pronounced as the phenolic substituent becomes more hydrophobic, as would be expected if substrate partitioning into the peroxidase's active site were disrupted by solvent hydrophobicity.

By taking advantage of the high solubilities of phenols in organic solvents, we have investigated the solvent effect on catalytic activity, V_{max}. Inspection of the data for *p*-cresol oxidation in aqueous and organic media (Figure 4) reveals that catalytic activity is stimulated in several organic solvents. For example, in both 80% dioxane and butyl acetate, *p*-cresol oxidation is over three-fold faster than in aqueous buffer. Hence, peroxidase retains its full catalytic power in a variety of non-aqueous solvents. Peroxidase does require added water for activity, however. No reaction is observed in water-miscible solvents in the absence of added water, and catalysis in water-immiscible solvents requires a

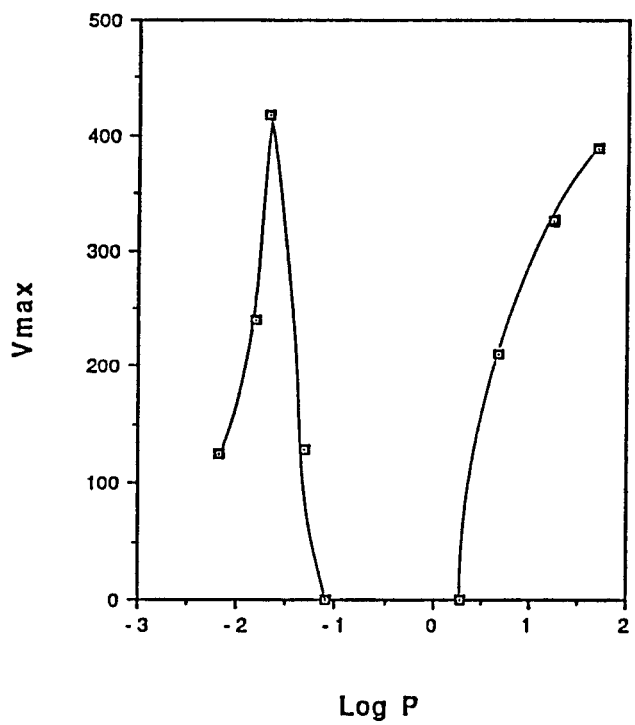

Figure 4. Effect of solvent hydrophobicity on the catalytic activity of horseradish peroxidase with *p*-cresol as substrate. The units of the maximal velocity are µmol/mg enzyme/sec.

small amount of water, usually an amount slightly below that required for saturation in that particular solvent (Zaks and Klibanov, 1988).

Our findings demonstrate that peroxidase catalysis in organic solvents can occur with full inherent catalytic turnover. The major effect of the solvent is to cause a dramatic increase in apparent K_m values. Furthermore, peroxidase catalysis in organic media follows a linear free energy relationship between catalytic efficiency and substrate and solvent hydrophobicities. Such a result allows us to mathematically describe peroxidase-catalyzed oxidation of alkyl phenols in organic solvents. Equation (2) represents an empirical mathematical description of peroxidase catalysis in organic media as a function of substrate and solvent hydrophobicities. This correlation is accurate to within 10% and enables one to predict catalytic efficiency of peroxidase given the substrate and organic solvent.

$$V_{max}/K_m = 680 \exp \{-[0.69(\log P) + 1.50] (\pi)\} \qquad (2)$$

We have developed several potential applications of peroxidases, two of which will be discussed in this paper — phenolic polymerizations and the development of a temperature abuse sensor.

PHENOLIC POLYMERIZATIONS

In nature, peroxidases catalyze the peroxidative coupling of phenols to give lignin (Sarkanen and Ludwig, 1971). In aqueous media, *in vitro*, peroxidase efficiently oxidizes a wide range of phenols in the presence of hydrogen peroxide to produce low molecular weight coupling products (Schwartz and Hutchinson, 1981), but higher molecular weight polymeric material is not produced due to the poor solubility of dimeric and trimeric phenols in water. This effectively causes the growing polymer chain to precipitate out of solution and polymerization to cease. Poor substrate solubilities also limit the productivity of phenolic polymerizations.

We have chosen to carry out phenolic polymerizations in nonaqueous media in order to overcome low substrate and product solubilities and low polymer molecular weights (Dordick et al., 1987). Using *p*-phenylphenol as an example, we have discovered that peroxidase-catalyzed polymerization in dioxane (containing 15% aqueous buffer) yields polymers with molecular weights nearly 50-fold higher than in aqueous media. Furthermore, the productivity of poly (*p*-phenylphenol) in dioxane is far higher than in aqueous media, which is due to the very high solubility of *p*-phenylphenol in 85% dioxane as compared to water. Using 1 M initial concentration of the phenol in 85% dioxane, 150 g/l poly (*p*-phenylphenol) are produced. In aqueous solutions the maximum solubility of *p*-phenylphenol is only 1.5 mM. The generality of this approach is highlighted in Table II. A wide number of phenols and aniline, an aromatic amine, are polymerized in organic media. Furthermore, other water-miscible solvents including acetone, methyl formate, and dimethylformamide, each containing a minimum of 5% aqueous buffer, support phenolic polymerizations.

Table II. *Average Molecular Weights of Phenolic Polymers Enzymatically Produced in 85% Dioxane* (Dordick et al., 1987)

Compound	Average Molecular Weight (daltons)
phenol	1400
p-methoxyphenol	2000
p-cresol	1900
p-chlorophenol	600
2,6-dimethylphenol	500
4,4'-biphenol	400
aniline	1700
1-naphthol	very high
2-naphthol	2000
p-tert-butylphenol	1900
p-phenylphenol	26,000

In addition to the increase in polymer yield and molecular weight, enzymatic polymerization in nonaqueous media offers the ability to maintain rigid selective control over the progress of the reaction. Once again using *p*-phenylphenol, we have found that the polymer size is highly dependent upon the water content in the dioxane (Figure 5). By simply adjusting the water content in dioxane, molecular weights from 500 to 26,000 D are afforded.

ENZYME-MEDIATED TEMPERATURE ABUSE SENSOR

The monitoring of temperature maintenance is important in the transport of frozen or chilled foods, pharmaceuticals, and other heat sensitive materials. A number of sophisticated and expensive electronic devices have been developed to monitor temperature abuse. These devices, however, are subject to tampering. We have developed an enzyme-mediated temperature abuse sensor for such applications that is very nearly tamper-proof (Boeriu et al., 1986). The basis of the sensor is the action of horseradish peroxidase in liquid vs. solid paraffins. We have shown (Boeriu et al., 1986) that peroxidase is over five million-fold more catalytically active in hexadecane at 25°C (a liquid) than at 4°C (a solid). In our prototype device, once the temperature rises above the melting point of the solvent, peroxidase catalyzes the facile oxidation of *p*-anisidine and color is produced. If the prototype, however, has been kept below the freezing point of the solvent, peroxidase remains inactive and no color is produced. By mixing various hydrocarbons, the melting point of the solvents can be varied.

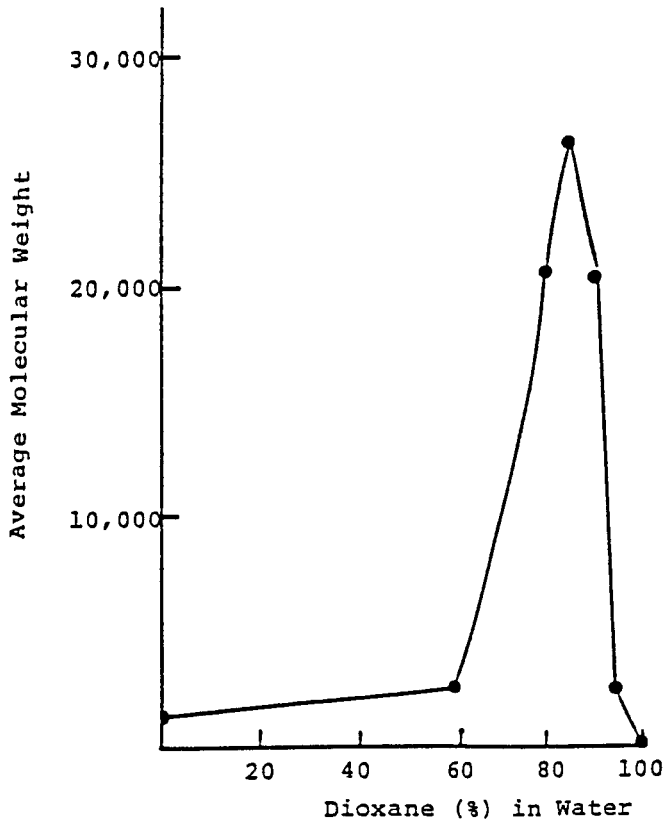

Figure 5. Effect of dioxane concentration on the average molecular weight of fractionated poly (*p*-phenylphenol).

SUMMARY

The application of enzymes in organic solvents has already had an impact on the way biochemists, chemists, and engineers view enzymology. The parameters that govern catalysis in nonaqueous media are beginning to be understood. It is clear that the role of water, the nature of the biocatalyst, and the effect of solvent hydrophobicity are several variables that dominate enzyme function in organic solvents. For the first time the physicochemical parameters of substrate and solvent hydrophobicity have been correlated to enzyme activity. Empirical quantitative relationships are being developed that will aid in the optimization of biocatalysis in organic media. Such information may provide clues to the behavior of enzymes in membranes where water activities are low. Specifically, the effect of gravity on cell membrane morphology and function may be simulated, to some extent, by studying enzymes in nonaqueous media and relevant transport phenomena.

From a practical standpoint, enzymatic catalysis in organic solvents has lead to numerous potential commercial applications. In this paper two such applications were highlighted — peroxidase-catalyzed phenolic polymerizations, and peroxidase-mediated temperature abuse sensor development. Phenolic polymers with molecular weights ranging from several hundred to over 25,000 D were prepared in organic solvents. Such a molecular weight range is impossible to achieve in conventional aqueous biocatalytic systems. In addition, the wide range of paraffin melting temperatures and even melting characteristics of the waxes has allowed a suitable and inexpensive temperature abuse sensor to be developed.

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