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Specific Effects in Acid Catalysis by Polymeric Sulfonic Acids in the Hydrolysis of the Amino Ester and Related Compounds

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The amino ester hydrolysis by polymeric sulfonic acids increased as the concentration ratio, $C_{\mathrm{ester}}/C_{\mathrm{acid}}$, was lowered, and a maximum in the plot of the rate constant, k, against the $C_{\mathrm{ester}}/C_{\mathrm{acid}}$ ratio was attained near the ratio=0.4 in the case of the β-alanine methyl ester as well as in the case of γ-methyl L-glutamate. At this optimum concentration, the catalytic effect of polyvinylsulfonic acid (A), for example, in the hydrolysis of the β-alanine methyl ester was 16 times greater than that of hydrochloric acid, while the effect of polystyrenesulfonic acid was about 7 times greater. The catalytic effects of hydrochloric acid and ethanesulfonic acid were the same in all cases, and there was no such specific behavior as in the former. Furthermore, the catalytic efficiency of polyvinylsulfonic acid in amino ester hydrolysis increased with an increase in the polymer molecular weight, in contrast with the case of the carboxylic ester, while no such correlation was found in the polystyrenesulfonic acid. Besides, the catalytic effect of partially-neutralized polymeric sulfonic acid, polyvinylsulfonic acid especially, decreased remarkably with the degree of neutralization. These different kinds of behavior can be interpreted as being due to the difference in the expansion in solutions and in the geometrical distances between nearest-neighboring sulfonic acid groups on the polymer chain.

A number of studies[2] have been carried out in recent years on the charge interaction in the reactions using polyelectrolytes in order to reveal the mechanism of enzyme action. When organic reactions are carried out between a polymeric molecule and a simple substrate, functional interactions not only between neighboring groups, but also between polymer and substrate, especially when electrically-charged, are very often responsible for a change in the reaction path, consequently, they provide additional information regarding the mode of action of the enzyme. Since polymeric sulfonic acids with ionic groups along the polymer chain show properties of both electrolytes and polymers, one possible mechanism of enzyme specificity may be expected to be due to the electrostatic attraction of the poly-anion with the cationic reagent, when it is used as a catalyst in the reaction with a basic substrate. In a previous paper,[3] we have

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[1] Dissertation submitted by Oh-Kil Kim in partial fulfillment of the requirements for the Ph. D. degree in the Graduate School of the University of Tokyo.

[2] a) H. Ladenheim, E. M. Loebl and H. Morawetz, *J. Am. Chem. Soc.*, **81**, 20 (1959); b) W. Kern and B. Scherhag, *Makromol. Chem.*, **28**, 209 (1958); c) R. L. Letsinger and T. J. Savereide, *J. Am. Chem. Soc.*, **84**, 114 (1962); d) C. G. Overberger, T. St. Pierre, N. Vorchheimer and S. Yaroslavsky, ibid., **85**, 3515 (1963).

[3] S. Yoshikawa and O.-K. Kim, This Bulletin, **39**, 1515 (1966).

demonstrated that the acid-catalyzed hydrolysis of carboxylic esters by polymeric sulfonic acid was governed by the hydrophobic interaction between the polymer catalyst and the substrate and, moreover, influenced by the molecular weight of the polymer.

The present work was undertaken in an attempt to find how the charge interaction of the polymeric acid on amino esters is operative with the hydrolysis rate in a homogeneous solution and to explain the mechanism of hydrolysis upon the basis of the charge density of the polymer acid. In this study we selected the hydrolysis of amino ester and related compounds using polyvinylsulfonic acid (PVSH), polystyrenesulfonic acid (PSSH) and sulfonated polystyrene sulfonic acid ((PS)SH), in addition to the monomeric analogue, ethanesulfonic acid, and hydrochloric acid, in order to compare the hydrolysis with that of uncharged carboxylic esters. The sulfonic acids were prepared by different polymerizing processes from their respective monomers (except for sulfonated polystyrene sulfonic acid), and all were water-soluble.

Experimental

Amino Esters.—Glycine methyl ester hydrochloride was prepared by refluxing a solution of glycine in absolute methanol containing anhydrous hydrogen chloride. α- and β-Alanine methyl esters, the γ-aminobutyric acid methyl ester and γ-methyl L-glutamate were prepared by treating each amino acid with methanolic hydrogen chloride. All the esters were recrystallized from anhydrous methanol-ether and dried in vacuo over sodium hydroxide. Acetylcholine chloride was obtained commercially.

Polymeric Sulfonic Acid.—The monomers, sodium vinylsulfonate and potassium *p*-styrenesulfonate, were prepared from sodium β-bromoethanesulfonate and sodium hydroxide by modifying the patent directions,[4] and from β-bromoethylbenzenesulfonyl chloride and potassium hydroxide according to the method of Wiley and Reed.[5] The polymerizations of sodium vinylsulfonate and potassium *p*-styrenesulfonate were carried out by three different methods.

Poly-(sodium vinylsulfonate) was obtained as follows:

PVSH(A).—Five grams of sodium vinylsulfonate in 4.9 ml. of water was poured into an ampoule, chilled in ice, and evacuated to about 2 mmHg, the evacuation and pressuring with nitrogen was repeated, and then the ampoule was sealed. The polymerization was carried out by exposing it to a ^{60}Co source, yielding about 2×10^5 r./hr., for 72 hr. at 0°C. The resulting sticky solid was dissolved in 30 ml. of water and precipitated by adding 45 ml. of methanol. Treating the gummy precipitate with methanol yielded 3.5 g. of straw-colored polymer after it had been dried in vacuo over phosphorus pentoxide. The intrinsic viscosity[6] of the polymer was 12.8, and the molecular weight[6] was 20000.

PVSH(R).—Twelve grams of a 50% aqueous solution of sodium vinylsulfonate containing 0.057 g. of potassium persulfate and 0.023 g. of sodium bisulfite was poured into an ampoule; the ampoule was then evacuated, repeatedly filled with nitrogen, and then sealed. After the ampoule had been tumbled in a bath at 18°C for 72 hr., the resulting viscous oil was dissolved in 30 ml. of water and precipitated with 45 ml. of methanol; 4.08 g. of polymer was obtained after drying in vacuo over phosphorus pentoxide. The intrinsic viscosity was 7.20, and the molecular weight was 12000.

PVSH(S).—Five grams of finely-powdered and dried crystalline sodium vinylsulfonate was sealed in an ampoule and then exposed to a ^{60}Co source, yielding about 2.5×10^5 r./hr., for 72 hr. at room temperature. The irradiated crystals, after precipitation with 70% aqueous methanol in the usual fashion in order to remove monomers, gave 2.5 g. of the polymer. The intrinsic viscosity and the molecular weight of the polymer were 1.70 and 3000 respectively.

Poly-(potassium *p*-styrenesulfonate) was obtained as follows:

PSSH(A).—Four grams of potassium *p*-styrenesulfonate in 16 ml. of water was poured into an ampoule. Polymerization reactions were then carried out in the same manner, with γ-irradiation, as in the case of PVSH(A), except that this was done at room temperature. The polymer formed was precipitated from 70% aqueous dioxane. After drying in vacuo over phosphorus pentoxide, 2.5 g. of the polymer was yielded. The intrinsic viscosity[7] of the polymer was 42.0.

PSSH(R).—Five grams of potassium *p*-styrenesulfonate in 15 ml. of water was placed into an ampoule, and then a solution of 1.35 g. of potassium persulfate and 0.66 g. of sodium bisulfite in 2 ml. of water was added as an initiator. The polymerization was carried out in the same manner as has been described in the case of PVSH(R). The resulting viscous oil was precipitated from 70% aqueous dioxane; 3.2 g. of polymer was thus obtained after drying in vacuo over phosphorus pentoxide. The intrinsic viscosity was 30.7.

PSSH(S).—Five grams of finely-powdered and dried crystalline potassium *p*-styrenesulfonate was sealed in an ampoule and exposed to radiation under the same conditions as have been described in the case of PVSH(S). The irradiated crystals yielded 2.3 g. of the polymer after precipitation with 75% aqueous methanol; the polymer was dried according to the usual fashion. The intrinsic viscosity was 18.09. All the sodium and potassium polymer sulfonates were completely converted into free acids by passing them through a column of cation exchange resin in a hydrogen form such as Amberlite IR-120.

(PS)SH (69%).*—The sulfonation of polystyrene ($\overline{M}_w=15\times10^4$) was carried out, using the addition-compound of sulfur trioxide and dioxane, by the method

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[4] D. B. Pat. 836491, Farbwerker Hoechst, *Chem. Abstr.*, **47**, 2766 (1953).

[5] R. H. Wiley and S. F. Reed, Jr., *J. Am. Chem. Soc.*, **78**, 2171 (1956).

[6] The value of intrinsic viscosity of poly-(sodium vinyl sulfonate) was determined in 0.5 N NaCl solution at 20°C and the molecular weight was calculated by K. Dialer and R. Kerber, *Makromol. Chem.*, **17**, 56 (1956).

* The degree of sulfonation.

[7] The value of initrnsic viscosity of poly-(potassium *p*-styrenesulfonate) was determined in 2 N NaOH solution at 20°C.

described by Signer and his co-workers.[8] To a finely-divided suspension of the adduct, consisting of 20 g. of sulfur trioxide and 26 ml. of dioxane in 140 ml. of dichloroethane, a solution of 26 g. of polystyrene in 140 ml. of dichloroethane was added in one portion, then the mixture was agitated for several minutes. The sulfonation proceeded to 69% after the mixture had stood at 20°C for 24 hr. The reaction was then stopped by the addition of water. The polymer was dissolved easily in water and neutralized with sodium hydroxide. The sulfonated polymer salt was dialyzed to remove sulfuric acid through a semipermeable membrane by keeping it in distilled water for one month. The dialyzed sulfonated polymer salt was converted into free acid through ion exchange resin in the hydrogen form by the usual method.

Ethanesulfonic acid (Et.-SO_3H) was prepared from ethyl bromide, sodium sulfite and water according to the directions of Houlton and Tartar.[9]

Hydrolysis Procedure.—All the kinetic studies reported here were carried out in aqueous solution at 45±0.01°C. The initial concentrations of amino acid ester hydrochlorides were adjusted to 0.20, 0.10, 0.05, 0.033, 0.025 and 0.020 mol./l., and the acid catalyst concentration, to 0.08 N. Three or four samples were used in each run, and each reaction mixture was made up by taking 4 ml. of the ester and an equal volume of the acid, both concentrations being initially adjusted. The reaction mixtures were contained in 10 ml. glass-stoppered bottles suspended in a thermostat controlled bath. The tubes were removed at suitable intervals and placed in an ice bath. After hydrolysis, the tubes were opened, and the contents were washed from the tube with water into a 100 ml. beaker; to this 10 ml. of the 37.5% formaldehyde solution, which had been neutralized with basic magnesium carbonate just before use, was added. A modified form of the Sörensen formol titration proposed by Dunn and Loshakoff[10] was used to determine the extent of hydrolysis. Titrations were made with 0.05 N sodium hydroxide, using an automatic titrator (Metrohm Co. potentiograph E336), in order to determine the end point. The titration of the known amino acid showed that this method gave satisfactory results, within ±0.2%. The hydrolysis rate constants were calculated using the first-order rate equation. The efficiency of the catalyst, q, was defined as the ratio of the specific rate of hydrolysis catalyzed by polymeric sulfonic acid to that catalyzed by hydrochloric acid.

Results and Discussion

Several series of experiments have been carried out, at a constant acid concentration (0.04 mol./l.), in order to examine the influence of the ester concentration on the order of the hydrolysis reaction. The experimental apparent first-order rate constant was evaluated from the slopes of the plots of the logarithm of the ester concentration against the time; it was found that the hydrolysis reaction proceeds through a first-order mechanism with respect to the ester concentration, as is shown in Fig. 1.

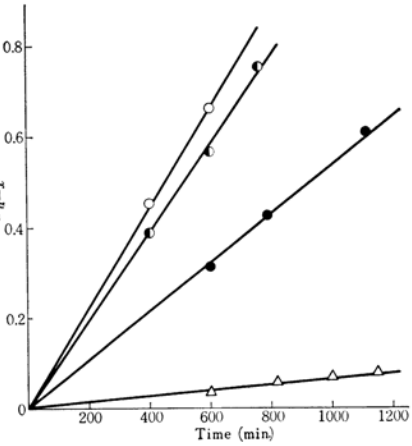

Fig. 1. Hydrolysis of β-alanine methyl ester catalyzed by 0.0405 N HCl and 0.0421 N PVSH-(A) in different ester concentrations (as indicated in the following parenthesis) at 45°C.
△ (0.0511 mol./l.) by HCl; ● (0.0511 mol./l.), ◐ (0.0255 mol./l.) and ○ (0.0170 mol./l.) by PVSH(A).

The effect of the structure of the amino ester on the rate of its hydrolysis can only be touched on here. One general point was that the α-amino ester tends to be less readily hydrolyzed than the β- or the γ-amino ester; this can be attributed to the fact that the former ester bond is nearer the positively-charged amino group in the amino ester. The results, given in Table I, indicate that all amino esters are hydrolyzed much more rapidly by polymeric sulfonic acids, especially PVSH(A), than by hydrochloric acid; the β-alanine methyl ester,

TABLE I. HYDROLYSIS RATES OF SEVERAL AMINO ACID METHYL ESTERS RELATING TO THE STRUCTURE OF AMINO ACIDS

$10^3 k$ (l.mol^{-1}min^{-1})

Ester** Acid*	Gly.Me.	β-Ala.Me.	γ-Amino n-Bu.Me.
HCl	1.10	3.46	10.63
PVSH(S)	3.52	16.91	39.80
PVSH(R)	7.01	25.48	66.31
PVSH(A)	8.25	30.21	76.70
PSSH(R)	5.81	15.13	42.76
(PS)SH (69%)	6.60	19.37	44.37

* The concentrations of acids were:
HCl, 0.0405 N; PVSH(S), 0.0428 N;
PVSH(R), 0.0396 N; PVSH(A), 0.0421 N;
PSSH(R), 0.0424 N; (PS)SH (69%), 0.0425 N.

** The concentrations of glycine-, β-alanine- and γ-aminobutyric acid methyl ester were 0.0500, 0.0511 and 0.0501 mol./l., respectively.

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[8] R. Signer, A. Demagistri and C. Müller, *Makromol. Chem.*, **18/19**, 139 (1956).

[9] H. G. Houlton and H. V. Tartar, *J. Am. Chem. Soc.*, **60**, 544 (1938).

[10] M. S. Dunn and A. Loshakoff, *J. Biol. Chem.*, **113**, 359 (1936).

TABLE II. RATE DEPENDENCE AS A FUNCTION OF THE RATIO OF THE CONCENTRATION OF THE ESTER TO THE ACID IN THE HYDROLYSIS OF SEVERAL KINDS OF AMINO ESTERS AND RELATED COMPOUNDS

a) Glycine methyl

10^3k (l.mol^{-1}min^{-1})

Ester / Acid*	0.0500	0.025	0.0167(mol./l.)
HCl	1.10	1.08	0.98
Et.-SO$_3$H	1.19	1.22	1.16
PVSH(S)	3.52	5.75	7.42
PVSH(R)	7.01	12.11	15.22
PVSH(A)	8.25	14.40	17.90
(PS)SH (69%)	6.60	10.56	14.47

b) β-Alanine methyl

10^3k (l.mol^{-1}min^{-1})

Ester / Acid*	0.0511	0.025	0.0170	0.0128(mol./l.)
HCl	3.46	3.80	3.98	
Et.-SO$_3$H	3.69	3.94	3.67	
PVSH(S)	16.91	25.25	27.73	
PVSH(R)	25.48	46.03	54.27	46.06
PVSH(A)	30.20	50.51	60.47	45.52
PSSH(R)	15.13	22.78	25.82	
PSSH(A)	15.63	23.91	25.90	
(PS)SH (69%)	19.37	27.21	29.75	27.29

c) L-Glutamic acid γ-methyl

10^3k (l.mol^{-1}min^{-1})

Ester / Acid*	0.0384	0.0192	0.0128(mol./l.)
HCl	2.84	3.50	3.76
Et.-SO$_3$H	2.75	3.21	3.50
PVSH(R)	35.10	52.84	46.12
PVSH(A)	38.74	60.60	55.55
PSSH(R)	19.83		
(PS)SH (69%)	20.27	35.10	42.86

d) Acetylcholine chloride

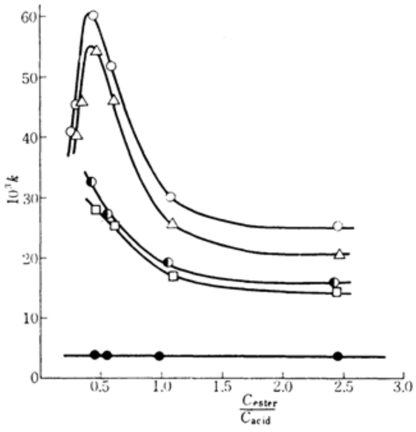
10^3k (l.mol^{-1}min^{-1})

Ester / Acid*	0.0501	0.0250(mol./l.)
HCl	9.72	10.60
Et.-SO$_3$H	9.94	9.78
PVSH(R)	57.78	111.18
PVSH(A)	67.10	116.50
PSSH(R)	47.29	78.05
(PS)SH (69%)	48.96	76.15

* The concentrations of acids used in all runs were:
HCl, 0.0405 N; Et.-SO$_3$H, 0.0420 N;
PVSH(S), 0.0428 N; PVSH(R), 0.0396 N;
PVSH(A), 0.0421 N; PSSH(R), 0.0424 N;
PSSH(A), 0.0413 N; (PS)SH (69)%, 0.0425 N.

for instance, was hydrolyzed 8.6 times faster by PVSH(A) than by hydrochloric acid.

Moreover, PVSH was a more effective catalyst than PSSH and (PS)SH (69%). A striking change was observed on varying the ester concentration in the hydrolysis of every amino ester with polymeric sulfonic acids, as is tabulated in Table II. The effect of the substrate concentration on the rates of hydrolysis with polymeric sulfonic acids at a constant acid concentration was determined. The hydrolysis rate constant increased with a decrease in the ester concentration (at a constant acid concentration), but subsequently rose to a maximum. It was reproduced in the plot of the ratio of the concentration of the ester-to-polymer catalyst, C_{ester}/C_{acid}, against the hydrolysis rate constant, k, with respect to the reaction of the β-alanine methyl ester, as is illustrated in Fig. 2. A maximum was attained near the concentration ratio of 0.4 in the case of the β-alanine methyl ester as well as in that of γ-methyl L-glutamate. No such specific behavior was, however, found in either the hydrolysis by hydrochloric acid or that by ethanesulfonic acid; the catalytic effects of these two acids were the same.



$[\eta]_{0.5NaCl}$ (ml.g^{-1})

Fig. 2. Rate dependence as a function of the ratio of the concentration of the ester to the acid in the hydrolysis of β-alanine methyl ester:
●, HCl; □, PVSH(S); △, PVSH(R);
○, PVSH(A); ◐, (PS)SH (69%).

This accounts for the fact that hydrolysis takes place more easily at the optimum concentration of the ester within the domain of the polymer molecules. In other words, a larger value of the concentration ratio (over 0.4) leads to a shortage of the catalytically available neighboring free acid group towards the polymeric acid-substrate complex, while a smaller one (below 0.4) leads to a shortage of the complex available to attack by the nieghboring free acid group. This phenomenon is of interest in discussing the reaction mechanism in connection with the findings that the solvolytic rates of potassium 3-nitro-4-acetoxybenzenesulfonate catalyzed by 0.010 base molar poly-(4-vinylpyridine) increase as the pH is lowered and that a maximum in the k - α plot (k is the rate constant, and α is the fraction of nitrogen present as the free base) is attained near $\alpha = 0.6$ (pH 3.6), as has been reported by Letsinger and Savereide.[2c]

This specific effect in acid catalysis by polymeric sulfonic acids proves especially interesting when the catalytic activity relating to the structure of the polymer catalyst is discussed. The above data support the hypotheses that a free amino group must be present on the substrate if the anion of polymeric sulfonic acid is to be catalytically active, and that a complex is formed between the amino group of the substrate and the charged sulfonate group of the polymer catalyst before hydrolysis takes place.

Moreover, it was found that a methyl-substituted amino group of the substrate, such as acetylcholine chloride, also underwent the same specificity. This means that the reaction is governed only by the charged interaction between the polymer catalyst and the substrate insensitive to the steric factor of the substituents on the amino group. These observations are supported by the fact that the catalytic effects of polymeric sulfonic acids in the hydrolysis of the acetylglycine methyl ester were nearly the same as that of hydrochloric acid; the rate constants of HCl, PVSH(R) and PSSH(R), under the same conditions as is shown in Table II except for the ester concentration (0.021 mol./1.), were 9.08×10^{-3}, 9.45×10^{-3} and 9.81×10^{-3} respectively, which leads to the conclusion that there is no interaction between the substrate and polymeric sulfonic acid.

Furthermore, the difference in the catalytic effects between PVSH and PSSH can be attributed to the difference in the expansion in solutions and in the geometrical distances between sulfonic acid groups on the polymer chain, as has been described in a previous paper[3] with respect to the hydrolysis of carboxylic esters. The molecules of PVSH in solutions are possibly not greatly expanded as compared to that of PSSH; therefore, the localized protons along the polymer are confined within an extremely limited region in the reaction system in proportion to the magnitude of the molecular weight of PVSH. This interpretation is probably closely related to the fact that the catalytic efficiency, q, of PVSH increases gradually with the magnitude of its molecular weight, as is shown in Fig. 3, though no such correlation was found with PSSH-type catalysts. This observation can be explained by the fact that the complex formed by the preferential binding of the poly-anion to the positively-charged amino group of the substrate was surrounded by a high proton concentration in the polymer coil arising from the limited expansion of PVSH compared to that of PSSH.

Fig. 3. Relation between the catalytic efficiency and the molecular weight of polyvinylsulfonic acid at constant C_{ester}/C_{acid} in the hydrolysis of β-alanine methyl ester (○) and L-glutamic acid γ-methyl ester (△).

Therefore, the local condensation of protons in the reaction system caused by the limited expansion of the polymer is largely responsible for the specific behavior of polymer catalysts, especially PVSH. The hydrolysis rate constants of the β-alanine methyl ester hydrolyzed by 30% neutralized PVSH(S) (0.0554 N) and by 30% neutralized (PS)SH (69%) (0.0620 N), both neutralized with barium hydroxide and where the ester concentration was 0.100 mol./1., were 6.84×10^{-3} and 12.57×10^{-3} (1. mol^{-1} min^{-1}) respectively, while the rate constants for the ester hydrolyzed by un-neutralized PVSH(S) and (PS)SH (69%) under the same conditions were 14.8×10^{-3} and 16.9×10^{-3} (1. mol^{-1} min^{-1}) respectively.

Such markedly different catalytic behavior of the two types of polymer catalysts can be attributed to the difference in the molecular expansion. The difference in the catalytic effects between sulfonated isotactic polystyrene sulfonic acid (70% sulfonation)[11] and the atactic one ((PS)SH(69%)) was, however, not very distinct for the series of amino esters; the hydrolysis rates with the former were 19.04×10^{-3} for the β-alanine methyl ester (0.0170 mol./1.), 35.16×10^{-3} for methyl γ-aminobutyrate (0.0501 mol./1.), and 44.10×10^{-3} for acetylcholine chloride (0.0501 mol./1.) at 45°C. These observations can be interpreted to mean that the difference in the geometrical distances between sulfonic acid groups on the polymer differing in configuration was not distinct enough in a solution to cause a difference in the catalytic activity.

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[11] Sulfonation of isotactic polystyrene ($\overline{M}_w > 5 \times 10^5$) was carried out in sulfuric acid for 6 hr. at 95°C using 10 g. of finely powdered isotactic polystyrene, 0.5 g. of silver sulfate and 100 ml. of concentrated sulfuric acid by modifying the directions by Takahashi and co-workers (*J. Chem. Soc. Japan, Ind. Chem. Sect.* (*Kogyo Kagaku Zassi*), **61**, 1617 (1958)).