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Decarboxylation of Oxalacetate by Polymeric Amines

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ABSTRACT: Decarboxylation of oxalacetate was examined in the presence of three polymeric amines: quaternized polyethylenimine, poly(L-lysine), and 3,6-ionene polymer (polybrene). Except polybrene, all polymers enhanced the rates of increase and then decrease of absorbance at 260 nm of the enol form of the substrate. By a modified Michaelis–Menten scheme which takes into account the keto–enol equilibria of both the substrate and the substrate–polymer complex, the rate constants of all steps could be estimated. From these values, it was shown that electrostatic interactions between the substrate and polymer and the hydrophobic environment of the polymer influence the keto–enol equilibria and decarboxylation steps. The entropy and volume of activation were larger than those for low molecular weight amine, suggesting the importance of a desolvation effect in the decarboxylation catalyzed by the functional polymers.

Decarboxylation is biologically a very important process[1] and many compounds have been synthesized as decarboxylase models.[2] Almost all of these have been designed only to bind substrate (nitrobenzisoxazole, for example) into a nonpolar environment. Examples include crown ethers,[3] micelles,[4] and polysoap domains.[5] Natural decarboxylase has been reported to carry out its role by involving a variety of factors: (1) the electron sink effects of coenzymes to obviate the necessity for forming a highly unstable carbanion: (2) stereochemical control of the enzyme-bound coenzyme–amino acid Schiff base to prevent the kinetically more favorable transamination: (3) a medium effect to make the active sites of enzymes less polar than the bulk phase; and (4) desolvation of the carboxyl group by removing the carboxyl group from contact with the solvent.[6] Previous model compounds not having a specific catalytic site were found to be unsuitable as decarboxylase models, except for a modified polyethylenimine examined by Spetnagel and Klotz[7] as mentioned later.

Decarboxylation of β-keto acids by amine catalysts has been examined by many researchers. For oxalacetic acid, primary amines were found to be most effective, and the reaction mechanism was suggested to be similar to enzymatic catalysis.[8] Spetnagel and Klotz introduced hydrophobic groups onto basic polyethylenimine and claimed to find a catalytic mechanism similar to that of the enzyme. In their analysis, however, they ignored an increasing absorbance at 260 nm, which was attributed by Kosicke et al.[9] to an increase in the concentration of enol-type substrate. In this paper we investigate this reaction system in detail using a more appropriate method including the keto–enol equilibria of both free substrate and substrate–catalyst complex. Furthermore, we try to evaluate the contribution of desolvation of the transition state by polymeric catalysts by estimating the entropy and volume of activation.[10] Desolvation mechanisms in enzyme action are interesting because the high efficiency of most enzyme reactions cannot be explained soley by the proximity of substrate to catalytic groups.[11] One of the best examples of the desolvation effect is the Asp-102 carboxylate of α-chymotrypsin which is buried in a nonpolar region near the catalytically important imidazole ring.[12]

Experimental Section

Materials. Oxalacetic acid (OAA) was purchased from Wako Pure Chemical Co. (Osaka, Japan) and recrystallized from acetone/benzene. Polyethylenimine (PEI, molecular weight 6000) was purchased from Nihon Shokubai Co. (Osaka, Japan) and dialyzed against distilled water using a Visking cellulose tube (cutoff molecular weight 3500) for 1 week to remove oligomeric ethylenimine. Dialyzed polyethylenimine was mixed with ethanol and condensed by evaporation. The ethanol mixing and evaporation were repeated several times to remove water azeotropically. Condensed polyethylenimine was dissolved in dimethylformamide and quaternized by an excess of butyl bromide (C_4PEI) or octyl bromide (C_8PEI) at 50 °C for 1 week. The quaternized polymer was precipitated by pouring into an excess of ethyl acetate. After filtration, all quaternized polymers were dried in vacuo, dissolved in water, and dialyzed for 1 week. Polybrene (3,6-ionene polymer, PB) from Sigma and poly(L-lysine) hydrobromide (PLL) from the Protein Research Foundation (Osaka, Japan) were also dissolved in water and dialyzed for 1 week. Hepes (*N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid) was obtained from the Dojin Chemical Laboratories, Kumamoto, Japan.

Dimethyl sulfoxide (Me_2SO) was a spectrophotometric grade reagent of the Dojin Chemical Laboratories. Deionized water was distilled before use. In the enzymatic method, lactate dehydrogenase (LDH, rabbit muscle, PL Biochemicals) was used.

The concentration of polymers and the degree of quaternization were determined by conductometric titration using a Wayne Kerr autobalance precision bridge (B-331).[13] The concentration was determined by back-titration using N/10 HCl and N/10 NaOH, and the degree of quaternization was determined with N/10 $AgNO_3$. The degrees of quaternization were 52% for C_4PEI and 31% for C_8PEI.

Kinetic Measurements. The decarboxylation reaction was monitored by the change in the absorbance at 260 nm. Fast and slow processes were found by using a stopped-flow apparatus (RA-1100, Union Engineering, Osaka, Japan) and a Union high-sensitivity spectrophotometer (SM-401). Reactions at high pressures were monitored with a Union high-pressure spectrophotometer to evaluate the volume of activation ($\Delta V^{\ddagger}$):

$$\Delta V^{\ddagger} = -RT(\mathrm{d} \ln k/\mathrm{d}P) \qquad (1)$$

where k is the rate constant, P the pressure, and RT the kinetic energy. Details of these apparatuses were described elsewhere.[10] In the case of enzymatic reaction, pyruvate, a product of decarboxylation of OAA, was reduced into lactate by LDH and the decrease in the absorbance of NADH at 340 nm was followed. The reaction was examined under the condition of the excess polymer in a pH 7.0 Hepes buffer. Hepes was used because its pK_a was insensitive to pressure (ΔpK_a from 1 to 1000 atm is 0.05).[14]

Results and Discussion

When an aqueous oxalacetate solution was mixed with a solution containing a polymer catalyst, the absorbance at 260 nm first increased rapidly and then slowly decreased. We first examined the effect of polymer on these two processes. Since the time orders of these processes differ by about 10^3 times, the relaxation time of each process could be evaluated independently by a conventional first-order analysis. For the first process, the maximal absorbance during the reaction was adopted as the value at $t \to \infty$. The effect of C_8PEI is shown in Figure 1, where the reciprocal values of the fast and slow relaxation times are given as $k_{f,obsd}$ and $k_{s,obsd}$, respectively. It

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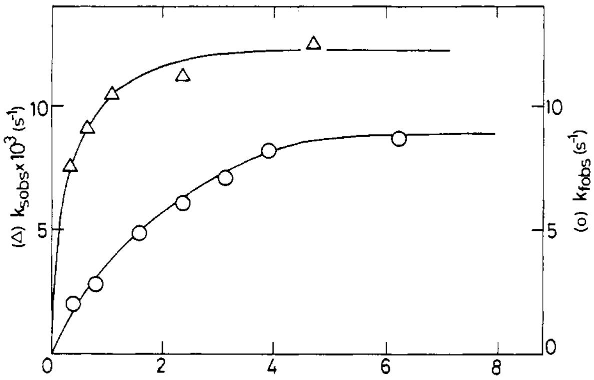


Figure 1. Effect of C_8PEI on the decarboxylation of oxalacetate in H_2O at 25 °C: (O) fast process; (Δ) slow process. [OAA] = 50 μM, pH 7.0, 0.1 M Hepes buffer.

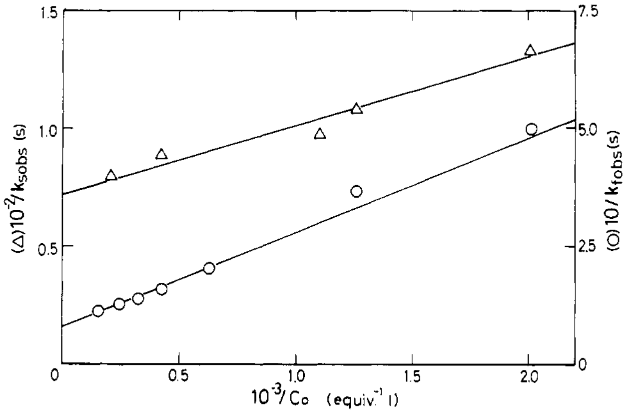
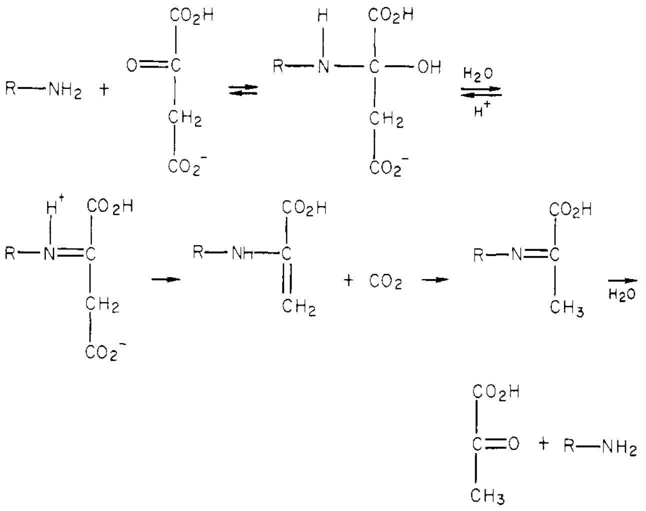


Figure 2. Double-reciprocal plots of the decarboxylation of oxalacetate in H_2O by C_8PEI at 25 °C: (O) fast process; (Δ) slow process. [OAA] = 50 μM, pH 7.0, 0.1 M Hepes buffer.

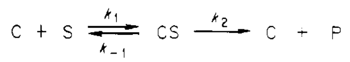
Scheme I



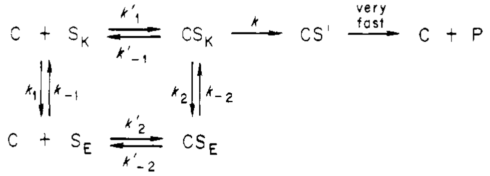
is clear that $k_{f,obsd}$ and $k_{s,obsd}$ values increase and then level off with increasing C_8PEI concentration. Similar tendencies were observed for all polyelectrolytes examined here. In Figure 2, $1/k_{obsd}$ vs. $1/C_0$ double-reciprocal plots of C_8PEI are given, where C_0 is the initial concentration of the polymer.

The reaction process of decarboxylation catalyzed by amines is shown in Scheme I. The carbonyl carbon atom of the keto form is attacked by amine and a Schiff base intermediate is formed. The CO_2 group is lost and finally pyruvic acid is produced by hydrolysis. Using an enzymatic method to follow the production of pyruvate established that the Schiff base formation step is rate determining and that the subsequent steps are very fast.[7]

Scheme II
Reaction Scheme Proposed by Spetnagel and Klotz



Scheme III[a]
Decarboxylation of Oxalacetate Catalyzed by Amines



[a] S_K = keto form of OAA; S_E = enol form of OAA; $v = d([S_E] + [CS_E])/dt$; $K_1 = k_1'/k_{-1}'$; $K_2 = k_2'/k_{-2}'$.

Table I
Kinetic Parameters for the Decarboxylation of Oxalacetate by Polymeric Amines at 25 °C[a]

polymer	$K_1 = K_2$, M^{-1}	k_2, s^{-1}	k_{-2}, s^{-1}	k, s^{-1}
PEI	1.1×10^2	17	7.0	2.1×10^{-3}
PLL	2.8×10^3	0.076	0.024	2.3×10^{-3}
C_8PEI	4.0×10^2	6.8	2.2	6.8×10^{-3}

[a] $k_1 = 0.036\ s^{-1}$; $k_{-1} = 0.15\ s^{-1}$.[16]

According to Spetnagel and Klotz's scheme (Scheme II), eq 2 and 3 are derived, where the Michaelis constant K_M

$$k_{f,obsd} = k_1C_0 + k_{-1} + k_2 \qquad (2)$$

$$k_{s,obsd} = k_2C_0/(C_0 + K_M) \qquad (3)$$

is equal to $(k_{-1} + k_2)/k_1$. Plots of $k_{f,obsd}$ should increase linearly with C_0, giving a Y intercept of $k_{-1} + k_2$. From Figure 1, it is apparent that Spetnagel and Klotz's scheme does not agree with the experimental data, and another scheme is necessary to interpret this reaction system.

We propose Scheme III as an alternative mechanism, where S_K and S_E are the keto and enol forms of substrate and CS_K and CS_E are complexes of polymer (C) with the keto and enol forms of substrate. Substrate comes to the vicinity of the cationic polymer by electrostatic interaction. Only the keto form substrate is decarboxylated.[15] We assume here that equilibrium binding processes K_1 and K_2 for the two substrates with the polymer are very fast because strong electrostatic bindings of anionic substrates with a cationic polyelectrolyte are involved. After the equilibria are established, the slower process $CS_K \rightarrow CS'$ is assumed to start. In this case, the $k_{f,obsd}$ is derived as $C_0(k_2 + k_{-2})/(C_0 + 1/K_1)$ under the condition that $k \simeq 0$ and $K_1 = K_2$. (Though the charge localization behaviors of the keto and enol form substrates are different from one another, we assumed that the difference is negligible and hence $K_1 = K_2$ here.) $k_{s,obsd}$ is derived to be

$$\frac{C_0k_{-1}k/(k + k_1 + k_{-1} + k_2 + k_{-2})}{C_0 + (k_1 + k_{-1})/(k + k_1 + k_{-1} + k_2 + k_{-2})K_1}$$

under the condition that $K_1 = K_2$ and $k_{-1} \gg k_{-2}k_2C_0$. The last condition was satisfied for all catalysts except PEI ($k_{-1} \simeq k_{-2}k_2C_0$). Since the percent of the free amine group of PEI is only 30%, we adopted the same analytic method as for the other catalysts here.

From the double-reciprocal plot, we could estimate K_1, k_2, k_{-2}, and k, and these are compiled in Table I. k_1 and k_{-1} were literatures values.[16] The k values obtained for

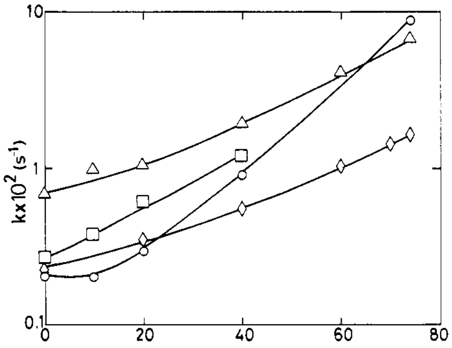


Figure 3. Effect of Me_2SO on k values plotted on a semilogarithmic scale at 25 °C: (○) PEI; (△) C_8PEI; (□) C_4PEI; (◇) PLL. [OAA] = 50 μM, 0.1 M Hepes buffer.

polymer catalyses were slightly different from the corresponding k_2 values estimated by the method of Spetnagel et al. (for C_8PEI, 6.8×10^{-3} s^{-1} and 1.38×10^{-2} s^{-1}, respectively). The k values of C_8PEI are larger than those of PEI and PLL. This means that the hydrophobic chain of C_8PEI enhances the reaction as was often observed in various decarboxylation reactions. K_1 of these polymers, the association constant, is in the order PLL > C_8PEI > PEI, because the amino group of PLL is almost protonated (p$K \simeq 9.8$[17] by CD measurement) and C_8PEI has quaternary charges (31% quaternized), whereas PEI does not.

In spite of the large K_1 and K_2 values, the catalytic effect of PLL for the fast step, $k_{\mathrm{f,obsd}}$, is small. The maximum value of $k_{\mathrm{f,obsd}}$ of PB, a fully quaternized polymer, is 0.3 s^{-1} and is smaller by 1 order of magnitude than those of PEI and C_8PEI. Those results show that not only electrostatic interaction but also the free amino group greatly influences the fast process.

Decarboxylation in Me_2SO–H_2O Mixture. The catalytic effect of polyelectrolytes in aqueous organic solvent is interesting because the role of the solvation and desolvation effect of activated complex or substrate by polyelectrolyte can be more easily studied.[18] Thus we observed here both medium and desolvation effects of polyelectrolyte catalysis in decarboxylation in water–dimethyl sulfoxide (Me_2SO) mixture. Me_2SO was reported to activate the nucleophilicity of the amino group through hydrogen bond formation between the Me_2SO oxygen and the NH_2 hydrogen.[18-20] Thus, addition of Me_2SO to this reaction system is expected to intensify the solvation–desolvation effect by polyelectrolyte.

The catalytic behavior of polymeric amines, such as a saturation phenomenon in the decarboxylation of oxalacetate, in H_2O–Me_2SO is the same as in water. In Figure 3, the Me_2SO effects on the k values of the decarboxylation of oxalacetate acid catalyzed by cationic polyelectrolytes are shown. Me_2SO accelerated the reaction rate in all cases partly because of the increase in nucleophilicity of the amino groups and partly because of the increase in reactivity of the substrate. The spontaneous decarboxylation was accelerated by the addition of Me_2SO because of a decrease in the dielectric constant of the reaction medium. The reaction rates of spontaneous decarboxylation (1.7×10^{-5} s^{-1} (water),[21] 1.31×10^{-3} s^{-1} (74% aqueous Me_2SO)) were considerably smaller than those of the polymeric amine catalyzed ones (2.6×10^{-3} s^{-1} (water), 3.2×10^{-2} s^{-1} (74% aqueous Me_2SO)) in the presence of 2.1 mequiv·L^{-1} of PLL. C_8PEI was most effective in the pure water system because of its octyl chain, whereas with increasing Me_2SO percent the catalytic effect of PEI increased substantially and finally became larger than that of C_8PEI. Though the difference in the conformational structure of C_8PEI in water and in H_2O–Me_2SO mixtures might be somewhat larger than that of PEI, we believe that both environmental and nucleophilicity effects are important for the polymer catalyses. In aqueous solution, octyl groups reduce the dielectric constant in the vicinity of polymer, whereas in the H_2O–Me_2SO mixture, the fixed charges of C_8PEI unfavorably increase the dielectric constant in the vicinity of polymer. Thus, the quaternized octyl group is more advantageous for catalysis in aqueous solution than in H_2O–Me_2SO mixture. In the case of PEI, however, there is no permanent charge in the polymer molecule, and the quantity of protonated amino group decreases (pH of the buffer solution increased from 6.9 to 7.3 with the addition of an equal amount of Me_2SO). At the same time, the nucleophilicity of the amino groups increases with the addition of Me_2SO through hydrogen bond formation between the Me_2SO oxygen and the NH_2 hydrogen. Thus the catalytic activity of PEI was greater than that of C_8PEI in H_2O–Me_2SO mixture.

Table II
Thermodynamic Parameters for the Decarboxylation of OAA by Amines[a]

catalyst	$\Delta G^{\ddagger}$,[b] kcal·mol^{-1}	$\Delta H^{\ddagger}$,[b] kcal·mol^{-1}	$\Delta S^{\ddagger}$,[b] cal·mol^{-1}	$\Delta V^{\ddagger}$,[c] mL·mol^{-1}
PEI	18.5 ± 0.2	14.0 ± 0.2	−15 ± 1	+11 ± 2
C_8PEI	18.7 ± 0.2	13.7 ± 0.2	−17 ± 1	
PLL	19.4 ± 0.2	13.1 ± 0.2	−21 ± 1	+5 ± 2
$BuNH_2$	21.1 ± 0.2	13.9 ± 0.2	−26 ± 1	−6 ± 2

[a] [OAA] = 5×10^{-5} M. [b] 74% (v/v) Me_2SO. [c] 40% (v/v) Me_2SO.

To clarify the desolvation effect by polymeric amine catalysts, we also estimated thermodynamic parameters for k values (not k_{obsd}) of this reaction system in H_2O–Me_2SO by varying the temperature of the reaction solution, since the water system is not suitable because of slow reaction rate. The results obtained are listed in Table II. The activation volume ($\Delta V^{\ddagger}$) was evaluated by using eq 1 in a 40% (v/v) aqueous Me_2SO mixture because the reaction in 74% (v/v) aqueous Me_2SO was too fast. These results were in good agreement with that obtained by the enzymatic method, which directly follows the production of pyruvic acid.

Thermodynamic parameters of a small amine, n-butylamine ($BuNH_2$), were estimated for comparison. Thermodynamic data for spontaneous decarboxylation of OAA were 23.6 kcal·mol^{-1} ($\Delta G^{\ddagger}$), 1.9 kcal·mol^{-1} ($\Delta H^{\ddagger}$), and −73 cal·mol^{-1}·deg^{-1} ($\Delta S^{\ddagger}$).[22] The extremely small value of $\Delta S^{\ddagger}$ was attributed to capture or orientation of solvent water by the transition state. $\Delta H^{\ddagger}$ of the catalytic reaction was much larger than that of the spontaneous reaction because of the necessity for the bond breaking and the release of oriented solvent water. From the table, it is apparent that the better catalytic activity of the polyelectrolyte relative to that of n-butylamine can be attributed to the increases of both entropy ($\Delta S^{\ddagger}$) and volume ($\Delta V^{\ddagger}$) of activation. These increases are believed to be caused by desolvation of the transition state by the polymer catalysts, that is, in the transition state, the substrate gathered in the vicinity of the polymer to form a Schiff base liberates water molecules around themselves because of strong electrostatic interaction between the anionic substrate and cationic charge in the neghborhood of the free NH_2 group of polymer. This results in enhancement of the reactivity of the substrate and facilitates attack of the polymer on the substrate. From the $\Delta H^{\ddagger}$ vs. $\Delta S^{\ddagger}$ plot, an isokinetic temperature for polymer catalyses was determined to be 150 K, lower than the experimental temperature (298 K). This phenomenon also proved that the catalytic reaction was entropy controlled.[23]

Cohen et al. suggested that α-chymotrypsin catalysis by desolvation of substrate in the neighborhood of hydrophobic residues of the enzyme molecule contributed a factor of 10^3 or so to the rate of catalysis.[24] Warshel also pointed out the importance of solvation energy on the energetics of enzymatic reactions.[25] Recently, we pointed out that "polymer catalysts" which provide reaction site in the polymer domain change reaction rates by solvation and/or desolvation of substrate and/or transition state.[10,18,26] The present work seems to corroborate these suggestions.

Registry No. OAA, 328-42-7; PEI, 9002-98-6; C$_4$PEI, 84680-84-2; C$_8$PEI, 84680-85-3; PLL, 25988-63-0; PLL, SRU, 26588-20-5; PB, 28728-55-4; butylamine, 109-73-9; Me$_2$SO, 67-68-5; decarboxylase, 9027-22-9.

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Determination of the Correlation between Molecular Weight Distribution and Chemical Composition Distribution in a High-Conversion Copolymer

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ABSTRACT: The dual distribution of molecular weight and chemical composition of a high-conversion copolymer was determined by combination of preparative gel permeation chromatography (GPC) and thin layer chromatography (TLC). A high-conversion statistical copolymer of styrene–methyl acrylate (S–MA) was separated into 11 fractions with respect to molecular weight by preparative GPC. The chemical composition distribution (CCD) of each fraction was determined by TLC. The three-dimensional distribution of molecular weight and chemical composition was constructed from those data. The CCD's of lower molecular weight fractions are not appreciably different from that of the original sample. Their CCD's have a main peak at a low MA content and a long tail toward the higher MA-content region. On the other hand, the CCD's of higher molecular weight fractions are shifted toward the higher MA-content region. These features of the dual distributions are well explained by analyzing two phenomena occurring simultaneously, i.e., the increase of molecular weight due to the so-called gel effect and the change of MA content with conversion.

Introduction

It is well-known that high-conversion copolymers have both molecular weight distribution (MWD) and chemical composition distribution (CCD) and a correlation exists between those two distributions. It is very important to know the correlation for the study of copolymerization kinetics and also for the study of the effect of MWD and CCD on physical properties of copolymers. Nevertheless, only a few papers dealing with the determination of the correlation have been published.[1-3]

By using an analytical GPC with dual detectors, we previously showed that a high-conversion copolymer of styrene (S) and methyl acrylate (MA) prepared in bulk has a broad MWD and the MA content of the fractions at point-by-point increases as the molecular weight (MW) increases.[4] The experimental data are reproduced in Figure 1. These facts were explained by the so-called gel effect in the copolymerization kinetics. That is, the MW of the components produced at high conversion is higher than that of the components produced at low conversion due to the gel effect, while the MA content of the components increases as the conversion increases. Consequently, a characteristic correlation is found between MW and chemical composition (CC) in high-conversion copolymers. However, this is simply a qualitative explanation.

In the present work, the dual distributions of MW and CC in a high-conversion sample of S–MA copolymer were determined by combination of a preparative GPC and TLC to confirm this explanation.

Experimental Section

Samples. The sample used in the present work is the same S–MA statistical copolymer (B-60) as used in our previous