BIOCHEMICAL ACTIVITY AND WATER: THE ACTIVITY OF HEME ENZYMES IN NON-AQUEOUS MEDIA*

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Recently, PIMENTEL *et al.* (1966) have challenged the time-honored concept linking water uniquely with life and life processes. After examining the several properties, dielectric constant, acid-base properties, thermodynamic values, etc., invariably listed in textbooks as biologically important, they conclude: "Careful reflection about each of the properties of water reveals that no matter how they are woven into the Earth-pattern of life, none of them seems to involve a functional uniqueness that would preclude life without that property." Although Pimentel *et al.* have opened the door to consideration of life elsewhere, we would contend that their argument can be supported experimentally with terrestrial systems even if they are the products of an aqueous evolutionary history. Recognizing fully that the operational characteristics of intact organisms and their isolated enzymes may bear little relation to one another, we nevertheless submit that if enzymes can retain their catalytic properties in highly modified solvent media (relative to the usual buffered aqueous solutions) then the notion of non-aqueous bio-systems must be taken more seriously and developed as a line of investigation.

1. Methods

The study of two enzymes is reported here. One was measured quantitatively, the other qualitatively.

The activity of crystalline horseradish peroxidase (Calbiochem) was followed photometrically using guaiacol and hydrogen peroxide as reactants. The oxidation of guaiacol in the presence of peroxidase has been discussed by SAUNDERS (1964), and it is sufficient to note that it involves the formation of highly colored dimeric quinones that absorb at ca 400 mμ. An aqueous reaction mixture at pH 6 was used as a standard for activity. It consisted of 2×10^{-8} moles of enzyme, 5×10^{-4} moles of guaiacol and 5×10^{-4} moles of hydrogen peroxide in 10 ml of water. The reaction was initiated by adding the peroxide to a premixture of all other reactants. At 25 °C in 30 sec, the quinone-oxidation products formed in this reaction had an optical density of 0.700 ± 0.011 at 400 mμ.

Catalase regulates decomposition of hydrogen peroxide into water and O_2. Although the course of decomposition may be followed by common oxidimetric titrations, colorimetry, or manometry, the presence of activity may be demonstrated

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simply by the evolution of O_2 bubbles. 10 ml reaction mixtures contained 5×10^{-3} moles of peroxide and 4×10^{-8} moles of catalase. The presence of bubbles after ca 15 min was scored as active (+), their absence as (−).

Prolonged boiling in water destroyed the catalytic activity of both enzymes under all subsequent test conditions.

2. Results and Discussion

The enzymes selected for this initial study are involved in electron-transfer processes and their activities are among the more readily observed, by color change in one case (peroxidase) and by gas evolution in the second (catalase). The inactivity of these enzymes after boiling indicates that the entire protein, not only the hemal moiety, was involved. Furthermore, heme alone at the experimental concentrations used was inactive.

The addition of various substances to peroxidase systems is known to alter their kinetics (SIEGEL and SIEGEL, 1960; SIEGEL, 1963) and modifications of activity by moderate changes in dielectric constant are known to influence several enzymes in aqueous solution (WEBB, 1965; EDSAL and WYMAN, 1958).

TABLE I

Activity of Peroxidase at 298°K in Solvent Systems

	Dielectric Constant	Peroxidase Activity	
		(Optical Units)	(Relative)
Protonic Solvents			
Water	78.5	0.700	100
Formic Acid	58.5	0.432	62
Acetic Acid	6.2	0.028	4
Propionic Acid	3.4	0.058	8
Methanol	32.6	0.134	19
Ethanol	24.3	0.030	4
Ethylene Glycol	37.7	0.092	13
Glycerol	42.5	> 2.00	> 286
Formamide	109.5	0.370	53
Aprotic Solvents			
Hexane	1.9	0.00	0
Benzene	2.3	0.00	0
Dioxane	2.2	0.00	0
Nitromethane	35.9	0.084	12
Binary Systems (Equal Vol.)			
Formic Acid-water		0.348	50
Acetic Acid-water		0.174	25
Propionic Acid-water		0.188	27
All Alcohols and Glycols-water		> 2.00	> 286
Formamide-water		> 2.00	> 286
Methanol-Formic Acid		0.076	11
Dioxane-Formic Acid		0.00	0
Hexane-Nitromethane		0.160	23
Benzene-Nitromethane		0.00	0
Dioxane-Nitromethane		0.054	8

Peroxidase was obviously active in all protonic solvents tested (Table I), most closely approaching the water activity level in formic acid, formamide and glycerol, the three media with the highest dielectric constants: These media are followed by methanol and ethylene glycol, also of relatively high dielectric constant.

Aprotic solvents of low dielectric constant support no activity but nitromethane, although also aprotic, possesses a dielectric constant comparable with glycol and supports a similar level of activity.

It is not to be supposed that either dielectric constant or ionic properties, or necessarily the two parameters in concert will determine the suitability of a solvent system. A comparison of methanol and ethylene glycol reveals about one-third less activity in the latter with a slightly higher dielectric constant. Of course, ethylene glycol is more viscous (diffusion limiting) than methanol, but glycerol should support even less peroxidative activity if viscosity were important, not well over twice the activity of water itself.

Addition of water to another solvent does not necessarily raise activity, but can do so strikingly in some cases.

Lacking additional physical-chemical information, one can only wonder at the state of solvation of the biochemically active protein in the non-aqueous systems.

Catalase is obviously more sensitive to solvent medium, but nevertheless was active in three alcohols, even at relatively low temperature (Tables II and III). In this case, the addition of a small quantity of water increased markedly the spectrum of solvents supporting activity, both at ordinary and low temperatures. In a sense, this constitutes a model that fits SALISBURY's (1962) suggestion that on a planet deficient in water, the latter may serve as a 'vitamin' or growth factor, Thiamin, nicotinamide, etc., earthly vitamins, are all required for the activity of specific enzymes.

On the basis of these data, we conclude that some enzymes of biochemical significance can remain operational in states quite modified from the usual, and suggest further experimentation with enzymes, enzyme systems and even metabolically important sub-cellular particles.

TABLE II

Evolution of O_2 after 15 min in the Catalase-H_2O_2 Reaction at 298°K and 233°K in Anhydrous Solvent Media

Solvent	O_2 Evolution	
	298°K	233°K
Formic Acid	–	–
Acetic Acid	–	–
Propionic Acid	–	–
Methanol	–	–
Ethanol	+	+
Ethylene Glycol	–	–
Glycerol	+	+
Formamide	–	–

TABLE III

Evolution of O_2 after 15 min in the Catalase-H_2O_2 Reaction at 298°K and 233°K in 90% Non-aqueous Solvents (10% H_2O)

90% Solvent	O_2 Evolution	
	298°K	233°K
Formic Acid	−	−
Acetic Acid	+	−
Propionic Acid	+	−
Methanol	+	+
Ethanol	+	+
Ethylene Glycol	+	+
Glycerol	+	+
Formamide	+	+

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