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Diffusional behavior of amino acids in solid-phase reaction field as studied by ^{1}H pulsed-field-gradient spin-echo NMR method

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Abstract

The diffusion coefficients (D) of *tert*-butyloxycarbonyl-L-phenylalanine (Boc-Phe) in Merrifield network polystyrene gels, used as a solid-phase reaction field have been determined as a function of the amino acid concentration over the temperature range from 30 to 50 °C by means of the ^{1}H pulsed-field-gradient spin-echo NMR method. From these experimental results, it was found that the D value of Boc-Phe in DMF-d$_7$ solution, in DVB 1 and 2% cross-linked network polystyrene gels depends on the amino acid concentration. The D value of Boc-Phe·Cs(*tert*-butyloxycarbonyl-L-phenylalanine cesium) salt in the solid-phase reaction field under chemical reaction was determined at 50 °C. Further, it was found that the D value depends on the NMR observation time, that is the applied two field-gradient pulse interval. Details of its analysis were discussed. © 2002 Published by Elsevier Science Ltd.

Keywords: Polymer gel; Diffusion coefficient; ^{1}H pulsed-field-gradient spin-echo NMR

1. Introduction

Solid-phase synthesis has been applied to combinatorial chemistry which has already become a standard part of the repertoire used in the search for new drugs. This approach has been used in the field of researching new organic materials. In general, it is known that the rate of reaction in the solid-phase reaction field is associated with the diffusional behavior of the reagents used. This depends on variables such as the diffusional behavior of solvents, dynamics and structure of matrix polymer, network size, particle size, concentration of reagents and reaction temperature [1–7]. Thus, it is important to understand the diffusional behavior of solvents and reagents and the dynamics of polymer gels.

NMR spectroscopy has given very useful information about the diffusional behavior of probe molecules and the structure and dynamics of polymer gel systems [8–14]. We have already reported the diffusional behavior of solvents in Merrifield network polystyrene gels and the dependence of the self-diffusion coefficient of amino acids in Merrifield network polystyrene gels on the degree of volume swelling (Q) and temperature as studied by the ^{1}H pulsed-field-gradient spin-echo (PFGSE) NMR method [15,16]. Further, it has been clarified that diffusion for amino acids in Merrifield network polystyrene gels with $Q = 2.5$ has many kinds of components for the diffusion such as slow diffusion components and multi-fast-diffusion-components, and the fraction of the slow diffusion component was about 0.6 with a measurement time scale of 10 ms.

In this work, we aim to elucidate the dependence of the self-diffusion coefficient of amino acids in network polystyrene gels on the amino acid concentration in order to understand deeply the diffusional behavior of amino acids in network polystyrene gels, and, further, to determine the diffusion coefficient of Boc-Phe·Cs(*tert*-butyloxycarbonyl-L-phenylalanine cesium) salt in the solid-phase reaction field. This permits us to clarify the diffusional behavior of reagents used in the solid-phase reaction field under chemical reaction. Further, we will discuss the restricted diffusion associated with the size of cavity of the gel network.

2. Experimental section

2.1. Materials

The Merrifield resin beads purchased from Nova Biochem Co. Ltd and Peptide Institute Inc. were used as network polystyrene samples. The network polystyrene

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resin is cross-linked by divinylbenzene (DVB) and has been functionalized with chloromethyl groups. Two types of resin beads were used with the fraction of cross-linking of 1% (resin A) and 2% (resin B). The average diameter of the dried beads was in the range of 75–150 μm. The gels of beads were prepared by soaking them in DMF-d_7 solution of amino acids for 3 days.

The degree of volume swelling of the network polystyrene gel (Q) is defined as the ratio of the volume of a swollen network polystyrene gel at room temperature (V_{swollen}) to the volume of a dried network polystyrene gel (V_{dry}) by $Q = V_{\mathrm{swollen}}/V_{\mathrm{dry}}$. These volumes are determined by the average diameter of the network polystyrene gel beads, which is measured by a microscope.

Boc-Gly(*tert*-butyloxycarbonyl-glycine) and Boc-Phe-(*tert*-butyloxycarbonyl-L-phenylalanine) were purchased from Peptide Institute Inc. *N*,*N*-Dimethylformamide-d_7 (DMF-d_7) was purchased from Merck Co. Boc-Phe·Cs-(*tert*-butyloxycarbonyl-L-phenylalanine cesium) salt was synthesized as follows. A solution of Boc-Phe (2.5 g) dissolved in water (5 ml) and methanol (50 ml) was placed in a 100 ml flask. By adding 20 wt% cesium carbonate aqueous solution, the reaction system was kept at pH 7. The crude products obtained were concentrated under reduced pressure, washed with 30 ml of DMF and then concentrated under reduced pressure again. Furthermore, this procedure was repeated twice with 20 ml of DMF. Then, it was dried over P_2O_5 under reduced pressure.

2.2. Measurements

The self-diffusion coefficient (D) measurements on amino acids in the network polystyrene gels were carried out over a wide range of temperatures by means of a JEOL GSX-270 NMR spectrometer operating at 270.1 MHz for ^{1}H with a homemade pulse gradient generator and a temperature controller [21–29]. The temperature control in these experiments was ±0.1 °C. A homemade pulsed-field-gradient generator is designed to generate a strong field-gradient pulse (with the maximum field strength of about 2000 G cm^{-1}) and to suppress the eddy currents induced. This has been successfully used in our previous works on diffusional behavior in polymer gel systems [15,30–33]. In this work, a field-gradient strength, G, of about 1360 G cm^{-1} was used. The spectral width and number of data points were 4.0 kHz and 4096, respectively. Only the free induction decay (FID) was recorded after the echo maximum and then the Fourier transformation (FT) was applied.

The D values were determined by using the relationship between echo signal intensity and 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 $\gamma^2 G^2 \delta^2(\Delta - \delta/3)$ give a straight line with a slope of $-D$. Therefore, the D value can be determined from its slope. When probe molecules have multi-components in diffusion on the measurement time scale, the total echo attenuation is given by a superposition 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, p_i is the fractional proton number of the ith component, and $\sum p_i = 1$. The fraction for the slow diffusion component can be determined from the intercept of the least-squares fitted straight line. The δ and the Δ values employed in these experiments are 0.001–1.0 and 10–50 ms, respectively.

The self-diffusion coefficient obtained decreases with an increase in diffusion time. Stejskal and Tanner discussed restricted diffusion in colloidal systems measured by the ^{1}H PFGSE NMR method. A general treatment of time dependent diffusion in a system of parallel planar barriers of arbitrary permeability has been performed, with emphasis on the results expected from NMR data [17,18]. Further, Von Meerwall and Ferguson have improved this theory [19]. For the diffusion of water in starch gels, by analyzing the data with this theory, the permeability of the skeletal structure of starch gels and the average size of water compartments and the diffusion coefficient of the compartmentalized water have been estimated [20]. These studies give information about the structure of the gel used. In this work, amino acids in network polystyrene gels were observed to have restricted diffusion. Further, we discuss the observation that the fraction for the slow diffusion component (averaged diffusion component) depends on the diffusing-time Δ which is related to the diffusion distance.

3. Results and discussion

3.1. Diffusional behavior of amino acids in network polystyrene gels

3.1.1. Dependence of the diffusion coefficients of Boc-Phe in network polystyrene gels on the concentration of amino acids

We are concerned with the dependence of the diffusion coefficients of amino acids in network polystyrene gels on the concentration of amino acids ($C_{\mathrm{amino\ acid}}$). As for Boc-Phe in DMF-d_7 solution, the plots of $\ln[A(\delta)/A(0)]$ against $\gamma^2 G^2 \delta^2(\Delta - \delta/3)$ are shown in Fig. 1. It is seen that the experimental data lie on a straight line. This shows that Boc-Phe in DMF-d_7 solution has a single-component of

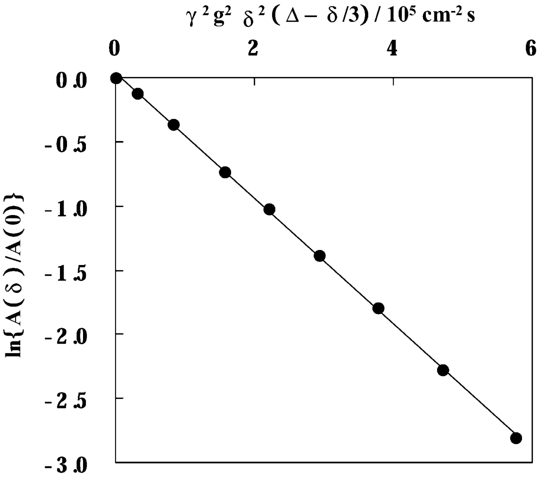

Fig. 1. Diffusional spin-echo attenuation of 10 wt% Boc-Phe in DMF-d_7 solution (●) by varying field gradient pulse duration at 30 °C.

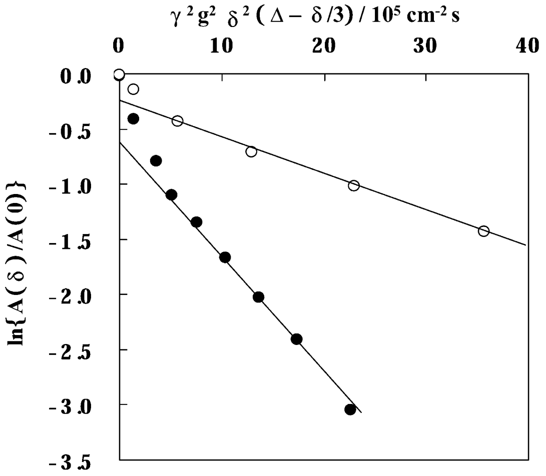

Fig. 3. Diffusional spin-echo attenuation of 10 wt% Boc-Phe in resin A gels (●) and resin B gels (○) by varying field gradient pulse duration at 40 °C.

diffusion during the observation time. Further, the plots of the diffusion coefficient ($D_{\text{Boc-Phe}}$) of Boc-Phe in DMF-d_7 solution in the absence of resin at 40 °C and the activation energy of diffusion (E) against the concentration $C_{\text{amino acid}}$ are shown in Fig. 2, where the activation energy of diffusion was obtained from the plots of $\ln D$ against the inverse of absolute temperature ($1/T$) (the Arrhenius plots) in the temperature range from 30 to 50 °C. From this figure, it is found that the $D_{\text{Boc-Phe}}$ value decreases with an increase in $C_{\text{amino acid}}$, and further that the $E_{\text{Boc-Phe}}$ value is kept constant (3.10 kcal mol^{-1}) within the $C_{\text{amino acid}}$ range from 4 to 12 wt%. The reduction of the $D_{\text{Boc-Phe}}$ value may be explained by a consideration that the Boc-Phe molecule interacts with itself with an increase in $C_{\text{amino acid}}$ in addition to interacting with DMF-d_7 and thus the diffusion of Boc-Phe is reduced. Further, we may explain the $C_{\text{amino acid}}$ independent activation energy of diffusion by a consideration that the strengths of intermolecular interactions between Boc-Phe molecules, and Boc-Phe and DMF-d_7 are close to each other.

As for Boc-Phe in resin A (with 1% cross-linking by divinylbenzene) gels and in resin B (2% cross-linking by divinylbenzene) gels at 40 °C, the plots of $\ln[A(\delta)/A(0)]$ against $\gamma^2 G^2 \delta^2(\Delta - \delta/3)$ are shown in Fig. 3. It is seen that the experimental data do not lie on a straight line. This shows that Boc-Phe in resin A and B gels have multi-component diffusion such as slow diffusion components and multi-fast-diffusion components during the observation time. In Figs. 4 and 5, the $D_{\text{Boc-Phe}}$ values as obtained from the slow diffusion components and the $E_{\text{Boc-Phe}}$ values of Boc-Phe in resin A and B gels at 40 °C are plotted against $C_{\text{amino acid}}$, respectively. By comparison with the experimental data as shown in Figs. 2, 4 and 5, it is found that the $D_{\text{Boc-Phe}}$ value in resin A is much smaller than that in DMF-d_7 solution in the absence of resin. This indicates that Boc-Phe interacts strongly with the polymer network. As

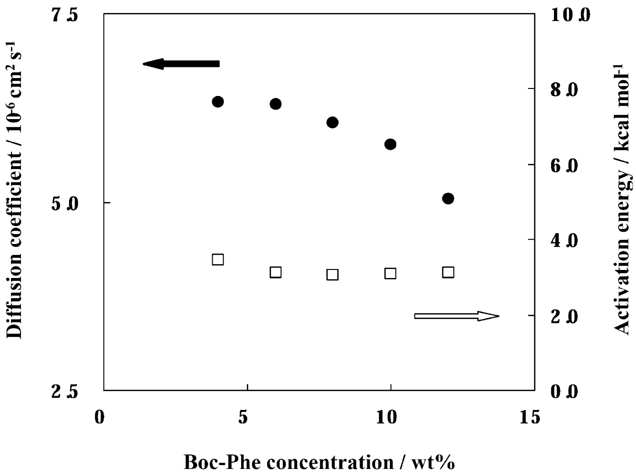

Fig. 2. The plots of the diffusion coefficient of Boc-Phe in DMF-d_7 solution (●) and activation energy of self-diffusion (□) against the concentration $C_{\text{amino acid}}$ at 40 °C.

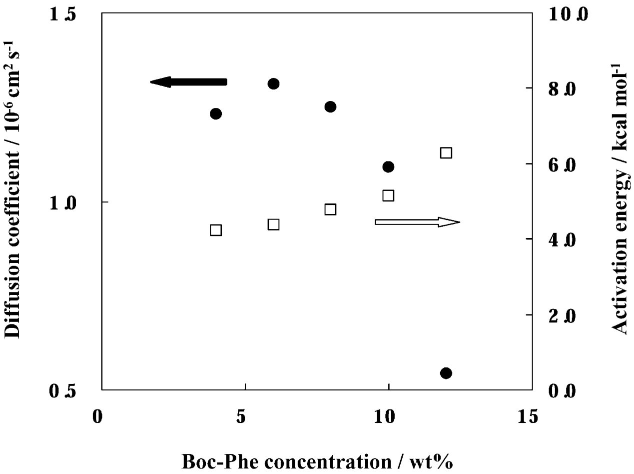

Fig. 4. The plots of the diffusion coefficient of Boc-Phe in resin A gels (●) with DMF-d_7 as solvent at 40 °C and activation energy of diffusion (□) against the concentration $C_{\text{amino acid}}$.

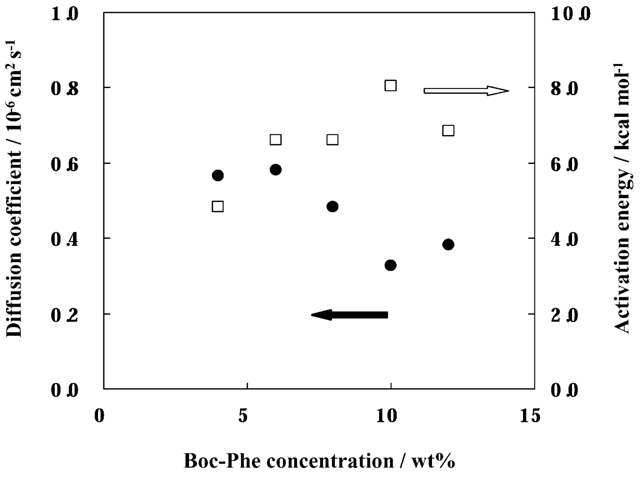

Fig. 5. The plots of the diffusion coefficient of Boc-Phe in resin B gels (●) with DMF-d_7 as solvent at 40 °C and the activation energy of diffusion (□) against the concentration $C_{\text{amino acid}}$.

seen from Fig. 4, the $D_{\text{Boc-Phe}}$ value in resin A gels below $C_{\text{amino acid}} = 10$ wt% is independent of $C_{\text{amino acid}}$, but decreases largely at $C_{\text{amino acid}} \geqq 10$ wt%. Such a reduction of the $D_{\text{Boc-Phe}}$ value may be explained by the consideration that the Boc-Phe molecule interacts strongly with the polymer network and interacts with itself with an increase in $C_{\text{amino acid}}$ in addition to interacting with DMF-d_7 and thus it leads to slow diffusion of Boc-Phe. On the other hand, the activation energy of diffusion for Boc-Phe increases with an increase in $C_{\text{amino acid}}$. This is very different from the observation made in the absence of resin. Such an increase in the activation energy of diffusion for Boc-Phe may come from an increase of intermolecular interaction with the polymer network. As reported previously [15], $E_{\text{Boc-Gly}}$ values in the $1.5 < Q < 2.5$ range increase with a decrease in Q. The Q values of resin A gels are 2.4 ± 0.1 at $4 < C_{\text{amino acid}} < 10$ wt%, but 2.1 at $C_{\text{amino acid}} = 12$ wt%. On the other hand, the $E_{\text{Boc-Phe}}$ value in resin A gels at $C_{\text{amino acid}} = 12$ wt% is larger than that of Boc-Phe at $4 < C_{\text{amino acid}} < 10$ wt%. The Q value of resin B gels is 1.7 at $\text{C}_{\text{amino acid}} = 4$ wt%, but is 1.5 at $C_{\text{amino acid}} = 12$ wt%. Thus, the $E_{\text{Boc-Phe}}$ value in resin B gels increases with an increase in $C_{\text{amino acid}}$.

Further, it is found from Figs. 4 and 5 that the $D_{\text{Boc-Phe}}$ value for the slow diffusion component in resin A gels at $C_{\text{amino acid}} = 12$ wt% (5.43×10^{-7} cm^2 s^{-1}) is close to the $D_{\text{Boc-Phe}}$ values in resin B gels at $C_{\text{amino acid}} = 4$ (6.21×10^{-7} cm^2 s^{-1}) and 6 wt% (5.82×10^{-7} cm^2 s^{-1}), and the fraction of the slow diffusion component in resin A gels at $C_{\text{amino acid}} = 12$ wt% is about 0.6, while the $D_{\text{Boc-Phe}}$ value for the slow diffusion component in resin B gels at $C_{\text{amino acid}} = 6$ wt% is 5.82×10^{-7} cm^2 s^{-1} and the fraction is about 0.8. Therefore, it can be said that there is difference in the diffusional behavior of Boc-Phe between resin A gels at $C_{\text{amino acid}} = 12$ wt% and resin B gels at $C_{\text{amino acid}} = 6$ wt%, but the rate of diffusion for Boc-Phe in the region close to polystyrene chains is nearly equal. From these experimental results, it can be said that the factors controlling diffusion of Boc-Phe in the resin A and B gels are $C_{\text{amino acid}}$ and Q.

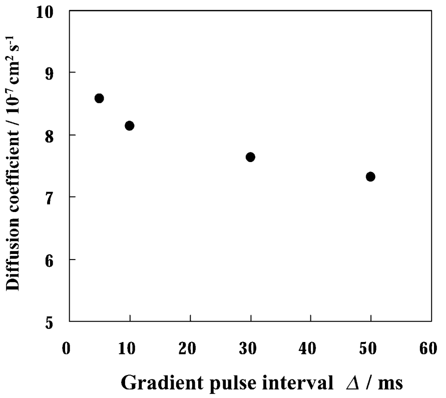

Fig. 6. Dependence of the diffusion coefficient of 10 wt% Boc-Gly in resin A gels (●) with DMF-d_7 as solvent on the gradient pulse interval Δ at 30 °C.

3.1.2. Diffusing-time dependence of diffusion coefficients of Boc-Gly in network polymer gels

The diffusion coefficient of 10 wt% Boc-Gly in network polymers with DMF-d_7 as solvent was measured as a function of diffusing-time (Δ) (that is, field-gradient pulse-interval time) by the field-gradient NMR method [17–20], in order to obtain from information about the polymer network size. The $D_{\text{Boc-Gly}}$ values obtained are plotted against Δ at 30 °C as shown in Fig. 6.

It is seen that the $D_{\text{Boc-Gly}}$ value depends on Δ. This shows that there exists restricted diffusion in this system. Extensive studies of restricted diffusion are very important in demonstrating the topological structure of the medium and the differences in the diffusion coefficient between shorter and longer Δ range measurements. To achieve this, a large number of diffusion coefficients must be measured by the PFG stimulated echo NMR method.

First, we are concerned with the result that the fraction of the slow diffusion component (averaged diffusion component) depends on the diffusing-time Δ which is related to the diffusion distance. It is assumed that Boc-Gly molecules are transported in some sites with different environments during the diffusing-time, in which intermolecular interactions between Boc-Gly and the polymer network are largely different from each other and then have multi-components for diffusion. This implies that by adjusting the diffusing-time, the diffusion of Boc-Gly molecules with different diffusion coefficients in different sites can be observed. In the corresponding PFGSE experiments, Boc-Gly in resin A gel has multi-components for diffusion such as the slow diffusion component and the multi-fast-diffusion-components. 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 polymer network contribute to the fast diffusion components. These interactions depend on the intermolecular distance between Boc-Gly and the polystyrene network. If probe molecules diffuse over a long Δ time, the observed diffusion coefficient may become an averaged value owing to the diffusion through the sites with different environments, which are near to or distant from the polymer network. In time scale of t, the self-diffusion coefficient D corresponds to a Gaussian distribution of a squared standard deviation as expressed by

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

where $\langle z^2 \rangle$ is the mean square displacement in the z direction from its starting point after the diffusion time Δ. In this measurement, t is Δ. As for Boc-Gly in resin A gels with DMF-d_7 as solvent, when $\Delta = 5$, 10, 30 and 50 ms, $\langle z^2 \rangle$ becomes 0.86, 1.63, 4.58 and 7.32 μm^2, respectively. These $\langle z^2 \rangle$ values give us information on the diffusion distance $\langle z \rangle$ which reflects the experimental results as expressed by the following equation.

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

Consequently, the experimental result obtained at $\Delta = 5$ ms leads to the diffusion distance $\langle z \rangle = 0.74$ μm, and at $\Delta = 50$ ms leads to the diffusion distance $\langle z \rangle = 2.16$ μm. The $\langle z \rangle$ values in these experiments are larger than the size of network (12–24 nm) but smaller than particle size of swollen gel beads (100–200 μm). The size of network in the equilibrium swollen state is estimated by using the fraction of DVB cross-linking. This is based on the assumption that polystyrene chains stretch in the equilibrium swollen state. The particle size of swollen gel beads was determined by microscope.

Therefore, the fraction of the slow diffusion component (f_{slow}) depends on the diffusion time Δ. Fig. 7 shows the dependence of f_{slow} on Δ at 30 °C. As seen from this figure, the f_{slow} value increases with an increase in Δ. The f_{slow} value at $\Delta = 5$ ms is about 0.1. On this time scale, diffusion distances for most of the Boc-Gly molecules in resin A gels are different from each other. The f_{slow} value at $\Delta =$ 50 ms is about 1.0. On this time scale, diffusion distances for all of the Boc-Gly in resin A gels are almost equal. Then, when the Boc-Gly molecules in resin A gels diffuse during $\Delta = 50$ ms, the diffusion coefficient observed is the single diffusion component. At Δ values below 50 ms, Boc-Gly molecules cannot diffuse to a large distance beyond a network cell, while at Δ values above 50 ms, all of the Boc-Gly molecules can diffuse from a given network cell to distant network cells.

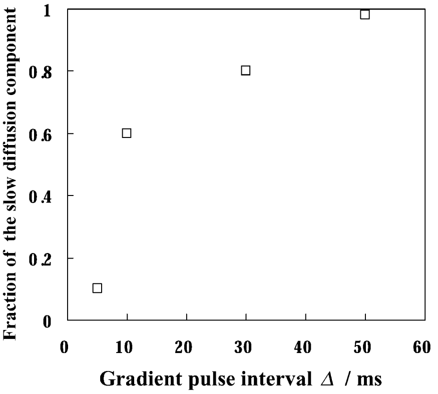

Fig. 7. Dependence of the fraction of the slow diffusion component (□) on the gradient pulse interval Δ at 30 °C.

3.2. *Diffusional behavior of amino acids in the solid-phase reaction field*

3.2.1. *Diffusion coefficients of Boc-Phe·Cs in network polystyrene gels as a solid-phase reaction field*

We are concerned with the diffusional behavior of Boc-Phe·Cs in network polystyrene gels which act as a solid-phase reaction field for the Boc-Phe group. The $D_{\text{Boc-Phe·Cs}}$ value is determined in resin A gels under chemical reaction at 50 °C at $\Delta = 10$ ms. The $D_{\text{Boc-Phe·Cs}}$ measurement starts after the resin A and Boc-Phe·Cs 10 wt% DMF-d_7 solution are mixed and then left for 3 h. Fig. 8 shows the diffusional spin-echo attenuation of the Boc-Phe in the network polystyrene gels and Boc-Phe·Cs in the solid-phase reaction field obtained by varying the field gradient pulse duration δ at 50 °C.

The D values are determined from the slopes of the plots. From this figure it is found that Boc-Phe·Cs in the solid-phase reaction field, as well as Boc-Phe in the network polystyrene gels, has multi-components for diffusion such as the slow diffusion component and the multi-fast-diffusion-components. The slow $D_{\text{Boc-Phe·Cs}}$ value and the slow $D_{\text{Boc-Phe}}$ value are determined as 1.06×10^{-6} and 1.42×10^{-6} cm^2 s^{-1}, respectively, and the f_{slow} of the corresponding diffusions are about 0.6. In the previous paper [15], it was shown that the diffusion coefficient of

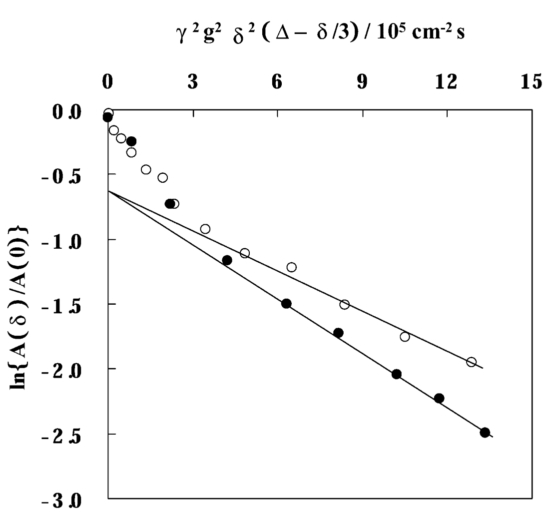

Fig. 8. Diffusional spin-echo attenuation of 10 wt% Boc-Phe in resin A gels (●) with DMF-d_7 as solvent and 10 wt% Boc-Phe·Cs in solid-phase reaction field (○) by varying field gradient pulse duration at 50 °C.

Boc-Phe in the network polystyrene gels is very similar to that of Boc-Gly at the same concentration in spite of the different molecular weight. Further, in Section 3.1, we have described that the $D_{\text{Boc-Phe}}$ value is decreased with an increase in $C_{\text{Boc-Phe}}$. When this measurement was performed, the $C_{\text{Boc-Phe·Cs}}$ value is smaller than 10 wt% because the solid-phase reaction proceeds, but the $D_{\text{Boc-Phe·Cs}}$ value is 0.75 times larger than the $D_{\text{Boc-Phe}}$ value. It can be said that there is a strong intermolecular interaction between the Cs group of Boc-Phe·Cs and the chloromethyl group on the phenyl rings of the network polystyrene gels in the solid-phase reaction field under chemical reaction. Further, from the experimental results that both of the f_{slow} values are almost equal, it can be said that except for diffusional behavior in the neighboring region of the polystyrene chains, diffusional behavior of Boc-Phe·Cs in the solid-phase reaction field is similar to that of Boc-Phe in network polystyrene gels.

4. Conclusions

The diffusion coefficients of Boc-Phe ($D_{\text{Boc-Phe}}$) in resin A and resin B gels and in DMF-d_7 solution in the absence of resin are determined by the ^{1}H PFGSE NMR method as a function of amino acid concentration over the temperatures range from 30 to 50 °C at $\Delta = 10$ ms. From these experimental results, it is found that the $D_{\text{Boc-Phe}}$ value in DVB 1% cross-linked network polystyrene gels closely depends on the concentration of Boc-Phe (C) in particular for high C values, and that the $D_{\text{Boc-Phe}}$ value in DVB 2% cross-linked network polystyrene gels decreases with an increase in C. It is clarified that the $D_{\text{Boc-Phe·Cs}}$ value in the solid-phase reaction field, under the reaction, is smaller than the $D_{\text{Boc-Phe}}$ value in the network polystyrene gels because of an interaction between Boc-Phe·Cs and the chloromethyl group on the phenyl rings of the network polystyrene gels. Further it is found that the fraction for the slow diffusion component (averaged diffusion component) depends on the diffusion time Δ which is related to the diffusion distance. Finally it is observed that the multi-components diffusion is averaged by a time of $\Delta = 50$ ms.

Acknowledgements

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