of 10^{-3} *M*. It is possible that a similar first-order reaction occurs in benzene also but clearly would be swamped by the faster process which we have described previously as dissociative.

The rapidly increasing rate must be connected with the mixed species formed as soon as even minor amounts of polyisoprenyllithium are present. The first product probably will be (*sec*-BuLi)$_3$PILi and the reaction curve can be fitted up to about 3% conversion (where more complex species can be neglected) on the assumption that it reacts about 12 times faster than *sec*-butyllithium tetramers. The increased initial rate with *t*-BuOLi present indicates that incorporation of other polar materials have a similar effect on the rate. An accelerating effect of alkoxide has been observed in the thermal decomposition of *sec*-BuLi[7,9] in aliphatic solvents but in other cases,[8] notably where a fractional external order in alkyl was observed, the effect is to decrease the rate, as was in fact observed at the propagation stage in the present experiments.

Further analysis of the reaction becomes too difficult because of increasing complex products. Thus the meaning of the apparent order of 0.7 at maximum rates is not clear and indeed may be in error because of normalization of the rates to unit *initial* monomer concentration whereas the actual monomer concentration may depart from this value to a varying extent over the range of initiator concentration. Unfortunately it is difficult by spectrophotometric means to analyze for monomer concentration simultaneously, under the conditions used in these experiments.

The change in mechanism and rate of the initiation reactions between benzene and hexane (or cyclohexane) as solvents suggests that the alkyl is much less easily dissociated in aliphatic solvents. It is interesting that this difference parallels Brown's observation[14] of a difference of a factor of 10^3–10^4 in rates of intermolecular exchange in the two types of solvent. The exchange rate between *sec*-BuLi and PILi in hexane is, however, much faster than between *t*-BuLi and trimethylsylylmethyllithium in cyclopentane. It was suggested[14] that the exchange rate is determined by the rate of dissociation of the less easily dissociated species, which in our case would probably be *sec*-BuLi. Rapid dissociation of PILi aggregates is necessary to explain the formation of polymers of narrow molecular weight distribution in anionic polymerization. The fact that the *sec*-BuLi–PILi system exchanges reasonably rapidly is not in disagreement with our postulate of slow dissociation of *sec*-BuLi alone. First, exchange can be promoted by dissociation to the dimer stage only, as pointed out by Brown, whereas our mechanism in benzene is dependent on dissociation to the monomeric form. Second, there exists the possibility that the exchange mechanism is no longer determined by the *sec*-BuLi in presence of the more polar, resonance stabilized carbanion pair of PILi. Some direct attack of dissociated PILi on *sec*-BuLi aggregates may take place.

Macromolecule–Substrate Complexation. A Saturation Phenomenon Exhibited by Poly(4(5)-vinylimidazole) and an Anionic Ester

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Received February 10, 1968

ABSTRACT: 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 ± 0.04 min^{-1}, respectively.

The selectivity and efficiency of an enzymic reaction are in part dependent upon a complexation of the substrate to the enzyme, followed by an "intramolecular" catalytic reaction.[2] It has recently been demonstrated that multifunctional catalysis, a unique characteristic of enzymatic reactions, can also be obtained when synthetic, imidazole-containing polymers are employed as catalysts.[3]

The binding sites of enzymes, necessary for a com-

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plexation with substrate, may be different from the catalytic sites, and the nature of the binding forces may vary from one enzyme to another. Model enzyme systems have demonstrated the efficiency of three main categories of binding substrate to catalyst: (i) covalent and electrostatic binding of a substrate to a catalyst which is a metal complex;[4,5] (ii) electrostatic binding of a charged substrate to (a) an oppositely charged polyion[6] and to (b) a macromolecule containing both charged groups and catalytically active functions;[3,7] (iii) hydrophobic bonding: (a) inclusion of substrates in cyclodextrin cavities[8] and (b) dissolution of lyophilic substrates in detergent micelles.[9]

For each category of binding mentioned above, kinetic evidence has indicated the formation of a catalyst–substrate complex in analogy with that of an enzyme–substrate complex. Regarding esterolytic catalyses, there have been two principal types of saturation phenomena when synthetic macromolecules were employed as catalysts. When the reactions of two low molecular weight species occur in the presence of polyions which contain no catalytically active groups, saturation effects have been observed when either substrate concentration, ionic strength, or catalyst concentration is increased.[6b,e,f] These processes would presumably differ from enzymic reactions because for the latter neither counterions are responsible for the catalytic reaction nor are the reactivities of the low molecular weight species dependent on their mutual attraction to the polyion. The second type of saturation effect was observed in a study of the solvolytic rates of ester functions in copolymers containing anionic sites catalyzed by partially protonated poly((N-vinylimidazole),[7d,e] a polymer possessing both charged and catalytically active groups. It was found that a limiting value to the rate, indicative of the association of catalyst and substrate, could be observed at high concentrations of either substrate or catalyst, a behavior rarely observed in enzyme systems. Although macromolecules are substrates for enzymes, only a specific portion of the macromolecular substrate takes part in the reaction. Therefore, the solvolysis of a polymeric substrate, where catalysis of the ester functions would be at random along the chain, would differ considerably from a natural enzyme process.

In the present work we have investigated the saturation effect in a system more closely resembling that encountered with natural enzymes, *viz.*, the solvolytic rates of low molecular weight substrates catalyzed by a polymer containing both charged groups and catalytically active functions under conditions in which the substrate concentration was in excess of the catalyst concentration. Polymers containing the imidazole group are of primary importance for biological study because the imidazole moiety of histidine is believed to be involved in the active sites of enzymes such as α-chymotrypsin,[10] acetylcholinesterase,[11] and bovine pancreatic ribonuclease.[12] The catalyst employed was poly(4(5)-vinylimidazole), and the substrates were the neutral ester *p*-nitrophenyl acetate (PNPA) and the negatively charged ester sodium 4-acetoxy-3-nitrobenzenesulfonate (NABS). Poly(4(5)-vinylimidazole) has been shown to be a considerably more efficient esterolytic catalyst than either of its isomers, *i.e.*, poly(N-vinylimidazole)[3a] and poly(2-vinylimidazole).[13] The esters PNPA and NABS were chosen for study because their binding to the partially protonated polymer would be expected to be of a different nature. The neutral ester PNPA, with no pronounced binding sites, could be expected to be attracted to the polymer by hydrophobic forces, whereas the negatively charged ester NABS would be strongly attracted to the protonated sites on the polymer chain by electrostatic forces.

Experimental Section

Poly(4(5)-vinylimidazole) and the substrates ***p*-nitrophenyl acetate** and **sodium 4-acetoxy-3-nitrobenzenesulfonate** were prepared as previously described.[3a,b,14]

Kinetic measurements were performed essentially as previously reported.[3a] A stock solution of poly(4(5)-vinylimidazole) was prepared in 28.5% ethanol–water. Samples of the polymer solution were diluted with an 0.02 *M* tris(hydroxymethyl)aminomethane and hydrochloric acid buffer at an ionic strength adjusted to 0.02 with potassium chloride. Kinetic measurements were obtained utilizing a Cary Model 14 recording spectrophotometer thermostated at 26°. The instrument was balanced at either 406 mμ for PNPA or 410 mμ for NABS. The substrate in 2-propanol (10–60 μl) was then added to 3 ml of the buffered catalyst solution, the cells were shaken briefly and returned to the thermostated compartment, and the recorder was started. Complete records of the increase in optical density with time were obtained, starting at approximately 20 sec after

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the addition of substrate, a period which is negligible compared to the long reaction times involved.

The initial slopes of the recorded plots of optical density as a function of time were constructed graphically (up to 5% reaction), converted to concentration changes as a function of time, and reported as the initial solvolysis rates (v_{measd}). Blank measurements (uncatalyzed reactions) of the initial rates (v_{blank}) were obtained in the same manner. For PNPA, the optical density *vs.* time plots were straight lines up to at least 10% reaction. In the case of NABS, the reaction appeared more complicated since appreciable curvature of the plots was noted quite early in the reaction. This is perhaps due to inhibition of the reaction by the anionic product. The actual concentration of substrate in a cell was obtained by measuring the absorption after the reaction was complete, *i.e.*, after at least ten half-lives. If necessary, solutions were diluted for this measurement. The pH values were determined before and after each reaction to ensure constancy.

The observed initial solvolysis rate, v_{obsd}, of the substrate is a composite of the initial rates of the catalyzed (v_{measd}) and uncatalyzed (v_{blank}) reactions, *i.e.*, $v_{obsd} = v_{measd} - v_{blank}$.

Results and Discussion

The solvolytic rate measurements of PNPA catalyzed by poly(4(5)-vinylimidazole) were performed at pH 8.0. At this pH value poly(4(5)-vinylimidazole) has been shown to be a more efficient catalyst than its monomeric analog imidazole.[3a,c,d] This enhanced effect was attributed to a cooperative interaction between two imidazole functions on the polymer chain and the substrate, the over-all reaction being pseudo second order. The kinetic data for the catalyzed solvolyses of PNPA at constant poly(4(5)-vinylimidazole) concentration of 5×10^{-5} *M* (in imidazole groups) were determined for concentrations of PNPA up to approximately 25 times that of catalyst. It was found that, at constant catalyst concentration, the initial rates are proportional to the substrate concentrations studied. The first-order observed rate constant for the reaction,[3a] employing the first-order rate equation $v_{obsd} = k_{obsd}$ (S), was determined to be 0.58×10^{-2} min^{-1}. Previously it was shown that at constant substrate concentration the reaction rate constants were also proportional to the catalyst concentration.[3a] Therefore it is apparent that, in the range of substrate to polymer proportions studied in this and previous investigations, the solvolysis of PNPA is an over-all second-order reaction, *i.e.*, first order in both substrate and catalyst. The lack of a limiting initial rate with increased substrate concentration could perhaps be accounted for by the fact that the substrate concentration was not sufficiently high and/or that hydrophobic forces are not sufficient to accumulate a high local concentration of the neutral ester in the vicinity of the polymer.

The catalytic behavior of poly(4(5)-vinylimidazole) toward NABS was quite different than that noted for PNPA. Rate measurements were performed for two concentrations of polymer at pH 7.1, since it has been shown that poly(4(5)-vinylimidazole) efficiently catalyzes the solvolyses of anionic esters at intermediate pH.[3a,d] The protonated sites on the polymer chain, which do not contribute to the solvolysis of PNPA, serve as binding sites for the anionic esters, thereby accumulating them in a high local concentration of catalytically active

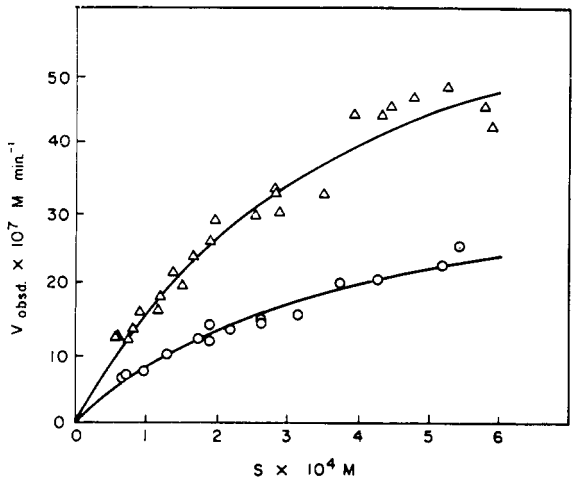

Figure 1. The observed initial rates of (○, 2.5×10^{-5} *M*; △, 5×10^{-5} *M*) poly(4(5)-vinylimidazole)-catalyzed solvolyses of NABS as a function of the substrate concentration, pH 7.1, 28.5% ethanol–water. The solid lines were calculated from the expression $v_{obsd} = V(S)/(K_m + (S))$ using the values of V and K_m given under Results and Discussion.

imidazole functions. This electrostatic effect would be expected to facilitate the saturation of the partially protonated polymer when high concentrations of substrate are employed. Such an electrostatic effect would be of a type similar to those previously reported.[6b,e,f,7d,e]

Figure 1 shows the plots of the observed initial rates against the substrate concentrations for the two concentrations of poly(4(5)-vinylimidazole) employed. The results of the uncatalyzed reaction, when treated by the least-squares method to the first-order rate equation $v_{blank} = k_{blank}$(S), gave the uncatalyzed rate constant k_{blank} of 0.28×10^{-3} min^{-1}. Although the data are somewhat scattered, a sufficient number of measurements were taken to illustrate the saturation effect. The general trend of the initial rates approaching a limiting value is quite pronounced. In its simplest form the kinetic expression for an enzymatic reaction can be represented by the Michaelis and Menten mechanism,[15] which can be written in the form of eq 1.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + Pr \qquad (1)$$

The mechanism is based on the assumption that the enzyme, E, reacts reversibly with the substrate, S, to form an enzyme–substrate complex, ES, which is then decomposed, giving back the free enzyme and a product or products, Pr.

Assuming that a similar mechanism applies in the present case, *i.e.*, the substrate first being complexed by the polymeric catalyst and then solvolyzed, and that there are no interactions between bound ester molecules which could affect the complexation of polyion and substrate, the rate expression derived from this mechanism can be written as in eq 2, where (P) is the

$$v_{obsd} = \frac{k_2(PS)}{K_m + (S)} = \frac{V(S)}{K_m + (S)} \qquad (2)$$

polymer concentration, K_m is the Michaelis constant

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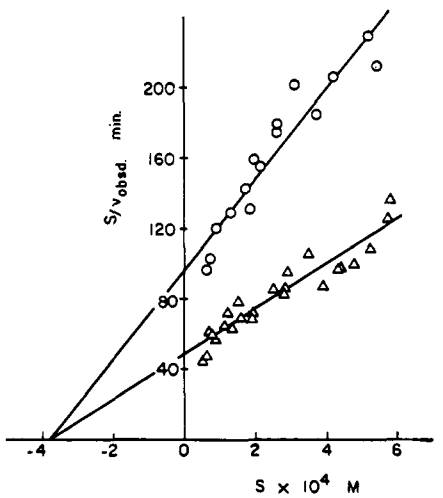

Figure 2. Determination of V and K_m for (○, 2.5 × 10^{-5} M; △, 5.0 × 10^{-5} M) poly(4(5)-vinylimidazole)-catalyzed solvolyses of NABS at pH 7.1, 28.5% ethanol–water.

($K_m = (k_{-1} + k_2)/k_1$) and V is the maximum velocity ($V = k_2(P)$).

Equation 2 can be rearranged to give eq 3. If

$$(S)/v_{obsd} = K_m/V + (S)/V \quad (3)$$

$(S)/v_{obsd}$ is plotted against (S), a straight line is obtained with a slope of $1/V$ and an intercept of the vertical axis of K_m/V. The negative value of K_m can also be obtained by extending the graph to the (S) axis. Figure 2 shows the data obtained from Figure 1 plotted in this manner for the two concentrations of poly(4(5)-vinylimidazole) studied. The solid lines were computed by a least-squares treatment. The values of V calculated for concentrations of poly(4(5)-vinylimidazole) of 2.5 × 10^{-5} M and 5.0 × 10^{-5} M are (3.95 ± 0.24) × 10^{-6} M min^{-1} and (7.82 ± 0.55) × 10^{-6} M min^{-1}, respectively. It is apparent that the values of V agree quite well, since the maximum rate should be proportional to the catalyst concentration. Values of K_m were determined to be (3.79 ± 0.20) × 10^{-4} M and (3.81 ± 0.22) × 10^{-4} M for catalyst concentrations of 2.5 and 5.0 × 10^{-5} M, respectively. Although the average Michaelis constant of (3.80 ± 0.21) × 10^{-4} M is lower than similar constants obtained for several neutral substrates of α-chymotrypsin-catalyzed reactions,[16] it is of the same magnitude as that of acetylcholinesterase with its positively charged substrate acetylcholine.[17] The latter reaction is known to be due in part to the electrostatic attraction of the positively charged portion of the substrate to an anionic site on the enzyme followed by a catalytic interaction from the esteratic site.[11] Indeed, in the present work, the determined value of K_m would appear to indicate a strong affinity between the anionic substrate and the partially protonated polymer.

The values of V determined in this study can be used to calculate the constant k_2, sometimes called the "turnover number," since V is defined by $k_2(P)$. The question which arises is the meaning of (P) in this system. In enzyme studies, the concentrations of enzyme are expressed as milligrams of nitrogen per milliliter or as units/milliliter. In the present case the composition of the catalyst is known, but the fraction of imidazole groups participating in bonding has yet to be determined. It can be assumed, somewhat arbitrarily, that this is equal to the fraction of protonated imidazole residues on the polymer chain which would account for the coulombic attraction of the substrate to the catalyst. A redetermined potentiometric titration of poly(4(5)-vinylimidazole) at an ionic strength of 0.02 afforded a value of 0.25 for the fraction of protonated imidazole residues at pH 7.[18] The value k_2 thus calculated is 0.63 ± 0.04 min^{-1}, a value of several orders of magnitude smaller than those of hydrolytic enzymes.

Although the present study has shown a high specificity of poly(4(5)-vinylimidazole) for the negatively charged ester NABS, as previous results have indicated,[3d] it is apparent that this system does not approach the catalytic efficiency encountered in enzymic reactions. It is generally believed that the tertiary structure of the enzyme is required for an intimate association and activation of the substrate molecule. The absence of a conformationally rigid tertiary structure in solution, characteristic of all synthetic macromolecular catalysts investigated to date, would be expected to preclude the efficiency of enzyme systems.

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