Diffusion Process of Amino Acids in Polymer Supports for Solid-Phase Peptide Synthesis as Studied by Pulsed-Field-Gradient Spin-Echo Proton Nuclear Magnetic Resonance

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ABSTRACT: The diffusion coefficient (D) values of *tert*-butyloxycarbonyl-glycine, *tert*-butyloxycarbonyl-L-tryptophan, *tert*-butyloxycarbonyl-L-phenylalanine (Boc-Phe), and 9-fluorenylmethoxycarbonyl-L-phenylalanine in Merrifield polystyrene (MPS) gels, poly(ethylene glycol)-grafted polystyrene (PEG–PS) gels, and crosslinked ethoxylate acrylate (CLEAR) gels, as used in solid-phase peptide synthesis, were determined by the pulsed-field-gradient spin-echo ^{1}H-NMR method. From these experimental results, it was found that the amino acids in MPS gels, PEG–PS gels, and CLEAR gels with *N*,*N*-dimethylformamide-d_7 (DMF-d_7) as a solvent had multidiffusion components within a measurement timescale of 10 ms. The D value of Boc-Phe in polystyrene gels (1% divinylbenzene crosslinked) with tetrahydrofuran-d_8 was much larger than that in the same gels with DMF-d_7. Furthermore, the required time in which an amino acid transferred from a reactive site to a reactive site was estimated, within which the solvents and amino acids in the polymer supports diffused in the swollen beads. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 89: 413–421, 2003

Key words: diffusion; resins; NMR; gels; synthesis

INTRODUCTION

Solid-phase peptide synthesis has widely been applied to solid-phase organic synthesis to produce combinatorial libraries for peptides, oligosaccharides, functional polymers, liquid crystals, and catalysts in addition to successful development for new medical drugs. Polystyrene (PS) network gels are the most popular polymer supports in solid-phase synthesis,[1,2] column chromatography,[3–5] ion-exchange resins, catalysts,[6–8] and so forth. These functionalities are closely associated with the diffusional behavior of solvents and probe molecules, the intermolecular interactions between networked polymer chains and probe molecules, and the structure and dynamics of polymer supports. A number of articles on solid-phase synthesis for combinatorial libraries have been reported, and reviews and monographs in this field have also appeared (e.g., see the annual report on combinatorial chemistry[9]), but there is little work on elucidating problems in designing a new series of solid-phase syntheses and in understanding the diffusional behavior of probe molecules in polymer supports. A solid-phase reaction field consists of solvents, reagents, networked polymer chains, reactive sites, and spacers. In general, it is known that the rate of a solid-phase reaction is closely associated with the diffusion rate of the reagents and reaction products.[10–16] However, shaking of the resins by gas bubbling does not affect the rate of reaction. Therefore, it is important to clarify the diffusion processes of reaction reagents in popular polymer supports.

NMR spectroscopy gives very useful information about the structure and dynamics of polymer gel systems.[17–23] Furthermore, the self-diffusion coefficient (D) of key probe molecules in the polymer network system considered in this work can be determined by the pulsed-field-gradient spin-echo (PFGSE) ^{1}H-NMR method. To study the diffusional behavior of molecules or polymers in polymer network gels with a wide range of diffusion coefficients, we have developed a high-field-gradient NMR system with a maximum strength of about 2000 G/cm. With this system, it is possible to measure the diffusion coefficient within the order of 10^{-5} to 10^{-10} cm^2/s. The use of this system leads to sophisticated knowledge on the diffusional behavior of probe molecules in polymer gels as a solid-phase reaction field.[24,25]

In previous works, we have reported that the diffusional behavior of solvents in PS network gels de-

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pends on the degree of volume swelling (Q) and temperature and that the diffusion coefficients of *tert*-butyloxycarbonyl-glycine (Boc-Gly; $D_{\text{Boc-Gly}}$) and *tert*-butyloxycarbonyl-L-phenylalanine (Boc-Phe; $D_{\text{Boc-Phe}}$) in PS network gels depend on Q, the amino acid concentration, and the temperature.[24,25]

With this background, we aim here to elucidate the diffusion process of Boc-Gly, Boc-Phe, *tert*-butyloxycarbonyl-L-tryptophan (Boc-Trp), and 9-fluorenylmethoxycarbonyl-L-phenylalanine (Fmoc-Phe) in polymer supports for solid-phase peptide synthesis with Merrifield polystyrene (MPS) network resin, poly(ethylene glycol)-grafted polystyrene (PEG–PS) network resin, and crosslinked ethoxylate acrylate (CLEAR) resin, which are popular polymer supports. Furthermore, we discuss the collision frequency between reactive sites and amino acids.

EXPERIMENTAL

Materials

MPS network resin beads were purchased from Nova Biochem Co., Ltd. (La Jolla, CA), and Peptide Institute, Inc. (Osaka, Japan); PEG–PS resin beads[26] were purchased from Argonaut Technologies, Inc. (Foster City, CA); and CLEAR resin beads[27–29] were purchased from Peptide Institute. They were used as polymer network samples for solid-phase peptide synthesis. The MPS resin was crosslinked by divinylbenzene (DVB) and was functionalized with chloromethyl groups. Two types of resin beads with 1% and 2% crosslinking were used. The PEG–PS resin had a PS backbone 1% crosslinked by DVB, which was grafted with poly(ethylene glycol) (PEG; degree of polymerization = 30–40) with a terminal amine. The CLEAR resin had a PEG backbone and was functionalized with amines. The diameter of these dried beads was 90–220 μm. The bead gels were prepared by being soaked in an aqueous solution of amino acids for 3 days.

Q for the polymer network gel is defined as the ratio of the volume of a swollen polymer network gel at room temperature (V_{swollen}) to the volume of a dried polymer network resin (V_{dry}; $Q = V_{\text{swollen}}/V_{\text{dry}}$). The volume of the gel was determined by the average diameter of the polymer network gel beads, which was measured with a microscope.

Boc-Gly (molecular weight = 175), Boc-Trp (molecular weight = 304), Boc-Phe (molecular weight = 265), and Fmoc-Phe (molecular weight = 387), purchased from Peptide Institute, were used as amino acids to be diffused in the gels. *N,N*-Dimethylformamide-d_7 (DMF-d_7) and tetrahydrofuran-d_8 (THF-d_8), purchased from Merck Co. (Darmstadt, Germany), were used as solvents. The amino acid concentration used in this work was fixed at 10 wt %.

Measurements

The D measurements on solvents and amino acids in the polymer network gels were carried out over a wide range of temperatures with a JEOL GSX-270 NMR spectrometer (Tokyo, Japan) operating at 270.1 MHz for ^{1}H with a homemade pulse gradient generator with a standard pulse sequence ($\pi/2$ pulse–τ–π pulse), the Hahn echo sequence.[30–38] In this work, a field-gradient strength (G) of about 1400 G/cm was used. This system has been used successfully in our previous works on diffusional behavior in polymer gels.[24,25,39–42]

The D values were determined with the relationship between the echo signal intensity and the field-gradient parameters:

$$\ln\left[\frac{A(\delta)}{A(0)}\right] = -\gamma^2 G^2 D \delta^2 \left(\Delta - \frac{\delta}{3}\right) \tag{1}$$

where $A(\delta)$ and $A(0)$ are the echo signal intensities at $t = 2\tau$ with and without the magnetic field-gradient pulse, which has the length δ, respectively. γ is the gyromagnetic ratio of the proton, and Δ is the gradient pulse interval. The echo signal intensity was measured as a function of δ. Plots of $\ln[A(\delta)/A(0)]$ against $\gamma^2 G^2 \delta^2(\Delta - \delta/3)$ gave a straight line with a slope of $-D$. Therefore, the D value could be determined from its slope. When probe molecules have multiple components of diffusion on the measurement timescale, the total echo attenuation is given by a superpositioning of contributions from the individual components:

$$\frac{A(\delta)}{A(0)} = \sum_i p_i \exp\left[-\gamma^2 G^2 D_i \delta^2 \left(\Delta - \frac{\delta}{3}\right)\right] \tag{2}$$

where D_i is the self-diffusion coefficient of the ith component and p_i is the fraction of the ith component ($\Sigma p_i = 1$). The fraction for the diffusion component can be determined from the intercept of the least-squares-fitted straight line at a large value of δ. The δ and Δ values employed in these experiments are 0.001–1.0 ms and 10–100 ms, respectively.

RESULTS AND DISCUSSION

Diffusion coefficients of amino acids in PS network gels with DMF-d_7 as a solvent

To determine the diffusion coefficient of Boc-Gly ($D_{\text{Boc-Gly}}$) in MPS gels with DMF-d_7 as a solvent at Q = 1.50 from 30 to 50°C, we have plotted $\ln[A(\delta)/A(0)]$ against $\gamma^2 G^2 \delta^2(\Delta - \delta/3)$ in Figure 1. The diffusion coefficient can be determined from the slope. The experimental data do not lie on a straight line. This shows that Boc-Gly in an MPS gel with Q = 1.50 has multiple components of diffusion, such as a slow-

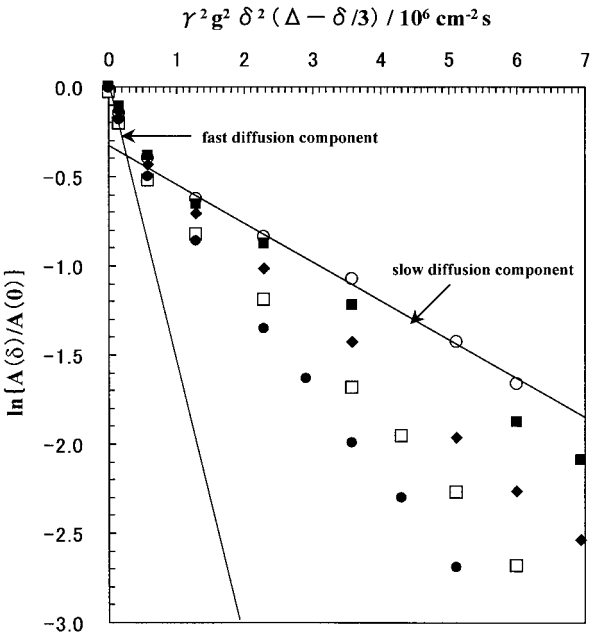

Figure 1 Diffusional spin-echo attenuation of Boc-Gly in MPS gels with DMF-d_7 at Q = 1.50 at (○) 30, (■) 35, (♦) 40, (□) 45, and (●) 50°C.

diffusion component and a fast-diffusion component, within the observation time, although Boc-Gly in a DMF-d_7 solution has a single component of diffusion. For convenience, a typical example with fast- and slow-diffusion components at 30°C is shown in Figure 1. The existence of strong and weak intermolecular interactions between the Boc-Gly and PS network leads to the existence of two diffusion components in the MPS gel. We are concerned with this problem, as described next.

Figure 2 shows the temperature dependence of $D_{\text{Boc-Gly}}$ for the slow-diffusion component in MPS gels at Q = 1.50 and 2.45 and of the fraction of the slow-diffusion component ($f_{\text{slow(Boc-Gly)}}$). It seems that Boc-Gly molecules interacting strongly with the polymer network contribute to the slow-diffusion component, and Boc-Gly molecules interacting weakly with the polymer network contribute to the fast-diffusion components. These interactions depend on the intermolecular distances between Boc-Gly molecules and PS network chains. The $D_{\text{Boc-Gly}}$ values for the slow-diffusion component in MPS gels at Q = 1.50 and 2.45 at 30°C are 1.79×10^{-7} and 8.14×10^{-7} cm^2/s, respectively. Their difference is very large. This comes from a large difference in the network size. To clarify this, we look at the activation energy of diffusion (E) for the diffusion process. The E value was obtained from the plots of ln D against the inverse of the absolute temperature ($1/T$; Arrhenius plots). The $E_{\text{Boc-Gly}}$ values for the slow-diffusion component in MPS gels at Q = 1.50 and 2.45 are 9.50 and 5.34 kcal/mol, respectively. The former is much larger than the latter. This shows that as an increase in the volume fraction of networked PS chains in MPS gels leads to a reduction of the PS network size and then a reduction of the intermolecular distance between Boc-Gly molecules and networked PS chains, intermolecular interactions between the Boc-Gly molecules and PS network are significantly increased. Therefore, the diffusion process and the diffusion rate are sensitively influenced by Q. Next, we look at $f_{\text{slow(Boc-Gly)}}$. The $f_{\text{slow(Boc-Gly)}}$ value for the slow-diffusion component in MPS gels at Q = 1.50 is slightly increased from 0.72 to 0.78 with an increase within the temperature range of 30–50°C; however, the $f_{\text{slow(Boc-Gly)}}$ value in MPS gels at Q = 2.45 is almost independent of temperature, being about 0.6. The $f_{\text{slow(Boc-Gly)}}$ value at Q = 1.50 is much larger than that at Q = 2.45. This suggests that Boc-Gly molecules interacting strongly with the PS network predominantly contribute to the slow-diffusion component and that their interactions depend on the intermolecular distance between the Boc-Gly molecules and PS chains. Therefore, the network size plays an important role in the intermolecular interactions. Consequently, it can be said that the large differences in the $D_{\text{Boc-Gly}}$, $E_{\text{Boc-Gly}}$, and $f_{\text{slow(Boc-Gly)}}$ values of Boc-Gly in MPS gels at Q = 1.50 and 2.45 come from the different PS network sizes.

$\ln[A(\delta)/A(0)]$ against $\gamma^2G^2\delta^2(\Delta - \delta/3)$ for Boc-Gly, Boc-Trp, and Fmoc-Phe in DMF-d_7 solutions in the absence of a resin at 30°C has been plotted (not shown). The experimental data lie on a straight line.

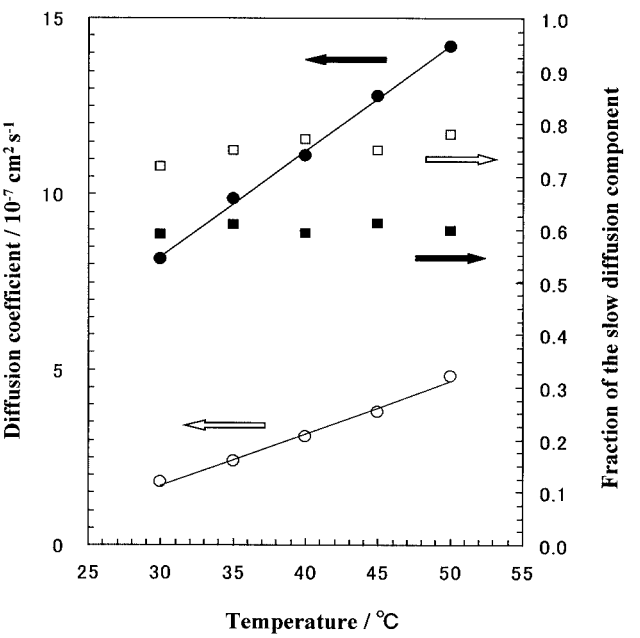

Figure 2 Temperature dependence of (●) the diffusion coefficient and (■) fraction of the slow-diffusion component of Boc-Gly in MPS gels with DMF-d_7 at Q = 2.45 and of (○) the corresponding diffusion coefficient and (□) fraction at Q = 1.50.

TABLE I
Diffusion Coefficients and Activation Energies of Amino Acids in MPS Gel[a]

Amino acids	MPS gel		Diffusion coefficient/10^{-7} cm^2 s^{-1} 30°C	35°C	40°C	45°C	50°C	Activation energy (kcal mol^{-1})
Boc-Gly	—[a]	D	48.3	52.6	59.6	61.5	67.2	3.18 ± 0.06
	1 mol % DVB	D_{fast}	17.7	17.3	20.8	22.1	22.0	2.65 ± 0.47
		D_{slow}	8.14	9.88	11.1	12.8	14.2	5.34 ± 0.05
	2 mol % DVB	D_{fast}	6.15	6.32	6.98	7.54	8.01	2.74 ± 0.06
		D_{slow}	1.79	2.38	3.09	3.78	4.81	9.50 ± 0.02
Boc-Phe	—[a]	D	48.4	53.1	57.6	61.7	66.9	3.10 ± 0.01
	1 mol % DVB	D_{fast}	21.4	18.7	19.9	23.3	26.7	2.54 ± 0.54
		D_{slow}	8.82	9.34	10.9	13.4	14.2	5.14 ± 0.20
	2 mol % DVB	D_{fast}	6.43	6.61	7.20	8.31	8.30	2.87 ± 0.23
		D_{slow}	2.08	2.59	3.28	3.84	4.77	7.99 ± 0.02
Boc-Trp	—[a]	D	42.1	45.4	48.8	53.3	57.5	3.05 ± 0.01
	1 mol % DVB	D_{fast}	12.0	12.8	14.5	15.2	16.1	2.95 ± 0.01
		D_{slow}	5.16	5.40	6.65	7.62	8.02	4.77 ± 0.21
	2 mol % DVB	D_{fast}	7.26	6.46	6.83	7.37	8.02	1.26 ± 0.77
		D_{slow}	2.43	3.02	3.57	4.16	4.94	6.77 ± 0.02
Fmoc-Phe	—[a]	D	36.1	41.2	43.9	46.9	51.6	3.29 ± 0.05
	1 mol % DVB	D_{fast}	11.5	12.3	13.0	15.0	15.1	2.89 ± 0.15
		D_{slow}	4.22	4.95	5.66	5.85	7.24	4.85 ± 0.18
	2 mol % DVB	D_{fast}	8.42	11.4	9.95	9.96	11.4	1.84 ± 1.18
		D_{slow}	2.40	3.11	3.95	4.13	4.80	6.52 ± 0.33

[a] In DMF-d_7 solution in the absence of resin.

This means that the amino acids have a single component of diffusion during the observation time. The $D_{\text{Boc-Gly}}$, $D_{\text{Boc-Trp}}$, and $D_{\text{Fmoc-Phe}}$ values were found to be 4.83×10^{-6}, 4.21×10^{-6}, and 3.61×10^{-6} cm^2/s, respectively. The magnitude of the D values depends on the molecular weights, that is, the molecular size. As the molecular weight is increased, the D value is decreased. The $D_{\text{Boc-Gly}}$, $D_{\text{Boc-Phe}}$, $D_{\text{Boc-Trp}}$, and $D_{\text{Fmoc-Phe}}$ values in the absence of a resin are shown as a function of temperature in the temperature range of 30–50°C in Table I. The D values are increased with an increase in temperature. Then, E for the amino acids was obtained, as shown in Table I. The E values determined for four kinds of amino acids are close to one another.

As for Boc-Gly, Boc-Trp, and Fmoc-Phe in 1% crosslinked MPS gels with DMF-d_7 as a solvent at 30°C, plots of $\ln[A(\delta)/A(0)]$ against $\gamma^2G^2\delta^2(\Delta - \delta/3)$ have been plotted (not shown). The experimental data do not lie on a straight line. This shows that Boc-Trp and Fmoc-Phe in 1% crosslinked MPS gels have multiple components of diffusion within the observation time: $Q_{\text{Boc-Gly}}$, $Q_{\text{Boc-Phe}}$, $Q_{\text{Boc-Trp}}$, and $Q_{\text{Fmoc-Phe}}$ are 2.45, 2.48, 2.55, and 2.50, respectively. Such a situation comes from significant intermolecular interactions between the amino acids and the network. The observed diffusion echo signal was approximately deconvoluted by two slow- and fast-diffusion components. Then, from such plots, the diffusion coefficients of the slow- and fast-diffusion components were determined, as shown in Table I. The determined $D_{\text{Boc-Gly}}$, $D_{\text{Boc-Phe}}$, $D_{\text{Boc-Trp}}$, and $D_{\text{Fmoc-Phe}}$ values for the slow-diffusion component are 8.14×10^{-7}, 8.82×10^{-7}, 5.16×10^{-7}, and 4.22×10^{-7} cm^2/s, respectively, and the $f_{\text{slow(Boc-Gly)}}$, $f_{\text{slow(Boc-Phe)}}$, $f_{\text{slow(Boc-Trp)}}$, and $f_{\text{slow(Fmoc-Phe)}}$ values are 0.6, 0.6, 0.5, and 0.5, respectively. However, the determined $D_{\text{Boc-Gly}}$, $D_{\text{Boc-Phe}}$, $D_{\text{Boc-Trp}}$, and $D_{\text{Fmoc-Phe}}$ values for the fast-diffusion component are 1.77×10^{-6}, 2.14×10^{-6}, 1.20×10^{-6}, and 1.15×10^{-6} cm^2/s, respectively, and the $f_{\text{fast(Boc-Gly)}}$, $f_{\text{fast(Boc-Phe)}}$, $f_{\text{fast(Boc-Trp)}}$, and $f_{\text{fast(Fmoc-Phe)}}$ values are 0.4, 0.4, 0.5, and 0.5, respectively. The diffusion coefficient for the fast-diffusion component of the amino acids is approximately twice as large as that for the slow-diffusion component, and the fractions of their two components are almost equal to each other. The $D_{\text{amino acids}}$ values in 1% crosslinked MPS gels from 30 to 50°C and the determined $E_{\text{amino acids}}$ values for the slow- and fast-diffusion components are shown in Table I. From these determined $D_{\text{amino acids}}$ values, it can be said that the $D_{\text{amino acids}}$ values in 1% crosslinked MPS gels are reduced with an increase in the molecular weight of the amino acids and that the four kinds of amino acids have very close values for $E_{\text{amino acids}}$, $f_{\text{slow(amino acids)}}$, and $f_{\text{fast(amino acids)}}$.

As for Boc-Gly, Boc-Trp and Fmoc-Phe in 2% crosslinked MPS gels with DMF-d_7 as a solvent at 30°C, plots of $\ln[A(\delta)/A(0)]$ against $\gamma^2G^2\delta^2(\Delta - \delta/3)$ have been plotted (not shown). The determined D values for the slow- and fast-diffusion components in 2% crosslinked MPS gels from 30 to 50°C and the determined E values are shown in Table I. The determined $D_{\text{Boc-Gly}}$, $D_{\text{Boc-Phe}}$, $D_{\text{Boc-Trp}}$, and $D_{\text{Fmoc-Phe}}$ values for the slow-diffusion component are 1.79×10^{-7}, 2.08

TABLE II
Diffusion Coefficients of Amino acids in MPS, PEG–PS, and CLEAR Gel[a]

Amino acids (molecular weight)	State		Diffusion coefficient (10^{-7} cm^2 s^{-1}, Q)			
			2 mol % DVB	1 mol % DVB	PEG–PS resin	CLEAR resin
Boc-Phe (265.30)	Solution	D		48.4		
	in gel	D_{fast}	6.43 (1.59)	21.4 (2.48)	14.2 (2.87)	17.3 (2.64)
		D_{slow}	2.08	8.82	9.17	12.5
Boc-Trp (304.34)	Solution	D		42.1		
	in gel	D_{fast}	7.26 (1.68)	12.0 (2.55)	12.3 (3.09)	17.1 (2.91)
		D_{slow}	2.43	5.16	7.04	10.8
Fmoc-Phe (387.43)	Solution	D		36.1		
	in gel	D_{fast}	8.42 (1.65)	11.5 (2.50)	11.4 (2.90)	14.0 (2.64)
		D_{slow}	2.40	4.22	5.61	8.46

$\times 10^{-7}$, 2.43 $\times 10^{-7}$, and 2.40 $\times 10^{-7}$ cm^2/s, respectively, and the $f_{slow(Boc\text{-}Gly)}$, $f_{slow(Boc\text{-}Phe)}$, $f_{slow(Boc\text{-}Trp)}$, and $f_{slow(Fmoc\text{-}Phe)}$ values are 0.8, 0.8, 0.8, and 0.6, respectively. However, the determined $D_{Boc\text{-}Gly}$, $D_{Boc\text{-}Phe}$, $D_{Boc\text{-}Trp}$, and $D_{Fmoc\text{-}Phe}$ values for the fast-diffusion component are 6.15 $\times$ 10^{-7}, 6.43 $\times$ 10^{-7}, 7.26 $\times$ 10^{-7}, and 8.42 $\times$ 10^{-7} cm^2/s, respectively, and the $f_{fast(Boc\text{-}Gly)}$, $f_{fast(Boc\text{-}Phe)}$, $f_{fast(Boc\text{-}Trp)}$, and $f_{fast(Fmoc\text{-}Phe)}$ values are 0.2, 0.2, 0.2, and 0.4, respectively. As expected from the network size of the gels, the $D_{amino\ acids}$ values of the slow-diffusion component in 2% crosslinked MPS gels are much smaller than those in 1% crosslinked MPS gels, for which $Q_{Boc\text{-}Gly}$, $Q_{Boc\text{-}Phe}$, $Q_{Boc\text{-}Trp}$, and $Q_{Fmoc\text{-}Phe}$ are 1.50, 1.59, 1.68, and 1.65, respectively. Furthermore, the $D_{Boc\text{-}Gly}$ and $D_{Boc\text{-}Phe}$ values are smaller than the $D_{Boc\text{-}Trp}$ and $D_{Fmoc\text{-}Phe}$ values even though the molecular weights of Boc-Gly and Boc-Phe are lower than those of Boc-Trp and Fmoc-Phe.

Next, we are concerned with E for these amino acids, as obtained from the temperature dependence of D. The $E_{Boc\text{-}Gly}$ and $E_{Boc\text{-}Phe}$ values for the slow-diffusion components are larger than the $E_{Boc\text{-}Trp}$ and $E_{Fmoc\text{-}Phe}$ values, although the $E_{amino\ acids}$ values determined for four kinds of amino acids in 1% crosslinked MPS gels are very close to one another. Such an experimental finding may be explained by a consideration of the $Q_{amino\ acid}$ behavior. In the 1% crosslinked MPS gels and 2% crosslinked MPS, the $Q_{Boc\text{-}Gly}$ and $Q_{Boc\text{-}Phe}$ values are smaller than the $Q_{Boc\text{-}Trp}$ and $Q_{Fmoc\text{-}Phe}$ values because $Q_{amino\ acid}$ depends on the crosslinking density and intermolecular interactions between the networked PS chains and amino acid or solvent.

Furthermore, we are concerned with E for the fast-diffusion component of the amino acids, as shown in Table I. The E values in the gels are 2.5–3.0 kcal/mol and are very close to those in solution (ca. 3 kcal/mol). This shows that there are very weak intermolecular interactions between the amino acids and PS network chains.

Diffusion coefficients of amino acids in PEG–PS network gels and CLEAR gels with DMF-d_7 as a solvent

In the PFGSE ^{1}H-NMR experiments on PEG–PS gels and CLEAR gels, in addition to MPS gels, the Δ value was fixed at 10 ms. The plots of $\ln[A(\delta)/A(0)]$ for Boc-Phe, Boc-Trp, and Fmoc-Phe in the gels against $\gamma^2G^2\delta^2(\Delta - \delta/3)$ do not lie on a straight line. Therefore, these amino acids have multiple components of diffusion within the observation time. The diffusion echo signal was approximately deconvoluted by two slow- and fast-diffusion components. In PEG–PS gels, the determined $f_{slow(Boc\text{-}Phe)}$, $f_{slow(Boc\text{-}Trp)}$, and $f_{slow(Fmoc\text{-}Phe)}$ values for the slow-diffusion component at 30°C are 0.7, 0.6, and 0.6, respectively. In CLEAR gels, the determined $f_{slow(Boc\text{-}Phe)}$, $f_{slow(Boc\text{-}Trp)}$, and $f_{slow(Fmoc\text{-}Phe)}$ values for the slow-diffusion component at 30°C are 0.7, 0.6, and 0.6, respectively. These $D_{amino\ acids}$ values for the slow-diffusion component in 1% crosslinked MPS gels, 2% crosslinked MPS gels, PEG–PS gels, and CLEAR gels at 30°C and the corresponding $Q_{amino\ acids}$ values are shown in Table II. In DMF-d_7 solutions, the order in the $D_{amino\ acids}$ value is Boc-Phe $>$ Boc-Trp $>$ Fmoc-Phe. As expected, amino acids with lower molecular weights diffuse more quickly. Also, in 1% crosslinked MPS gels, PEG–PS gels, and CLEAR gels, the order in the $D_{amino\ acids}$ value is Boc-Phe $>$ Boc-Trp $>$ Fmoc-Phe. Therefore, it can be said that the $D_{amino\ acids}$ value is sensitively dependent on the molecular weight, that is, the molecular size, of corresponding amino acids, except for 2% crosslinked MPS gels at $Q < 2.0$. As for the diffusion process of the amino acids in the four kinds of polymer gels, the order in the $D_{amino\ acids}$ values of Boc-Phe, Boc-Trp, and Fmoc-Phe in the gels is CLEAR gels $>$ PEG–PS gels $>$ 1% crosslinked MPS gels $>$ 2% crosslinked MPS gels, although the magnitude of the $Q_{amino\ acids}$ value in the gels is PEG–PS gels $>$ CLEAR gels $>$ 1% crosslinked MPS gels $>$ 2% crosslinked MPS gels. These experimental results are different from those reported previously,[24,25] in which the $D_{amino\ acids}$

value in MPS gels is increased with an increase in $Q_{\text{amino acids}}$. In MPS gels and PEG–PS gels with PS backbones, such a trend agrees with the previous experimental results, as shown in Table II. However, the $D_{\text{amino acids}}$ values in CLEAR gels with PEG backbones are much larger despite small Q values in comparison with PEG–PS gels with PS backbones. It seems that in PEG–PS gels, amino acid molecules strongly interact with the phenyl groups of PEG–PS networks, and so their diffusion is more greatly restrained than for the case of CLEAR gels. Furthermore, this may be explained as follows. MPS gels and PEG–PS gels have bulky phenyl groups with six π electrons. When the corresponding gels have the same Q value, the cavity size in the gels with PS backbones is predicted to become much smaller than that in CLEAR gels with PEG backbones because of the existence of the bulky phenyl rings.

Next, the D_{fast} values of Boc-Gly, Boc-Phe, Boc-Trp, and Fmoc-Phe are shown in Table II. The D_{fast} values in polymer gels are two or three times larger than the D_{slow} values. PEG–PS gels and CLEAR gels, as well as MPS gels, have broad distributions of the diffusion rate, and so the D_{fast} values in these gels are different from one another. However, some amino acid molecules have similar diffusion rates in different gels. This is a remarkable point.

Diffusion coefficients of Boc-Phe in PS network gels with THF-d_8 as a solvent

In a previous work,[24] we have reported that the D_{THF} value of THF in PS gels is much larger than the D_{DMF} value of DMF in PS gels with the same Q value and that the volume swelling of PS network resins with THF is much larger than that with DMF. Therefore, it can be assumed that the $D_{\text{amino acid}}$ value of amino acids in MPS gels with THF as a solvent is larger than that in MPS gels with DMF as a solvent. The diffusion coefficients of Boc-Phe in THF-d_8 as a solvent and in DMF-d_7 as a solvent were determined. As for Boc-Phe in a THF-d_8 solution in the absence of the resin, Boc-Phe in 1% crosslinked MPS gels with THF-d_8 as a solvent, and Boc-Phe in 2% crosslinked MPS gels with THF-d_8 as a solvent at 30°C, $\ln[A(\delta)/A(0)]$ against $\gamma^2G^2\delta^2(\Delta - \delta/3)$ lies on a straight line (not shown) when the Boc-Phe concentration is 10 wt % and $Q_{1\ \text{mol\%DVB/THF}}$ and $Q_{2\ \text{mol\%DVB/THF}}$ are 3.71 and 2.98, respectively. In the PFGSE NMR experiments, the Δ value was fixed at 10 ms. Boc-Phe in MPS gels with THF-d_8 as a solvent, as well as Boc-Phe in a THF-d_8 solution, has a single component of diffusion within the observation time. The $D_{\text{THF solution}}$, $D_{1\ \text{mol\%DVB/THF}}$, and $D_{2\ \text{mol\%DVB/THF}}$ values are 8.51×10^{-6}, 2.57×10^{-6}, and 2.29×10^{-6} cm^2/s, respectively, at 30°C; however, the $D_{\text{DMF solution}}$, $D_{1\ \text{mol\%DVB/DMF}}$, and $D_{2\ \text{mol\%DVB/DMF}}$ values are 4.84×10^{-6}, 8.82×10^{-7}, and 2.08×10^{-7} cm^2/s, respectively, at 30°C. The diffusion rate of the amino acid in the MPS/THF gel system in the solid-phase peptide reaction field is much faster than that in the MPS/DMF system.

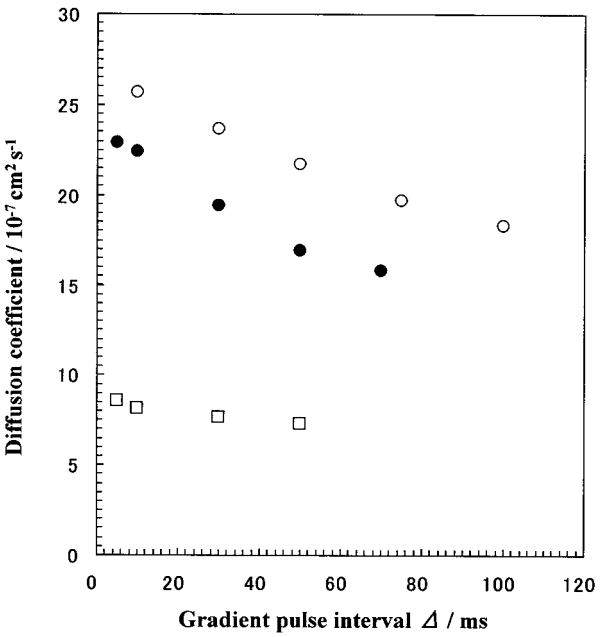

Figure 3 Dependence of $D_{\text{Boc-Phe}}$ in MPS gels with THF-d_8 at (○) Q = 3.71 and (●) Q = 2.98 and in MPS gels with DMF-d_7 at (□) Q = 2.48 in Δ and at 30°C.

In Figure 3, the determined $D_{1\ \text{mol\%DVB/THF}}$, $D_{2\ \text{mol\%DVB/THF}}$, and $D_{1\ \text{mol\%DVB/DMF}}$ values at 30°C are plotted against Δ, that is, the observation timescale. In the previous work, we have reported that the $D_{\text{Boc-Gly}}$ value in 1% crosslinked MPS gels with DMF-d_7 as a solvent depends on Δ, the $f_{\text{slow(Boc-Gly)}}$ value depends on Δ, which is related to the diffusion distance, and the multiple components of diffusion are averaged over the observation time within Δ = 50 ms.[25] However, Boc-Phe in MPS gels with THF-d_8 as a solvent and Boc-Phe in a THF-d_8 solution have a single component of diffusion within the observation time in the Δ range of 5–100 ms, and the effect of the heterogeneity of gels on diffusion could not be observed. Furthermore, the Δ dependence of $D_{1\ \text{mol\%DVB/THF}}$ is different from that of $D_{1\ \text{mol\%DVB/DMF}}$.

Required time for which solvents and amino acids in polymer supports for a solid-phase peptide synthesis diffuse between average diameters of swollen beads

We are concerned with the reaction rate of solid-phase synthesis, as reported previously.[43–46] The reaction rate of 9-fluorenylmethoxycarbonyl deprotection with 25-μm TentaGel particles is more than three times faster than that with 90-μm TentaGel particles.[11,46] This indicates that a reduction of the particle size of

the polymer support leads to a dramatic increase in the reaction rate. Therefore, the rate of reaction depends on the diffusion rate. Furthermore, the reaction rate in 1% crosslinked MPS gels is much faster than that in 2% crosslinked MPS gels. From these experimental findings, it can be said that the reaction rate depends on the diffusion rate associated with the collision frequency between amino acids and reactive sites.

In a series of solid-phase peptide syntheses, the solvent plays an important role in carrying amino acids to reactive sites in the polymer matrix and in removing reaction products, unreacted starting materials, and impurities from gels. For these reasons, it seems that the diffusion rate of a solvent in polymer gels is closely related to the required time in a series of syntheses. Here, T_r is defined as the required time in which solvents diffuse in a polymer gel with d_{swollen}, which is the average diameter of polymer gel beads. In this work, MPS gels with an average diameter of 150 μm in the dried state are employed. Then, d_{swollen} in the gel at $Q = 2.0$ is 189 μm, and d_{swollen} in the gel at $Q = 3.0$ is 216 μm. When the Q value is large, solvent molecules must move a long distance. In that case, the diffusion rate becomes fast. Therefore, T_r depends on D and Q. The T_r value was estimated as follows. In the measurement timescale of Δ, D corresponds to a Gaussian distribution of a squared standard deviation:

$$\langle z^2 \rangle = 2D\Delta \tag{3}$$

where $\langle z^2 \rangle$ is the mean-square displacement in the z direction from its starting point after the diffusion time Δ. The $\langle z^2 \rangle$ value gives us information on the diffusion distance $\langle z \rangle$, which reflects the experimental results:

$$\langle z \rangle = 2\sqrt{\frac{D\Delta}{\pi}} \tag{4}$$

Therefore, T_r becomes Δ if $\langle z \rangle$ is equal to d_{swollen} in eq. (4). Here, the obtained D values in our previous work[24] were employed. To determine T_r exactly, we must determine the D value on the timescale of Δ if $\langle z \rangle$ is equal to d_{swollen} because of restricted diffusion, and so the obtained D value at $\Delta = 10$ ms was employed. In practice, it is very difficult to measure the D value of probe molecules in swollen beads on a timescale of minutes with the NMR method. As for DMF-d_7 in MPS gels at 30 and 50°C and for THF-d_8 in MPS gels at 30°C, the T_r dependence of Q is shown in Figure 4. At 30°C, the $T_{r(\text{DMF})}$ value decreases with an increase in Q, and especially in the range of $Q < 2.0$, the change in the $T_{r(\text{DMF})}$ value is very large. In the range of $Q > 3.0$, the $T_{r(\text{DMF})}$ value is independent of Q. At 50°C, the Q dependence of the $T_{r(\text{DMF})}$ value is similar to that

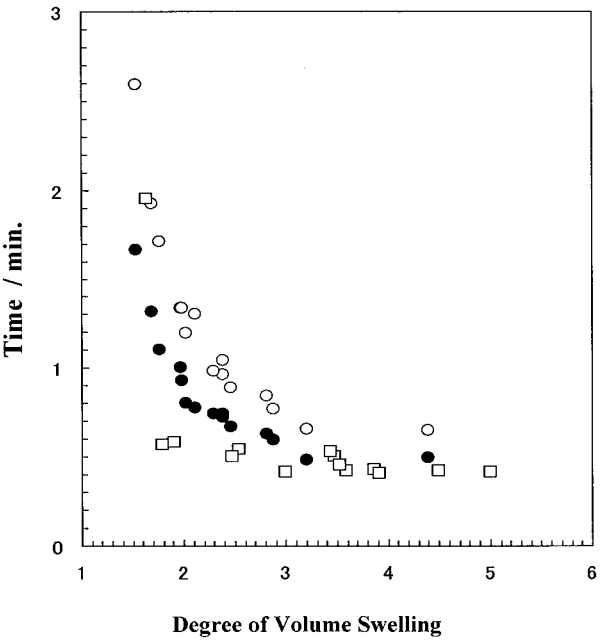

Figure 4 Dependence of T_r for solvents in MPS gels between d_{swollen} on Q: in DMF-d_7 at (○) 30 and (●) 50°C and in THF-d_8 (□) at 30°C.

of the $T_{r(\text{DMF})}$ value at 30°C. As shown in Figure 4, the $T_{r(\text{THF})}$ value is somewhat smaller than the $T_{r(\text{DMF})}$ value at the same Q value. Furthermore, the $T_{r(\text{THF})}$ value is independent of Q in the range of $Q > 2.0$. From these results, it is found that the required time of a series of solid-phase syntheses cannot simply be reduced with gels at large Q, but it seems that the required time of a series of solid-phase syntheses increases with a decrease in Q in the range of $Q < 2.0$.

The reaction rate depends on the collision frequency and the collision energy. It is not easy to determine the collision frequency. Nevertheless, a parameter related to the collision frequency is discussed next. We discuss the required time within which amino acids transfer from a reactive site to a reactive site. For dried beads, the distance between the two reactive sites is assumed to be a. When a dried bead with d_{dry} is swollen by a solvent at equilibrium, the diameter of a bead gel becomes d_{swollen}. In the swollen bead gel, the distance between the two reactive sites is assumed to be l. Therefore, l is expressed by the following equation:

$$l = a \times \frac{d_{\text{swollen}}}{d_{\text{dry}}} \tag{5}$$

It can also be expressed with Q:

$$l = a \times \sqrt[3]{Q} \tag{6}$$

To relate this equation to the collision frequency, we may use the required time ($T_{t(\text{amino acids})}$) in which

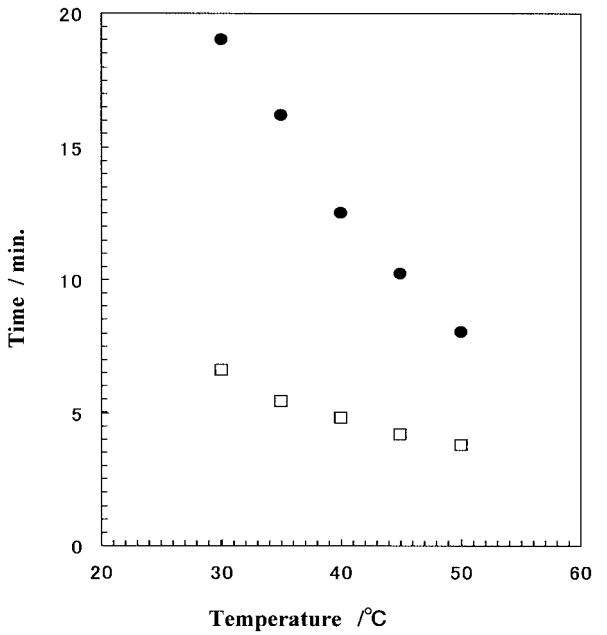

Figure 5 Temperature dependence of $T_{t(\text{Boc-Phe})}$ for Boc-Phe (□) in 1% crosslinked MPS gels with DMF-d_7 and (●) in 2% crosslinked MPS gels from 30 to 50°C.

amino acids in polymer gels diffuse within d_{swollen}, instead of the required time in which amino acids transfer from a reactive site to another reactive site. Therefore, $T_{t(\text{amino acids})}$ is expressed by the following equation:

$$T_{t(\text{amino acids})} = (d_{\text{swollen}})^2 \times \frac{\pi}{4D} \tag{7}$$

d_{swollen} can be expressed by eqs. (5) and (6):

$$d_{\text{swollen}} = d_{\text{dry}} \times \sqrt[3]{Q} \tag{8}$$

Figure 5 shows the temperature dependence of $T_{t(\text{Boc-Phe})}$ in 1% crosslinked MPS gels and 2% crosslinked MPS gels from 30 to 50°C. The $d_{\text{swollen/1 mol\%DVB}}$ value of 1% crosslinked MPS gels is 202 μm, and the $d_{\text{swollen/2 mol\%DVB}}$ value of 2% crosslinked MPS gels is 172 μm. The smaller $T_{t(\text{Boc-Phe})}$ is, the greater the collision frequency is between amino acids and reactive sites. As seen in Figure 5, the collision frequency in 1% crosslinked MPS gels is greater than that in 2% crosslinked MPS gels. Furthermore, the collision frequency in 2% crosslinked MPS gels can be increased greatly by temperature.

As for Boc-Phe, Boc-Trp, and Fmoc-Phe in MPS gels, PEG–PS gels, and CLEAR gels, the $T_{t(\text{amino acids})}$ values were estimated in the same way as Boc-Phe in MPS gels. Figure 6 shows $T_{t(\text{amino acids})}$ values at 30°C. MPS1 indicates 1% crosslinked MPS gel, and MPS2 indicates 2% crosslinked MPS gel. As for Boc-Phe, the magni-

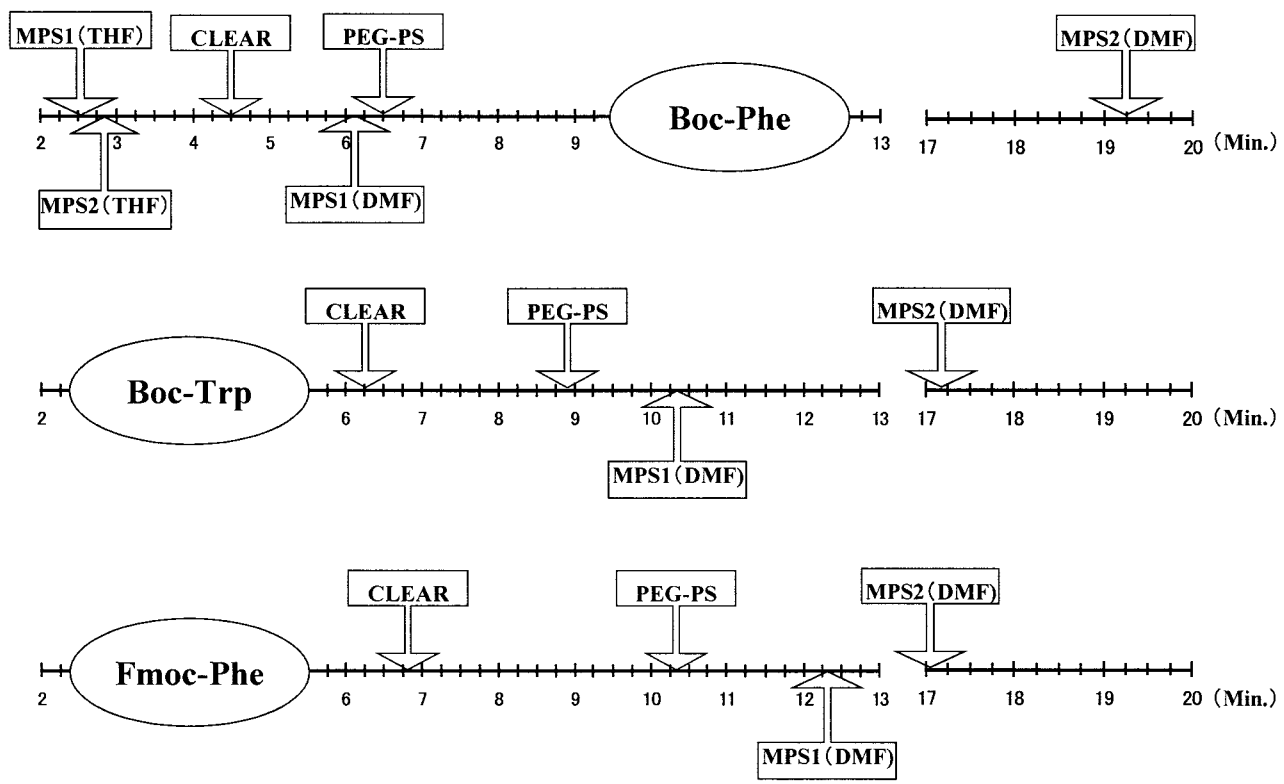

Figure 6 $T_{t(\text{amino acids})}$ at 30°C. MPS1 is 1% crosslinked by DVB, and MPS2 is 2% crosslinked by DVB.

tude of the $T_{t(\text{Boc-Phe})}$ values in the gels is as follows: MPS1 gel with THF as a solvent ė MPS2 gel with a THF solution $<$ CLEAR gel with a DMF solution $<$ MPS1 gel with a DMF solution ė PEG–PS gel with a DMF solution $\ll$ MPS2 gel with a DMF solution. It is interesting that the collision frequency for the MPS2 gel is very low. As for Boc-Trp and Fmoc-Phe, similar tendencies were obtained. As seen in this figure, the collision frequency between amino acids and reactive sites has the following order in the gels: CLEAR gel $>$ PEG–PS gel $>$ MPS1 gel $>$ MPS2 gel.

As mentioned previously, the collision frequency for MPS, PEG–PS, and CLEAR solid-phase peptide reaction fields can roughly be estimated by diffusion coefficients. In general, the reaction rate of the solid-phase synthesis depends on the rate of diffusion for the amino acid. In practice, it seems that the solid-phase reaction rate depends on the dynamics of the reactive sites in addition to the diffusional behavior of the solvents and reagents. The reactive sites of the MPS resin are in the neighboring region of the main chain, but that of the PEG–PS resin is at a distance from the main chain because of the existence of the spacer of PEG groups. Therefore, the mobility of the reactive sites in the PEG–PS gel is better than that of the reactive sites in the MPS gel.

CONCLUSIONS

The D values of Boc-Gly, Boc-Trp, Boc-Phe, and Fmoc-Phe in MPS gels, PEG–PS gels, and CLEAR gels were determined with the PFGSE ^{1}H-NMR method. From these experimental results, the dependence of the diffusion coefficients of amino acids in various polymer gels on the nature and molecular weight of the amino acids was elucidated and was found to be sensitive to Q, and the effect of heterogeneity on the network size was significantly clarified. In addition, it was demonstrated that the PFGSE ^{1}H-NMR method with a strong field-gradient system could give very useful information about the diffusion rates of reagents in solid-phase reaction fields.

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