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MECHANISM OF ENZYME ACTION

Semiannual Report

by

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THE UNIVERSITY OF MICHIGAN
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Foreword

The work described in this report was authorized under Contract No. DAAA-15-67-C-0567 entitled "Mechanism of Enzyme Action." This work was started in January and completed in June 1968.

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Reactions of synthetic polymers with small reagents have revealed several analogies with enzymic processes, particularly with polymers that contain catalytically active imidazole nucleophiles.

We have continued our investigation of the cooperative interactions between pendent functional groups on a vinyl polymer chain and a reactive substrate. Interactions of this type are of interest owing to their analogy to the functioning of an enzyme's active site. As a model for acetylcholinesterase, we have investigated the solvolysis of the positively charged substrate 3-acetoxy-N-trimethylanilinium iodide (ANTI) catalyzed by copolymers containing "esteratic sites" and "anionic sites." In these cases copolymers of 4(5)-vinylimidazole with acrylic acid and with vinylsulfonic acid were employed. In contrast to the inhibited behavior of the imidazole-sulfonic acid copolymer toward ANTI, the imidazole-acrylic acid copolymer has been found highly reactive. These results are indicative of cooperative interactions between pendent imidazole and carboxylate groups.

In a continuation of polymeric and monomeric 1,2,4-triazole catalyzed solvolyses, esterolytic reactions were performed in varying alcohol-water contents. Results indicate these reactions to be ionic in character, where anionic triazole is the most catalytically active species. A number of new 1,2,4-triazoles are reported.

Evidence has been obtained supporting strong hydrophobic interactions between neutral or ionic substrate and poly-4(5)-vinylimidazole. With regard to paraffinic chain, ionic substrates, the rates of solvolyses by polymeric imidazole appear to be as rapid as an enzyme catalyzed process. This appears to be the first reported example of a synthetic, polymeric catalyst approaching the efficiency of an enzymic system. Effects of hydrophobic interactions have also been revealed in dimeric model systems, although the results are not as dramatic as in the polymeric case.

In order to further understand the inactivation of cholinesterases by toxic organic phosphorous compounds, we have synthesized the positively charged tri(choline chloride) phosphate and are currently investigating its hydrolysis in the presence of partially ionized poly(acrylic acid). This system could be expected to be analogous to the attraction of acetylcholine to the anionic carboxylate sites in acetylcholinesterase.
MECHANISM OF ENZYME ACTION

Results and Discussion

I. COOPERATIVE INTERACTION OF IMIDAZOLE AND CARBOXYLATE GROUPS

A cooperative electrostatic effect involving pendent imidazole and carboxylate groups was reported for the solvolysis of the positively charged ester 3-acetoxy-N-trimethylanilinium iodide (ANTI) catalyzed by a copolymer of 4(5)-vinylimidazole and acrylic acid (I, R=COOH).\(^{(1)}\) Although this copolymer was a less effective catalyst than imidazole in solvolyzing the neutral ester \(\text{p-nitrophenyl acetate (PNPA)}\) and the negatively charged ester 4-acetoxy-3-nitrobenzoic acid (NABA), marked selectivity toward ANTI was realized at high pH values. The selectivity of the copolymer was rationalized by the electrostatic attraction of the positively charged ester to the anionic carboxylate groups on the polymer that accumulate the substrate in a high concentration of imidazole nucleophiles. This type of cooperative effect is believed to be in part similar to that of the acetylcholene-esterase catalyzed hydrolysis of its positively charged substrate, acetylcholine.\(^{(2)}\) An illustration of the selectivity of polymers containing pendent imidazole and carboxyl groups toward ANTI is given in Figure 1. The second-order catalytic rate constants \((k_{\text{cat}})\) are given as a function of the neutral imidazole content \((\alpha_1)\) for an alternating copolymer of 4(5)-vinylimidazole and maleic acid \((I, y=\text{HOOC-CH-CH-COOH})\), a copolymer of 4(5)-vinylimidazole and acrylic acid \((46.3 \text{ mole } \% \text{ imidazole})\), a dimeric imidazole-carboxylic acid model, \(\gamma-4(5)-\text{imidazoylbutyric acid (II)}\), and imidazole. It is evident that both copolymers markedly enhance the solvolytic
Figure 1

Solvolysis of ANTI catalyzed by an alternating copolymer of 4(5)-vinylimidazole and maleic acid (□), a copolymer of 4(5)-vinylimidazole and acrylic acid (46.3 mole % imidazole) (△), γ-4(5)-imidazoylbutyric acid (○) and imidazole (○); 28.5% ethanol-water, ionic strength 0.02, 26°C.
rate of ANTI, the former copolymer, containing more anionic sites, doing so to a greater extent. These copolymers are found to be less reactive than imidazole in catalyzing the solvolyses of PNPA and NABA, particularly for the negatively charged substrate. With regard to the neutral substrate, the imidazole-maleic acid copolymer and a series of imidazole-acrylic acid copolymers display almost identical reactivities for analogous contents of neutral imidazole. This behavior reveals that a neutral ester is not excluded from a catalytically active poliony as the negative charge density is increased. This fact is in contrast to that previously reported (see previous Semianual Report, March 1968) for the exclusion of PNPA from anionic polyacrylic acid. Upon reinvestigation of the latter effect, it was observed that anionic polyacrylic did not alter the reactivity of either PNPA or NABA.

In order to study in more detail the characteristics of the ANTI selective solvolysis, the effects of composition (mole % imidazole) on the solvolytic rate of ANTI catalyzed by copolymers of 4(5)-vinylimidazole with acrylic acid and with vinylsulfonic acid (I, R=SO$_3$H) were investigated. It could be anticipated that if the carboxylate groups in the imidazole-carboxylic acid copolymers serve only to electrostatically attract the cationic ester to the polion, then a copolymer of 4(5)-vinylimidazole and vinylsulfonic acid should reveal a similar catalytic activity. This behavior is not observed (Figure 2). At pH 9, the solvolytic rate of ANTI catalyzed by the imidazole-carboxylic acid copolymers reaches a maximum at approximately 47 mole % imidazole, and decreases in the region of high and low imidazole contents. Apparently at a high imidazole content there are insufficient anionic sites to accumulate ANTI around the polymer. Conversely, at a low imidazole content the polymer begins to behave principally as a poliony. Through such an effect, the polyanion could accumulate the positively charged ester in its vicinity and repel the catalyzing hydroxide ions. This would then lead to an inhibition of the solvolytic rate because of the decrease in collision frequency of the ionic reagents. Indeed, in the last Semianual Report (March 1968), such an inhibitory effect was found for the solvolysis of ANTI in the presence of anionic polyacrylic acid.

Inspection of the data for the 4(5)-vinylimidazole-vinylsulfonic acid catalyzed solvolysis of ANTI reveals that a bell-shaped curve is absent. Instead, the reaction is apparently inhibited at imidazole contents less than 50 mole %. This suggests that the imidazole-sulfonic acid copolymer is behaving simply as a polyanion toward ANTI.

Therefore, it seems probable that in the imidazole-acrylic acid copolymer catalyzed solvolysis of ANTI, some bifunctional catalysis between the pendent, neutral imidazole groups and carboxylate groups does exist. Besides functioning as binding sites for ANTI, the carboxylate groups can be involved in the solvolytic process in much the same fashion as the previously reported pendent phenoxide groups (I, R=C$_6$H$_5$O$^-$).

(4, 5, 6)
Figure 2

Solvolyis of ANTI catalyzed by copolymers of 4(5)-vinylimidazole with acrylic acid (O) and with vinylsulfonic acid (△); pH 9.0, 28.5% ethanol-water, ionic strength 0.02, 26°C.
II. CATALYTIC ACTIVITIES OF MONOMERIC AND POLYMERIC TRIAZOLE

In the previous Semiannual Report (March 1968) it was reported that in 28.5% ethanol-water the nucleophilic reactions of neutral 1,2,4-triazole and poly-3-vinyl-1,2,4-triazole with neutral, negatively and positively charged phenyl esters were rather inefficient. On the other hand, the reactivities of both monomeric and polymeric triazole functions increased dramatically as the fraction of anionic triazole functions increased. Indeed, it was found that the rates of these esterolytic catalyses were linearly dependent upon the fraction of anionic triazole groups. This type of behavior is characteristic of poly-5(6)-vinylbenzimidazole at high pH values. (7)

Since poly-3-vinyl-1,2,4-triazole is water soluble, it was of interest to study esterolytic catalyses in solvents of varying water-ethanol contents. With the negatively charged ester sodium \(4\)-acetoxy-3-nitrobenzenesulfonate (NABS) at pH 9.0, it was found that both polymeric and monomeric catalyses were seven times more efficient in 100% water than in 40% water. This fact indicates that the solvolytic process is ionic in character, such as previously indicated.

Owing to the nucleophilic character of anionic triazole, it was believed that a copolymer of \(4\)(5)-vinylimidazole and 3-vinyl-1,2,4-triazole should exhibit very efficient catalysis if the nucleophilic attack of neutral imidazole on substrate is catalyzed by anionic triazole acting as a general base (III or IV). These processes could be similar to those which are believed to occur in

![III](image1.png)  
![IV](image2.png)
poly-4(5)-vinylimidazole,\(^{(8,9)}\) poly-5(6)-vinylbenzimidazole,\(^{(7,9)}\) and copoly[4(5)-vinylimidazole-\(2\)-vinylphenol]\(^{(6)}\) catalyzed solvolyses at high pH values.

Since the usual preparation of 3-vinyl-1,2,4-triazole from the dehydration of 3-\(\beta\)-hydroxyethyl-1,2,4-triazole gives immediate polymer formation, it was necessary to investigate other preparations of the stable monomer. It was believed that the most promising route would be decarboxylation of V, the triazole derivative of urocanic acid VI (precursor to 4(5)-vinylimidazole).

\[
\begin{align*}
\text{CH}=\text{CH}-\text{COOH} & \quad \text{CH}=\text{CH}-\text{COOH} \\
\text{V} & \quad \text{VI}
\end{align*}
\]

The following sequences of reactions were employed to prepare V:

\(\text{(1)}\)

\[
\begin{align*}
\text{C} & \quad + \quad \text{H} & \quad \text{N} & \quad \text{N} \\
\text{O} & \quad \text{O} & \quad \text{C} & \quad \text{NH}_2 \\
\text{H} & \quad \text{N} & \quad \text{N} & \quad \text{SH} \\
\text{VII} & \quad \text{VIII}
\end{align*}
\]

\(\text{KOH (aq.)}\)

\[
\begin{align*}
\text{O} & \quad \text{O} & \quad \text{N} & \quad \text{N} \\
\text{H} & \quad \text{N} & \quad \text{N} & \quad \text{SH} \\
\text{VII} & \quad \text{VIII}
\end{align*}
\]

\(\text{HNO}_3\)

\[
\begin{align*}
\text{O} & \quad \text{O} & \quad \text{N} & \quad \text{N} \\
\text{H} & \quad \text{N} & \quad \text{N} & \quad \text{HNO}_3 \\
\text{VIII}
\end{align*}
\]

\(\text{Na}_2\text{CO}_3\)

\[
\begin{align*}
\text{V}
\end{align*}
\]
Only by route 1 could V be prepared. Diazotization of IX was unsuccessful. Unfortunately, it was found that decarboxylation of V gave monomer, which polymerized very rapidly. Consequently, it has been difficult to prepare a copolymer of 3-vinyl-1,2,4-triazole with 4(5)-vinylimidazole. Several attempts at copolymerization have given polymers in which the carbon, hydrogen, and nitrogen contents were approximately 84%. This is not yet understood.

We are currently investigating the behavior of 3-isopropyl-1,2,4-triazole and N-methyl-1,2,4-triazole. The former compound serves as a more appropriate model for poly-3-vinyl-1,2,4-triazole than does 1,2,4-triazole, and the latter compound should indicate reduced catalytic activity due to a lack of anionic sites. Furthermore, we are also investigating the effects of the related 1,2,3-triazole and 1,2,3-benzotriazole on ester solvolyses.

III. HYDROPHOBIC INTERACTIONS IN POLYMERIC CATALYSIS

In recent years it has become increasingly evident that hydrophobic (apolar) interactions play a crucial role in enzymic reactions, particularly in the binding of a substrate at an active site.

Since poly-4(5)-vinylimidazole has displayed several reactions analogous to enzymes, it was of interest to determine if hydrophobic bonding could occur in polymeric imidazole catalyses. The substrates chosen were neutral phenyl esters related to p-nitrophenyl acetate, but possessing long paraffinic chains. In Table 1 are listed a comparison of the reactivities of polymeric and monomeric
Table I(a)

SOLVOLYSES OF p-NITROPHENYL n-ALKANOATES BY IMIDAZOLE AND POLY-4(5)-VINYLIMIDAZOLE IN 60% n-PROPANOL-WATER

<table>
<thead>
<tr>
<th>H-(CH₂)n-COO-NO₂</th>
<th>Imidazole k_{cat(Im)}</th>
<th>Poly-4(5)-vinylimidazole k_{cat(PVIm)}</th>
<th>k_{cat(PVIm)} / k_{cat(Im)}</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5.15</td>
<td>3.31</td>
<td>0.64</td>
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<tr>
<td>6</td>
<td>1.48</td>
<td>0.86</td>
<td>0.58</td>
</tr>
<tr>
<td>11</td>
<td>1.46</td>
<td>0.65</td>
<td>0.46</td>
</tr>
<tr>
<td>17</td>
<td>1.46</td>
<td>0.66</td>
<td>0.45</td>
</tr>
</tbody>
</table>

(a) At pH 7.95, ionic strength 0.02; k_{cat} in l/mole·min.

Imidazole catalyzed solvolyses of p-nitrophenyl n-alkanoates. These reactions were initially performed at pH 8 (where most of the imidazole functions are in the neutral form) in 60% propanol-water (where hydrophobic interactions would be at a minimum because of the high solubilities of catalyst and substrate). It is seen that as n increases (Table I), the catalytic rates of imidazole and poly-4(5)-vinylimidazole both decrease. This suggests that in 60% propanol-water both of these processes are dependent upon steric effects. Upon repetition of the above reactions in 24% n-propanol-water (Table II), however, a drastic change in reactivities is observed when the first-order observed rate constants are compared. It is noted that as the hydrophobic character of the substrate increases in a solvent of higher polarity, poly-4(5)-vinylimidazole becomes a much more efficient catalyst than does imidazole.

Table II(a)

SOLVOLYSES OF p-NITROPHENYL n-ALKANOATES BY IMIDAZOLE AND POLY-4(5)-VINYLIMIDAZOLE IN 24% n-PROPANOL-WATER

<table>
<thead>
<tr>
<th>H-(CH₂)n-COO-NO₂</th>
<th>Imidazole k_{obs(Im)}</th>
<th>Poly-4(5)-vinylimidazole k_{obs(PVIm)}</th>
<th>k_{obs(PVIm)} / k_{obs(Im)}</th>
</tr>
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<tbody>
<tr>
<td>n</td>
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<td>11.00</td>
<td>1.28</td>
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<td>6.83</td>
<td>2.43</td>
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<td>2.21</td>
<td>9.48</td>
<td>4.29</td>
</tr>
<tr>
<td>17</td>
<td>2.21</td>
<td>24.4</td>
<td>11.0</td>
</tr>
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(a) At pH 7.95, ionic strength 0.02, k_{obs} in min⁻¹; [Imidazole] = 5.5 x 10⁻⁴ M, [PVIm] = 6.0 x 10⁻⁴ M.
In order to obtain more information concerning the mechanistic pathway of hydrophobically activated solvolytic reactions, the solvolysis of p-nitrophenyl laurate (n=11) was performed by varying the concentration of poly-4(5)-vinylimidazole. An unexpected concentration dependence of rate was observed in that saturation kinetics were obtained. This is an unusual occurrence when the polymer concentration is in excess of the (low molecular weight) substrate concentration, although such behavior has been noted in the interactions of a polymeric substrate.\(^{(10)}\) Previously, we have observed saturation effects, indicative of catalyst-substrate complexation, in the solvolysis of the negatively charged ester NABS catalyzed by partially protonated poly-4(5)-vinylimidazole (substrate concentration in excess of polymer concentration); no such effect was found for the solvolysis of the neutral ester PNPA (n=1).\(^{(11)}\) These data indicated that electrostatic forces were sufficient to concentrate NABS in the vicinity of the polymer chain, while hydrophobic forces were not sufficient to accumulate PNPA in the polymer environment. It is now apparent that increasing the hydrophobic character of the neutral substrate facilitates an apolar complexation with a polymeric catalyst. An effect of this type has recently been reported by Klotz and Stryker.\(^{(12)}\)

In order to ascertain if electrostatic and hydrophobic forces can combine to increase the catalytic efficiency of a vinyl polymer, the solvolysis of the anionic substrate 4-lauryloxy-3-nitrobenzoic acid (NLBA), a long chain analog of NABA, was investigated in propanol-water and ethanol-water solutions. In solvents of high alcohol contents, the reactivities of polymer and monomeric analog approach each other, although the polymer is slightly more reactive than imidazole. At low alcohol contents, however, the polymeric reaction becomes so rapid as to approach the efficiency of an enzymic process; no such effect occurs in the imidazole catalyzed reaction. The velocity of the polymeric reaction at low alcohol contents by far exceeds our ability to measure the reaction rate using our current instrumentation. This effect is one of the most exciting discoveries yet encountered in synthetic, polymeric catalysis, and may aid in the understanding of the driving force for enzyme-substrate complexation, which has been shown to be, in part, due to hydrophobic interactions.

At the present time we are studying the solvolysis of NLBA in solvents containing 30% ethanol. Using this alcohol concentration, the polymer is approximately 350 times more efficient than imidazole and it is possible to obtain reproducible measurements. With lower alcohol contents the polymeric reaction is instantaneous and measurements cannot be followed by conventional spectrophotometers.

IV. PHOSPHATE ESTER HYDROLYSES

It is well known that certain trisubstituted esters of phosphoric acid are very toxic and generally cause death by respiratory paralysis. This behavior has been attributed to the inhibition, through phosphorylation, of the cholineesterase enzymes which causes an accumulation of acetylcholine in the membranes of nerves.\(^{(2)}\)
In order to utilize our knowledge of the catalytic properties of synthetic macromolecules, we have begun investigating the solvolyses of trisubstituted phosphate esters catalyzed by nucleophilic polymers. In this manner it may be possible to prepare a polymer which is capable of reacting quickly with either the toxic agent or with the inhibited cholinesterase to ensure the life process.

Our usual method of studying solvolytic rates has been by employment of ultraviolet spectroscopy to follow the liberation of (substituted) p-nitrophenol; however, since many cholinesterase inhibitors are nonaromatic compounds, we have constructed two pH-stat instruments. These versatile instruments will allow us to follow, by means of titration, the solvolysis of any organic phosphorous compound. For the purpose of calibration of the pH-stat we have studied the solvolysis of tris(p-nitrophenyl phosphate), a nontoxic agent, which hydrolyzes quickly. This rapid hydrolytic reaction enabled us to deduce the reproducibility of the pH-stat over a broad range of pH.

The active center of acetylcholinesterase is believed to be composed of two sites, an anionic site, which is responsible for specificity, and an esteratic site, which causes hydrolysis of the substrate. Preferential substrates for acetylcholinesterase normally possess the positively charged trialkylammonium group, presumably because of the opposite charge of the anionic site.

Compounds related to acetylcholine are of obvious interest in cholinesterase inhibition owing to the attraction to the active center. Recently, several organophosphorous compounds related to acetylcholine were synthesized and their toxicities were investigated. Compounds of the type shown below (X) were found to exhibit strong cholinesterase inhibiting and toxic properties if R^3 was either an alkyl or alkoxy group and R^2 was a fluoro group. If a thiocholine were employed instead of choline, the diethoxy derivative was then found to have an LD50 of 0.14. If R^1 and R^2 in X were both ethoxy groups, the LD50 was found to be 495. These facts clearly show that the affinity of the phosphate ester for the active site of the cholinesterase is quite different from its ability to phosphorylate.

Since electrostatic effects are very pronounced in synthetic, polymeric catalysts, it seemed reasonable to investigate the hydrolysis of a phosphate ester related to acetylcholine catalyzed by an oppositely charged polyanion. The substrate chosen for study was tri(choline chloride) phosphate [X, R^1=R^2=0-CH_2-CH_2-N(CH_3)_3] (TCCP), since its three positive charges would ensure a rapid attraction to a polyanion. TCCP was prepared using the procedure of Jackson by reacting trimethylamine with tri-ß-chloroethyl phosphate.
At the present time we are investigating the hydrolysis of TCCP in the presence and absence of anionic polyacrylic acid. This polymer was chosen for its high negative charge density and the fact that carboxylate ions, though weak nucleophiles, can displace chloride from phosphorochloridates.\(^{(15)}\) In the absence of polyanion, the first-order (blank) rate constant is \(1.0 \times 10^{-3} \text{ min}^{-1}\) at \(pH\) 9.0. In the presence of polyanion there is an instantaneous decrease in \(pH\) as TCCP is added which causes a rapid addition of base to maintain \(pH\). This effect is either due to a very rapid hydrolysis of TCCP to \(\text{di(choline chloride)}\) phosphoric acid or to a change in ionization of the polyanion by the highly charged substrate. When this reaction is conducted at an ionic strength of 0.02 no such phenomenon seen.

We are currently investigating this effect in detail and shall employ other charged and neutral substrates as well as other polymeric catalysts that contain either phenoxide, amino, pyridinyl, imidazoyl, hydroxyl, or oxime groups.

V. PREPARATION AND ESTEROLYTIC BEHAVIOR OF DIMERIC ANALOGS

We have attempted to ascertain if cooperative interactions between imidazole and phenol groups, such as occurs on a polymer chain,\(^{(5,6)}\) can occur in the dimeric model compound, \(4(5)-[\text{9(3-hydroxyphenyl)nonyl} \text{imidazole (XI). Solvolytic reactions}}\]

\[
\text{XI}
\]

of FNPA, NABS, and ANTI with XI were initially performed in 80% methanol-water because this solvent system had been used for the highly reactive copolymer of \(4(5)-\text{vinylimidazole and p-vinylphenol,}\(^{(5,6)}\) In this solvent it was found that XI revealed no enhanced catalytic effects toward any substrate. This indicates that cooperative, catalytic interactions can occur to a significant extent only when a high local concentration of catalytically active groups is present, a circumstance that occurs on a polymer chain.

Although no cooperative effects were realized with this dimeric compound in 80% methanol-water, we have found evidence for hydrophobic interactions between XI and a neutral substrate. With \(\text{p-nitrophenyl acetate, the imidazole-phenol dimer is approximately twice as reactive as imidazole; however, employing the ester} \text{p-nitrophenyl palmitate, a substrate with a long paraffinic chain, the imidazole-phenol dimer is approximately five times more reactive than imidazole. This effect is not caused by bifunctional imidazole-phenol catalysis because similar enhanced rates occur when the terminal hydroxyl group in X is methylated.}\)
It could be expected that the effectiveness of catalytic groups on a model compound would be greatly increased if the functional groups are in juxtaposition for a concerted attack on substrate. Consequently, another imidazole-phenol dimeric model compound, 4(5)-(2-hydroxyphenyl)imidazole\(^{16,17}\) (XII) was prepared by the following procedure:

\[
\begin{align*}
\text{CH}_3 & \quad \text{CuBr}_2 & \quad \text{O} & \quad \text{N} \\
\text{OH} & \quad \text{C} & \quad \text{CH}_3 & \quad \text{Br} \\
\text{OH} & \quad \text{C} & \quad \text{H}_2 & \quad \text{N} \\
\text{H} & \quad \text{N} & \quad & \quad \text{H}
\end{align*}
\]

XII

Catalytic studies with XII will shortly be in progress.

VI. LITERATURE CITED


(2) For a review see N. Englehard, K. Prchal, and M. Nenner, Angew. Chem. internat. Edit., 6, 615 (1967).


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MECHANISM OF ENZYME ACTION

Semianurnal Report—January–June 1968


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Life sciences basic research in support of material

Edgewood Arsenal Research Laboratories
Edgewood Arsenal, Maryland 21010
(J. Epstein, Proj. O. Ext. 25114)

The objectives of this contract are to synthesize polymeric catalysts and to study their reactivities toward neutral and charged phosphate esters and carbon esters. Copolymers of 4(5)-vinylimidazole with acrylic acid and with vinylsulfonic acid were studied for their catalytic effects on a positively charged phenyl ester 3-acetoxy-N-trimethylenilinium iodide. Results indicated a cooperative interaction between pendant imidazole and carboxylate groups. Evidence has been obtained indicating that strong hydrophobic interactions occur between paraffinic substrates and poly-4(5)-vinylimidazole. Several of these hydrophobic reactions appear to be as efficient as enzymic processes. The compound tri(choline chloride) phosphate has been synthesized and its hydrolysis in the presence and absence of anionic polycrlyclic acid is under investigation.
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<td>Enzyme-substrate complexation</td>
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<td>Phosphate ester</td>
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<td>Tri(choline chloride) phosphate</td>
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<td>Toxic agent</td>
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<td>Anionic polyacrylic acid</td>
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<td>Dimeric analogs</td>
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