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MECHANISM OF ENZYME ACTION

Terminal Report

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## Foreword

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## MECHANISM OF ENZYME ACTION

### I. Publications List

- (1) Synthesis of 5(6)-Vinylbenzimidazole and of 2-Vinylbenzimidazole. C. G. Overberger, B. Kösters, and T. St. Pierre, *J. Polymer Sci.*, Part A-1, Vol. 5, 1987-92 (1967).

The syntheses of 2- and 5(6)-vinylbenzimidazole are described. These compounds were characterized by appropriate chemical reactions and physical properties. Incidental to this work, 3,4-diaminostyrene was also prepared.

- (2) Cooperative Effects in the Esterolytic Action of Synthetic Macromolecules Containing Imidazole and Hydroxyl Functions. C. G. Overberger, J. C. Salamone, and S. Yaroslavsky, *J. Amer. Chem. Soc.*, 89, 6231-6236 (1967).

The imidazole group of histidine and the hydroxyl group of serine are the purported participants in the active site of  $\alpha$ -chymotrypsin. Copolymers of 4(5)-vinylimidazole with vinyl alcohol and with p-vinylphenol were prepared in order to investigate whether interactions between two different functional groups within a vinyl polymer chain would occur in esterolytic catalyses. The substrates investigated were neutral and negatively and positively charged phenyl esters. Rates of solvolyses were determined in either 28.5% ethanol-water or in 80% methanol-water. The copolymer of 4(5)-vinylimidazole and p-vinylphenol was found a highly superior catalyst at high pH values toward each substrate investigated than any of its monomeric or polymeric analogs. These rate enhancements are not associated with the apparent dissociation constants of the imidazole or phenol groups, but can be attributed to bifunctional catalysis by imidazole and anionic phenol. One probable mechanism is the nucleophilic attack of imidazole on the substrate, followed by reaction of anionic phenol acting as a general base on the tetrahedral intermediate. Considerable rate enhancements were also observed for reactions catalyzed by copolymers of 4(5)-vinylimidazole and vinyl alcohol.

- (3) Selective Catalytic Effects of Strongly Ionizing Polycations on Ester Solvolysis. C. G. Overberger, H. Morawetz, J. C. Salamone, and S. Yaroslavsky, *J. Amer. Chem. Soc.*, 90, 651 (1968).

The cationic polyion, poly(1-vinyl-3-methylimidazolium iodide) (PVMI), was found to enhance the solvolytic rates of the negatively charged esters

4-acetoxy-3-nitrobenzoic acid and sodium 4-acetoxy-3-nitrobenzenesulfonate in 28.5% ethanol-water solutions. These rates were unaffected by the monomeric analog of the polyion, 1,3-dimethylimidazolium iodide. At high pH values, the enhanced solvolytic rates of the negatively charged esters may be accounted for by the high local concentration of hydroxyl counterions in the vicinity of the polymer chain. At intermediate pH values, the large catalytic effect is attributed to an enhanced susceptibility of the anionic ester to direct water attack in the vicinity of the polycation. The solvolyses of uncharged esters (p-nitrophenyl acetate and p-nitrophenyl hexanoate) were not accelerated, indicating that hydrophobic forces are insufficient to concentrate the esters in the neighborhood of the polyion. A copolymer of 1-vinyl-3-methylimidazolium iodide (VMI) containing 86 mol % vinyl alcohol residues catalyzed the hydrolysis of anionic esters with an efficiency similar to that of the PVMI homopolymer, if the two were compared at equal stoichiometric concentrations of imidazolium groups. On the other hand, a copolymer of VMI containing 63 mol % p-vinylphenol residues was about twice as effective as PVMI. This is due to the fact that the polycation draws the anionic ester into a region with a high local concentration of the phenoxide nucleophile.

- (4) Macromolecule-Substrate Complexation. A Saturation Phenomenon Exhibited by Poly(4(5)-vinylimidazole) and an Anionic Ester. C. G. Overberger, R. Corett, J. C. Salamone, and S. Yaroslavsky, *Macromolecules*, Vol. 1, 331 (1968).

The rates of solvolyses of the neutral ester p-nitrophenyl acetate (PNPA) and the negatively charged ester sodium 4-acetoxy-3-nitrobenzenesulfonate (NABS), each catalyzed by poly(4(5)-vinylimidazole), were investigated under conditions in which the substrate concentration was in excess of the catalyst concentration. In contrast to the solvolytic behavior of the neutral ester PNPA, the reaction of the negatively charged ester NABS gave rise to a kinetic scheme similar to that of hydrolytic enzymes in that the initial solvolysis rate reached a limiting value at high substrate concentration. This would appear to indicate the saturation of the polymeric catalyst with anionic substrate. The lack of a saturation effect exhibited by PNPA in the concentration range and pH value investigated can perhaps be accounted for by the inadequacy of hydrophobic forces to sufficiently concentrate the neutral ester in the vicinity of the polymer chain, whereas at intermediate pH the anionic substrate would be strongly attracted to the protonated imidazole sites on the polymer by electrostatic forces. Employing the basic Michaelis-Menten kinetic system, values of  $K_m$  and  $k_2$  for the solvolysis of sodium 4-acetoxy-3-nitrobenzenesulfonate were found to be  $(3.8 \pm 0.2) \times 10^{-4}$  M and  $0.63 \pm 0.04 \text{ min}^{-1}$ , respectively.

- (5) Optically Active Imidazole-Containing Polymers. C. G. Overberger and Iwhan Cho, *J. of Polymer Sci., Part A-1*, 6, 2741 (1968).

In order to investigate the stereospecificity of enzyme-catalyzed reactions, an optically active copolymer of 4(5)-vinylimidazole and 2,5(S)-dimethyl-1-hepten-3-one was synthesized, and its effects on the solvolytic rates, in ethanol-water, of the p-nitrophenyl and 4-carboxy-2-nitrophenyl esters of 3(R)- and 3(S)-methylpentanoic acid and of the commercially available N-carbobenzoxy-(R)- and (S)-phenylalanine p-nitrophenyl esters were investigated. The optically active comonomer was prepared by thermal decomposition of solid (+)-1-piperidino-2,5(S)-dimethylheptan-3-one hydrochloride, which was obtained from the reaction of 2(S)-methylbutyllithium with 3-piperidino-2-methylpropionitrile. The 3(R)-methylpentanoic acid was prepared in 92% optical purity from L-alloisoleucine via diazotization in concentrated hydrobromic acid and subsequent reductive debromination with zinc amalgam in dilute hydrochloric acid. In the optically active copolymer-catalyzed solvolyses of the optically active esters performed at pH values of 6-8 no significant differences between the solvolytic rates of (R) and (S) isomers of substrates were observed. Poly-L-histidine was also employed as a catalyst for the solvolyses of these substrates. At pH 6.0 in ethanol-water the latter catalyst also failed to exhibit specificity toward (R) and (S) substrates.

- (6) Esterolytic Catalyses by Triazoles. C. G. Overberger and P. S. Yuen, J. Amer. Chem. Soc., in press.

The catalytic effects of 1,2,4-triazole, 1,2,3-triazole and several of their respective derivatives in the solvolyses of neutral, negatively and positively charged phenyl esters were investigated. Similar studies were performed with poly-3-vinyl-1,2,4-triazole. For all systems it was observed that their catalytic effect exhibited a linear dependence on the concentration of their respective anionic forms. A comparison of catalytic behavior of the triazole and imidazole systems was made. The difference of their respective catalytic behavior was shown to arise from their  $pK_a$  values rather than the nature of the catalyzing species. An empirical relationship between catalytic ability and  $pK_a$  value was obtained for these system, and can be expressed by the equation  $\log k_i = npK_i + \log C$ , where  $n$  and  $\log C$  were determined to be 0.612 and -2.97, respectively. This equation could possibly be used to predict the catalytic effect of a closely related five-membered nitrogen heterocycle.

- (7) Cooperative Effects Involved in Esterolytic Reactions Catalyzed by Imidazole-Carboxylic Acid Copolymers. C. G. Overberger and H. Maki, Macromolecules, 3, 214 (1970).

In order to elucidate the occurrence of cooperative interactions between pendent imidazole and carboxylate groups in 4(5)-vinylimidazole-acrylic acid copolymer-catalyzed esterolytic reactions, copolymers of 4(5)-vinylimidazole and vinylsulfonic acid were prepared and their catalytic activities were investigated. The latter copolymers, rich in vinylsulfonic acid, had no catalytic activity, indicating the occurrence of cooperative interactions between pendent imidazole and carboxylate groups in the former copolymers.

- (8) Esterolytic Catalyses by Copolymers Containing Imidazole and Carboxyl Functions. C. G. Overberger and H. Maki, *Macromolecules*, 3, 220 (1970).

The electrostatic interactions involved in the imidazole-carboxylic acid copolymer-catalyzed solvolyses of  $\beta$ -acetoxy-N-trimethylanilinium iodide (ANTI), p-nitrophenyl acetate (PNPA) and  $\beta$ -nitro-4-acetoxybenzoic acid (NABA) were studied and compared with the monomeric analog  $\gamma$ -4(5)-imidazolebutyric acid. The effects of copolymer composition of the 4(5)-vinylimidazole-acrylic acid copolymers on their catalytic activities were investigated in detail. These effects became apparent by inspecting the dependencies of their activities on the monomer sequence distributions, which were found to control the overall catalytic activities of the copolymers for the charged esters. The most catalytically active species toward ANTI is the carboxylate-imidazole-carboxylate triad.

- (9) Synthesis of (R)- $\beta$ -Methylpentanoic Acid. C. G. Overberger and Iwhan Cho, *J. Org. Chem.*, 33, 3321 (1968).
- (10) The Effect of Ethanol-Water Solvent Composition on Poly-4(5)-Vinylimidazole Catalyzed Esterolytic Reactions. C. G. Overberger, M. Morimoto, I. Cho and J. C. Salamone, *Macromolecules*, 2, 553 (1969).
- (11) Esterolytic Action of Synthetic Macromolecules. C. G. Overberger and J. C. Salamone, *Accounts of Chem. Res.*, 2, 217 (1969).
- (12) Esterolytic Activities of Copolymers Containing Imidazole Groups. C. G. Overberger, J. C. Salamone, I. Cho, and H. Maki, *Annals of the New York Academy of Sciences*, Vol. 155, Article 2, pp. 431-446 (1969).
- (13) Specific Catalytic Action of Large Molecules Containing Imidazole Groups. C. G. Overberger, J. C. Salamone, and S. Yaroslavsky, *Pure and Applied Chem.*, Vol. 15, 453-464 (1967).
- (14) Cooperative Effects in the Esterolytic Action of Synthetic Polymers Containing Pendent Imidazole Groups. C. G. Overberger, H. Maki, and J. C. Salamone, *Svensk kemisk tidskrift*, 80:5 (1968).



## II. Objectives

The objectives of this work have been to synthesize new catalytically active, synthetic macromolecules and to study their activities toward carbon and phosphate esters. In these investigations it was hoped that a careful study of the reaction kinetics would allow certain analogies to enzyme behavior to be drawn. Further, it was believed the field of macromolecular reactions was in itself interesting and quite different from those reactions of low molecular weight materials.

### III. Introduction

During the past decade there has been a considerable advance in understanding the reactivities of catalytically active, synthetic macromolecules with low molecular weight substrates.<sup>(1)</sup> This area of macromolecular research has been of considerable importance because of possible analogies with enzyme catalyzed processes. Although the synthetic macromolecular catalysts lack the tertiary structure, and often the secondary structure, of naturally occurring proteins, it has been possible to achieve certain behavioral patterns of enzymes. For example, synthetic polymeric species can be characterized by (a) higher reactivities than corresponding monomeric systems,<sup>(2)</sup> (b) specificity of substrate hydrolysis (particularly if the substrate is of a charge opposite to that of the charged groups on the polymer),<sup>(2-4)</sup> (c) competitive inhibition by substances similar to the reactive substrate,<sup>(5,6)</sup> (d) bifunctional catalysis involving the interactions of two pendent functional groups and substrate,<sup>(2,4,7-9)</sup> and (e) saturation (complexation) by high and low molecular weight reagents.<sup>(5,10-14)</sup> However, until recently, it has not been possible to achieve the high catalytic efficiency that is usually associated with an enzyme catalyzed reaction.

#### IV. Discussion

In our previous reports we have described that the behaviors of synthetic polymers toward low molecular weight compounds are of two different types. First, if two low molecular weight ionic species are the reactive reagents, a polymer with charged groups will tend to concentrate and/or repel one or both low molecular weight reagents in its vicinity and, consequently, will function as either an inhibitor or an accelerator of the reaction. Secondly, if catalytically active functions are added to a polymer which contains charged groups, the polymer itself, and not its counterions, is able to react with either charged or neutral reagents. Several workers have investigated enhanced or inhibited catalytic actions of polyions that contain no catalytic functions (Type 1) on the reactivities of similarly and/or oppositely charged, low molecular weight reagents. (11-13,15-18) This effect has been theoretically treated by considerations of the distribution of the electrostatic potential (11,13,15,17) and by investigation of the primary salt effect. (18) Reactions which involve polymers of the second type would apparently be more related to those of hydrolytic enzymes, since the charged groups can serve as electrostatic binding sites thereby accumulating a substrate in a high local concentration of catalytically active substituents. This cooperative effect would lead to enhanced catalytic action. (2-5,19)

Using a polymeric reaction system of Type I, we have recently reported (20) that anionic polyacrylic acid or polyvinylsulfonic acid could effectively inhibit the hydrolysis of the positively charged phosphate ester tris (choline chloride) phosphate (TCCP), an analog of the biologically important acetylcholine. Furthermore, from this investigation we were able to ascertain by a mathematical treatment the number of negative charges on the polymer backbone which are necessary for complete inhibition of the solvolytic rate and the binding constant of the substrate to the polymer.

The implications of this work are that it may be possible to use synthetic macromolecules to either accumulate or inhibit the hydrolysis of a phosphate ester. Although the above example indicated how a negatively charged polyion could accumulate a positively charged phosphate and then inhibit its hydrolysis from the catalyzing hydroxyl ions (or any other hydrolyzing species) it should be possible to incorporate other groups on the polymer chain which could facilitate hydrophobic interactions with a neutral phosphate ester. By this technique, therefore, it is conceivable that a synthetic polymer could be utilized to accumulate and possibly hydrolyze a toxic agent, such as certain phosphotriesters, and thereby protect vital enzymes from becoming phosphorylated.

Unfortunately, in this investigation we were not able to find a synthetic oxygen- or nitrogen-containing nucleophilic polymer which would attack and hydrolyze TCCP. This investigation should be continued, since if such a polymer

could be found, then the possibility of attracting a phosphotriester by either electrostatic or hydrophobic interactions, and then allowing certain nucleophilic groups to attack and hydrolyze the triester, would result in a novel and perhaps biologically important macromolecule. This type of macromolecule could be efficacious in ensuring the life process from certain toxicological agents.

Another area which has been of considerable interest to us is in the possible elucidation of enzyme action through the use of synthetic macromolecules of type II in which charged groups can electrostatically attract a substrate into the vicinity of catalytically active groups on the polymer chain. In this regard, we have been particularly interested in elucidating the effect of cooperative, multifunctional interactions between catalyst groups at the active site of an enzyme and a reactive substrate by using a synthetic macromolecule of the abovementioned type. Indeed, we have found that several types of bifunctional interactions can occur between pendent groups in either poly-4(5)-vinylimidazole or poly-5(6)-vinylbenzimidazole.<sup>(1)</sup> Investigations of this type are of considerable importance owing to the fact that the high catalytic efficiency of enzyme reactions is in part dependent on such cooperative, multifunctional interactions.

In our solvolytic work to date, we have determined that bifunctional interactions of the following types could occur<sup>(1)</sup>:

- (1) cooperative interaction of one neutral and one anionic pendent benzimidazole group in the poly-5(6)-vinylbenzimidazole catalyzed solvolysis of the anionic esters sodium 3-acetoxy-4-nitrobenzenesulfonate (NABS) and 4-nitro-3-acetoxybenzoic acid (NABA), and the neutral ester p-nitrophenyl acetate (PNPA) at high pH.
- (2) cooperative interaction of two neutral pendent imidazole groups in the poly-4(5)-vinylimidazole catalyzed solvolysis of PNPA at high pH and of two neutral pendent benzimidazole groups in the solvolysis of NABS at intermediate pH.
- (3) cooperative interaction of one neutral and one protonated pendent imidazole group in the poly-4(5)-vinylimidazole catalyzed solvolyses of NABA and NABS at intermediate pH and in the poly-5(6)-vinylbenzimidazole catalyzed solvolysis of NABS at low pH.
- (4) cooperative interaction of one neutral, pendent imidazole and one anionic, pendent phenol in the copolymer of 4(5)-vinylimidazole and p-vinylphenol catalyzed solvolyses of PNPA, NABA, NABS and the positively charged ester 3-acetoxy-N-trimethylanilinium iodide (ANTI).
- (5) cooperative interaction of one neutral, pendent imidazole and one anionic, pendent carboxylate in the copolymers at 4(5)-vinylimidazole and acrylic acid catalyzed solvolysis of ANTI.

Although a variety of cooperative interactions between pendent groups in the synthetic imidazole containing polymers have been elucidated, it had not been possible to achieve a high catalytic activity, such as that which would be characteristic of an enzymic process.

Since all the above cooperative interactions were obtained for reactions that were second-order, i.e., first-order in polymer and first-order in substrate, we attempted to better compare our synthetic system with an enzymic system by "saturating" poly-4(5)-vinylimidazole with a low molecular weight substrate. This effect of saturation had previously never been reported with a synthetic macromolecule and a low molecular weight substrate. When the neutral ester PNPA was employed, we were unable to saturate (or complex) poly-4(5)-vinylimidazole. These results suggested that hydrophobic interactions were insufficient to accumulate PNPA in the vicinity of the catalytically active polymer. However, when the negatively charged ester was employed with a partially protonated poly-4(5)-vinylimidazole, we did achieve saturation. In fact, by this reaction we were able to obtain a kinetic system of the Michaelis-Menten type, which is used so often in enzyme reactions, and we determined that the Michaelis constant ( $K_m$ ) was the same order of magnitude as that of certain enzyme substrate reactions.<sup>(14)</sup> Although this similarity, however, was of considerable interest, the turnover number for this process was very small, indicating that the overall catalytic process was not particularly efficient.

The above reaction did show that it was possible to complex a synthetic, catalytically active macromolecule with a reactive substrate. The method of attraction in this case was primarily through electrostatic forces. Since enzyme-substrate complexation presumably involves more hydrophobic or non-polar interactions than electrostatic interactions, we have begun an investigation of nonpolar interactions in poly-4(5)-vinylimidazole catalyzed reactions.

When the long-chain, anionic ester 4-nitro-3-dodecanoyloxybenzoic acid (NDBA) was employed as a substrate and poly-4(5)-vinylimidazole was the catalyst, a remarkable catalytic efficiency was achieved when the solvent system had a low concentration of ethanol. In fact, it was determined that the polymeric reaction was approximately 2000 times faster than that of the monomeric analog imidazole.<sup>(21)</sup> These extremely rapid rate constants, on the order of certain enzymic reactions, had to be determined by stopped-flow spectroscopy. By this system we were able to achieve turnover numbers which would be characteristic of an enzyme catalyzed hydrolysis of a nonspecific substrate. This is the first reported example of a synthetic polymer which not only complexed with the substrate, but also achieved a remarkably high solvolytic rate.

Perhaps the most significant point of this investigation was the pronounced effect of the ethanol-water solvent composition on the solvolytic rate of NDBA. It was found that the maximum rate was achieved in 30% ethanol-water (v/v). In this ratio it has been reported that the ethanol-water solvent system achieves a maximum "structuredness."<sup>(21)</sup> When the temperature of this reaction is varied, it is found that a maximum rate occurs near room temperature.

This is a characteristic behavior of enzymic systems, and it is highly unusual to find this with a synthetic, macromolecular catalyst.

These results suggest that if the structuredness of the solvent is important in synthetic systems, then a similar effect may occur in enzymic systems; i.e., an ordering of the water at the enzyme's active site may be necessary for high catalytic efficiency.

## V. Future Research

It is apparent that the reactions of synthetic polymers are extremely interesting, particularly with regard to the very unusual catalytic effects which can be achieved by a proper choice of reaction conditions.

In this regard we wish to continue our investigation of nonpolar interactions in these synthetic, macromolecular reactions. For example, the studies with NDBA have all been performed below the critical micelle concentration (cmc) of this substrate. As one approaches the cmc, the apolar interactions between poly-4(5)-vinylimidazole and substrate should increase dramatically, possibly leading to high catalytic rates. It is also of interest to synthesize a positively charged, long chain phenyl ester and to study its solvolysis. It is known that the short-chain, positively charged ester ANTI is not solvolyzed very effectively by poly-4(5)-vinylimidazole, perhaps because of the electrostatic repulsion which is involved. It could be expected, however, that a long chain, positively charged ester would overcome this electrostatic barrier, since the apolar interactions with polymer would presumably outweigh any electrostatic effects.

These studies will lead to a better understanding of apolar interactions between macromolecules (either natural or synthetic) and substrates, an area of extreme importance in reactions which involve complexation before a hydrolytic reaction.

We are also interested in utilizing poly-4(5)-vinylimidazole to help elucidate the role of ribonuclease (RNase) in the hydrolysis of RNA.<sup>(20)</sup> Since RNase is believed to contain two imidazole functions at its active site, poly-4(5)-vinylimidazole might effectively cleave ribonucleotides in much the same fashion as RNase. This area would add a new dimension to the reactions of catalytically active, synthetic polymers.

Another new area in which catalytically active, synthetic macromolecules may find application is in the hydrolysis of amide bonds. Although a considerable amount of research has been performed on the hydrolyses of (carbon) esters, very little attention has been given to the studies of amide hydrolyses by macromolecular catalysts. Of particular interest in this regard is the fact that Bruice<sup>(22)</sup> has shown that the protonated imidazole function is a catalytically active species in an intramolecular amide catalyzed hydrolysis. This process strongly suggests that a partially protonated poly-4(5)-vinylimidazole may be a very effective catalyst for amide cleavage. Furthermore, should this reaction prove feasible, it also should be possible to study the hydrolysis of a protein catalyzed by poly-4(5)-vinylimidazole.

As suggested before, with a proper choice of reaction conditions it should be possible to design a synthetic polymer for a specific catalytic process. We now know that one could use electrostatic forces to accumulate a charged substrate to an oppositively charged polymer. It is also possible to incorporate hydrophobic sites on a polymer chain which would then allow a substrate to be attracted to the polymer by apolar interactions. Furthermore, the electrostatic interactions and/or the apolar interactions could be used to bind the substrate to the polymer, resulting in a polymer-substrate complex. We also know that multifunctional interactions can exist between pendent groups on the synthetic polymer in their interactions with substrate, thereby leading to enhanced catalytic action. The cooperative interactions involved could exist between one type of functional group or between two different functional groups. In addition, these interactions could be between a protonated and a neutral group, a neutral and a neutral group, or a neutral and an anionic group. From our recent studies on solvent effects, it seems clear that there is also a question of "structuredness" of the solvent medium which must also be considered.

In conclusion, although synthetic macromolecules will undoubtedly never replace an enzyme for a specific hydrolysis reaction, it should be possible, based on the previously discussed effects, to design a hydrolytic system in which a synthetic polymer will function as a very efficient catalyst.



## VI. Glossary

TCCP	tris (choline chloride) phosphate
NABS	sodium 4-acetoxy-3-nitrobenzenesulfonate
NABA	4-nitro-3-acetoxybenzoic acid
PNPA	<u>p</u> -nitrophenyl acetate
ANTI	3-acetoxy-N-trimethylanilinium iodide
K <sub>m</sub>	Michaelis constant
NDBA	4-nitro-3-dodecanoyloxybenzoic acid
v/v	volume/volume
cmc	critical micelle concentration
RNase	ribonuclease
RNA	ribonucleic acid

## VII. Literature Cited

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13. ABSTRACT  The objectives of this work have been to synthesize new catalytically active, synthetic macromolecules and to study their activities toward carbon and phosphate ester. In these investigations it was hoped that a careful study of the reaction kinetics would allow certain analogies to enzyme behavior to be drawn. Further, it was believed the field of macromolecular reactions was in itself interesting and quite different from those reactions of low molecular weight materials. During the period of this contract we have found several extremely interesting catalytic effects displayed by synthetic imidazole containing polymers. Indeed, in one investigation it was found that very rapid solvolytic rates could be achieved, and that these rates of reaction were comparable to those of certain enzymic reactions. In this report are also described several new areas where synthetic macromolecules may again reveal very unusual catalytic effects.			

14.

KEY WORDS

LINK A

LINK B

LINK C

ROLE

WT

ROLE

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ROLE

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Bifunctional Catalysis

Saturation

Complexation

Catalytic Activity

Phosphotriesters

Electrostatic

Hydrophobic

Interactions

Nucleophilic

Michaelis Constant

Turnover Number

Nonpolar

Apolar





