

Structural Transitions and Enzymatic Function of Ribonuclease A Encapsulated in Transparent Porous Silica Gel

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Transparent porous silica gel encapsulating ribonuclease A (RNase A) in nanoscale pores was prepared on the wall of a quartz cell. The UV and CD spectral changes upon heating showed that the protein unfolds in the pores with apparent heat denaturation temperatures (T_m) at 60 to 64 °C, which are slightly lower than that in solution. The unfolding was irreversible due probably to strong interaction of the unfolded species with the surface of the pores upon prolonged treatment at high temperatures. However, when the gel was subjected to repeated temperature-jump experiments between 40 and 75 °C, RNase A reversibly unfolded and refolded in the pores. The unfolding was not apparently decelerated but the refolding was remarkably slowed. The protein refolded in the porous gel exhibited enzymatic activity toward cytidine 2′,3′-cyclic monophosphate (c-CMP), indicating that small molecules, such as c-CMP and its hydrolyzed species, can go through the holes of the silica gel. These results demonstrated not only that the wet porous gel is useful for the heat denaturation study of proteins but also that the gel encapsulating a protein can be applied as a soft material with enzymatic function.

The structure of a protein is an essential factor for the function as an enzyme, a sophisticated nanoscale biocatalyst. However, the folded structure is not rigid in cells but always fluctuating so that the protein can bind the substrate highly efficiently and selectively.[1] The reversible global structural transformation to the unfolded state is also possible in vitro for the case of small monomeric proteins.[2] These dynamic motions usually take place in a short time scale from ps to s. Therefore, experimental observations of the dynamic processes are not easy tasks without the use of state-of-art techniques, such as ultra-fast solution mixing,[3] fluorescence resonance energy transfer (FRET),[4] laser-induced temperature jump relaxation spectroscopy,[5] etc.[6]

Encapsulation of protein molecules in nanopores of wet gel would be another useful method for observation of rapid conformational transformation processes because the molecular motions should be restricted and hence decelerated in the nanoporous environment. Taking advantage of transparent wet silica gel, Shibayama and Saigo[7–10] pioneered this methodology and have shown that conformational transitions of human adult hemoglobin[7] and sperm whale myoglobin[8] in the binding and releasing of oxygen significantly slow down in porous gel. Investigation of folding intermediates of cytochrome c[9] and β-lactoglobulin[10] has also been successful recently. Similar methods were employed by other research groups as well to investigate unfolding and/or refolding processes of proteins, such as green fluorescent protein[11] and carbonmonoxymyoglobin.[12] In these preceding studies however, the structural transitions were usually triggered by changing the solution pH or the denaturant concentration. Utilities of nanoporous silica gel for thermally induced structural transitions of proteins have not been investigated, to the best of our knowledge.

Conformational folding of bovine pancreatic ribonuclease A (RNase A) (EC 3.1.27.5),[13] a small monomeric enzyme consisting of 124 amino acid residues, has been well studied in solution with the four native disulfide (SS) bonds intact[14] as well as reduced.[15] Herein, we applied porous-gel encapsulation to the SS-intact thermal unfolding and refolding of RNase A in order to demonstrate usefulness of the methodology for investigation of thermal structural transitions of a protein. The enzymatic function of RNase A encapsulated in the porous gel was also assayed by using cytidine 2′,3′-cyclic monophosphate (c-CMP) as the substrate.

Results

Heat Denaturation of RNase A Encapsulated in Porous Silica Gel. Transparent porous silica gel, encapsulating RNase A in nanoscale pores, was synthesized on the wall of a quartz cell according to a literature method.[7] Figure 1 shows a side view (A) and a top view (B) of the quartz cell. Light path I was used for measurement of the UV and CD spectra of RNase A encapsulated in the gel, while light path II was used for checking the leak of RNase A from the gel to the buffer solution as well as for measurement of the spectral change of c-CMP, a small substrate used for the enzymatic assay of RNase A.[16] The gel was thoroughly washed with water and then an appropriate buffer solution so that methanol, which was liberated by polymerization (gelation) of tetramethyl orthosilicate (TMOS), and the protein, which was adsorbed on the surface of the gel, were not detected through light path II.

Mono-layered porous silica gel soaked in a pH 7.0 phosphate buffer solution was employed to investigate heat denaturation of RNase A encapsulated in the gel. Series of UV and CD spectra obtained by gradual elevation of the

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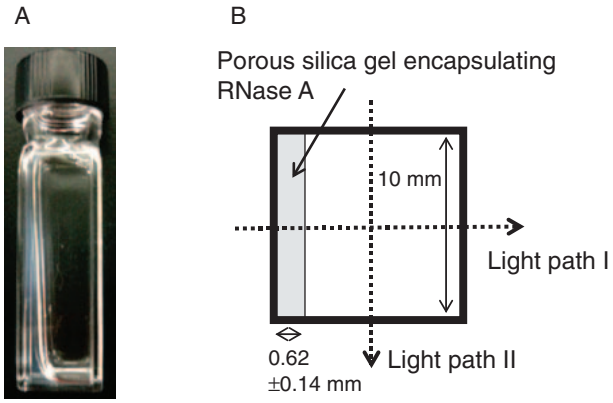

Figure 1. A side view (A) and a top view (B) of the mono-layered transparent porous silica gel, encapsulating RNase A, prepared on the wall of a quartz cell.

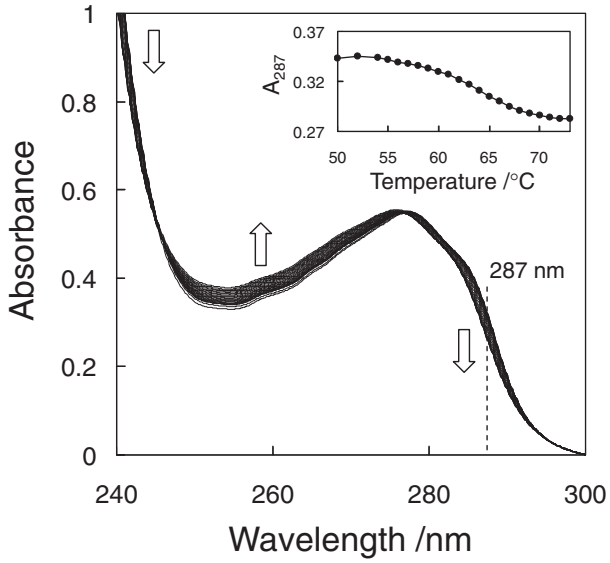

Figure 2. UV spectra of RNase A encapsulated in mono-layered porous silica gel by changing the temperature gradually from 50 to 73 °C in a pH 7.0 phosphate buffer solution. The inset is the absorption change at 287 nm as a function of the temperature.

temperature from 40 to 76 °C are shown in Figures 2 and 3, respectively. In the UV spectrum, the absorption at 287 nm decreased and the absorption at around 260 nm increased at high temperatures with isosbestic points at 277 and 246 nm, while in the CD spectrum the signal at 216 nm decreased with an isosbestic point at 229 nm. The spectral changes were similar to those observed for RNase A in a pH 7.0 phosphate buffer solution upon heating, suggesting that the RNase A encapsulated in the porous gel maintains the native structure and thermally unfolds following a pseudo two-state structural transition. Strictly speaking, distinct differences were seen between the differential UV spectra observed in the gel (Figure S1) and those in the solution: the UV absorption increase at around 260 nm upon heating was more enhanced in the gel, while the UV absorption decrease at 287 nm was attenuated by ca. 33% in the gel. In contrast, the differential CD spectra observed in the gel (Figure S2) were approximately

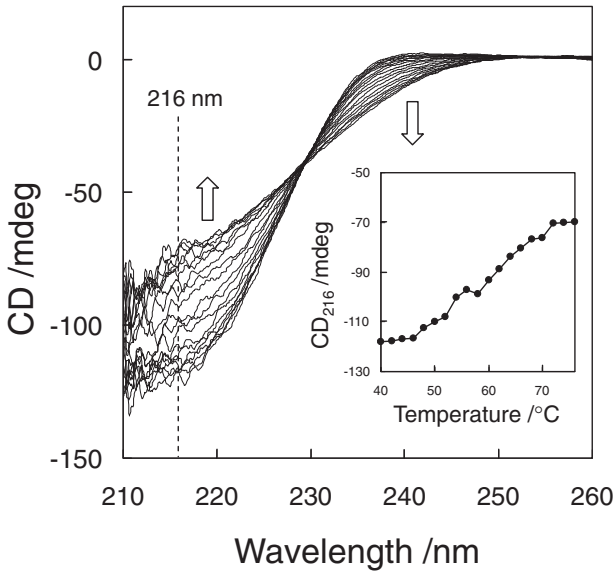

Figure 3. CD spectra of RNase A encapsulated in mono-layered porous silica gel by changing the temperature gradually from 40 to 76 °C in a pH 7.0 phosphate buffer solution. The inset is the CD change at 216 nm as a function of the temperature.

identical to those in the solution: no significant attenuation of the spectral change at around 220 nm was observed in the gel. By analyzing the intensities of the UV absorbance at 287 nm and the CD at 216 nm as a function of the temperature, apparent heat denaturation temperatures (T_m) of the encapsulated RNase A were determined to be 64 and 60 °C, respectively. The temperatures were 2 to 6 K lower than those determined in solution.[17] It also appeared that the unfolding of RNase A observed in Figures 2 and 3 was not reversible: the folded structure was not recovered at low temperatures after the spectral measurement at 76 °C. These observations suggested that unfolded RNase A has strong interaction with the surface of the pores upon prolonged treatment at high temperatures and the interaction would cause slight stabilization of the unfolded state in the porous environment relative to that in the solution.

Kinetic Analysis of Temperature-Jump Experiments. To investigate whether the native structure of RNase A can be reformed from the unfolded state in the porous gel, temperature-jump experiments between 40 and 75 °C were carried out by using triple-layered, instead of mono-layered porous silica gel. RNase A was encapsulated in the middle layer of the porous silica gel, and blank silica gel was prepared as the bottom and top layers: the bottom layer facing the quartz cell and the top layer facing the buffer solution. We employed this triple-layered gel for the temperature-jump experiments because the sudden temperature changes made cracks in the single-layered gel. The temperature was kept at 40 °C for 30 min and at 75 °C for 10.5 min, and the temperature-jump was repeated several times.

Figure 4 shows the UV spectra obtained at 40 and 75 °C in the repeated temperature-jump experiments and the differential spectra. At 75 °C unfolded RNase A (U; a broken line) was observed, while folded RNase A (F; a solid line) was observed at 40 °C. Although the folded structure was not completely

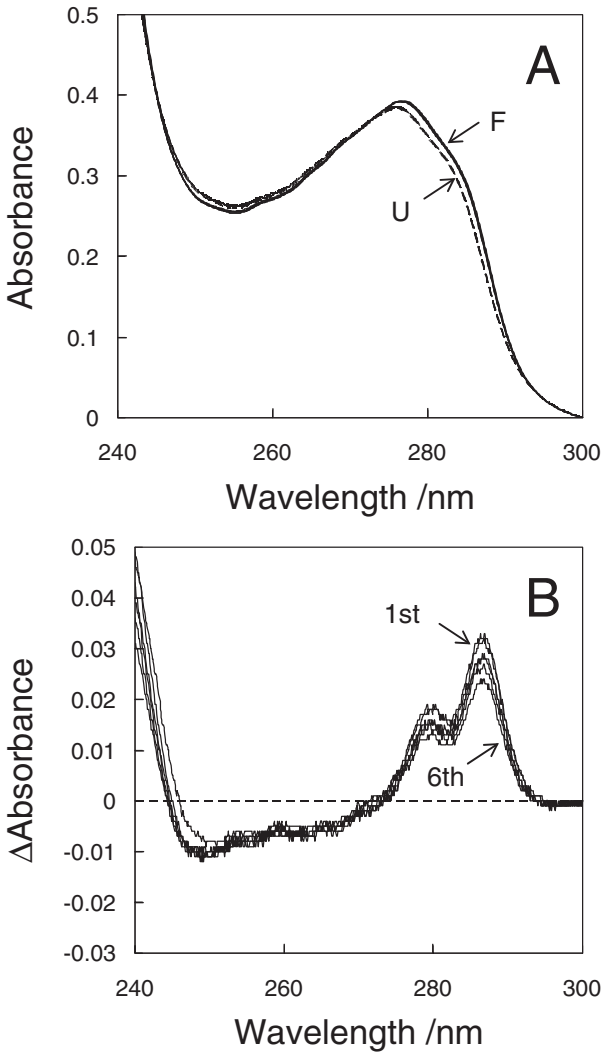

Figure 4. UV spectra (A) and the differential spectra (B) observed for RNase A encapsulated in triple-layered porous silica gel by temperature-jump experiments repeated 6 times in a pH 7.0 phosphate buffer solution. U and F are the superimposed UV spectra observed before and after the temperature jump from 75 to 40 °C.

recovered, the results clearly showed that RNase A can repeatedly unfold and refold in the porous silica gel. Thus, the porous silica gel methodology would be useful for heat denaturation and renaturation study of a protein unless the unfolded protein is not incubated in the porous gel for a long period of a time at high temperatures. However, since the gel had been treated at 75 °C for 30 min twice in pre-heating, about 50% of RNase A was already unfolded irreversibly in the gel on the basis of a magnitude of the absorption change at 287 nm in the solution. It should also be noted that the UV spectra of unfolded RNase A shown in Figures 2 and 4 are not the same. This suggests conformational differences of the unfolded states observed in the gradual heat denaturation and temperature-jump experiments.

Subsequently, the reaction kinetics of unfolding and refolding of RNase A in the porous gel was analyzed by following the UV absorbance at 287 nm as a function of time (Figure 5).

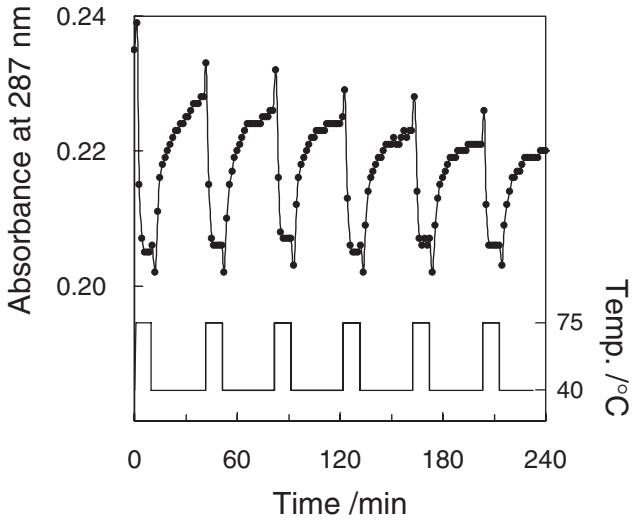

Figure 5. UV absorption changes at 287 nm observed for RNase A encapsulated in triple-layered porous silica gel by temperature-jump experiments in a pH 7.0 phosphate buffer solution. The UV spectra were recorded at intervals of 1.5 min.

When the temperature was rapidly increased from 40 to 75 °C, the UV absorbance shifted up by ca. 0.005 and then decreased quickly, whereas it shifted down by ca. 0.005 and then increased slowly when the temperature was rapidly decreased from 75 to 40 °C. The small shifts of the UV absorbance would be due to swelling of the gel at 75 °C. Comparison of these observations with those obtained from the reference experiment, in which a similar temperature-jump experiment was done for RNase A dissolved in a pH 7.0 phosphate buffer solution (Figure S3), revealed that the unfolding of RNase A is not decelerated apparently in the porous gel more than the dead time required for thermal equilibration (ca. 6 min) but refolding obviously slows in the porous gel.

Since the refolding in the porous gel was not fully reversible and the thermal equilibration was slow, refolding did not follow exact first-order reaction kinetics. However, the observed absorption changes shown in Figure 5 suggest that the refolding velocity of RNase A in the porous gel does not change significantly with increasing repetition of the temperature-jump. On the other hand, the ratio of native RNase A recovered in the gel decreased with increasing repetition more significantly than that in solution (Table 1). This tendency is in accord with the presumption that a portion of the unfolded RNase A species would be stuck on the surface of the pores with prolonged treatment at high temperatures. When the refolding time at 40 °C was increased from 30 to 60 min, the first recovery ratio of the native RNase A was enhanced by ca. 10%, but such obvious increment of the recovery ratio was not observed for the subsequent repeats.

Enzymatic Activity of RNase A Encapsulated in Porous Silica Gel. The UV spectral changes observed in the experiments (Figures 4 and 5) indicated that the native state of RNase A was apparently regenerated in the porous silica gel from the unfolded state. To confirm the formation of the native folded structure, enzymatic activity of RNase A refolded in the

Table 1. Ratios of Native RNase A Recovered in the Triple-Layered Porous Silica Gel at 40 °C after the Temperature-Jump Experiments

Repeats of the temperature-jump[a)]	Native state recovered/%[b)]	
	In gel	In solution
1	79	98
2	73	91
3	67	85
4	64	77
5	58	—
6	55	—

a) See Figures 5 and S3 and the text for details of the experimental conditions. b) The ratios were calculated based on the UV absorbance change at 287 nm observed for the first temperature jump.

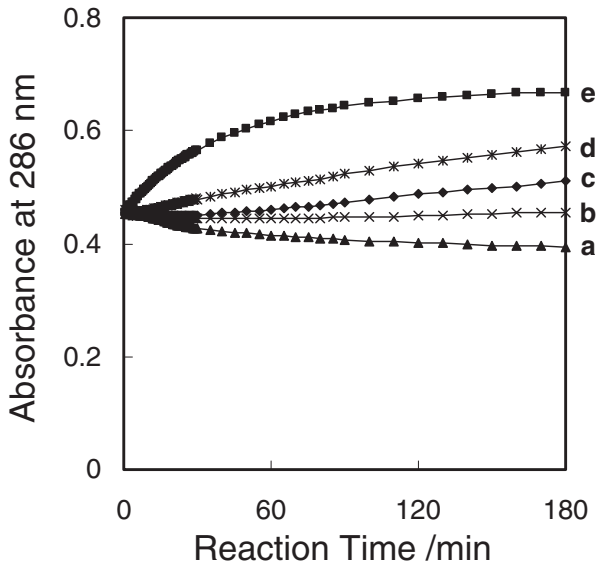

Figure 6. The enzymatic assay of RNase A by using c-CMP (0.18 mM) at pH 7.0 and 25 °C. (a) A blank triple-layered silica gel without RNase A soaked in a Tris buffer solution. (b) The phosphate buffer solution that was taken away from a triple-layered gel with RNase A after two repetitions of the temperature-jump experiment. (c) RNase A encapsulated in a triple-layered porous silica gel soaked in a Tris buffer solution after two repetitions of the temperature-jump experiments. (d) RNase A encapsulated in the porous gel corrected with respect to the blank gel. (e) RNase A (0.29 μM) in the Tris buffer solution.

porous gel was measured by using c-CMP as the substrate.[16] The gel was soaked in a Tris pH 7.0 buffer solution containing c-CMP, and the reaction progress for hydrolysis of the substrate was measured by the UV absorbance change at 286 nm through light path II. The results are shown in Figure 6.

When a blank gel without encapsulating RNase A was used, UV absorption at 286 nm slightly decreased with time (Figure 6a). This would be because c-CPM gradually defused into the pores of the silica gel, which caused the decrease of the concentration of c-CMP in the solution. On the other hand, the triple-layered gel encapsulating RNase A in the middle layer exhibited a low but distinct enzymatic activity as indicated by the increase of the UV absorption at 286 nm (Figures 6c and 6d). The activity decreased with repetition of the temperature change, and gel that had been heated at 75 °C for 4 h did not exhibit any enzymatic activity. Although the activity of RNase A refolded in the gel was significantly lower than that of RNase A dissolved in the buffer solution (Figure 6e), this confirmed that the small substrates, i.e., c-CMP and its hydrolyzed species, can go through the holes of the silica gel and also that RNase A can refold to the native structure in the restricted space in the pore. It should be noted as well that the buffer solution, to which the gel was soaked, did not show any enzymatic activity (Figure 6b). This excluded the possibility of the leak of RNase A from the gel to the solution.

Discussion

Unfolded and Refolded States of RNase A in Porous Silica Gel. The unfolding and refolding of RNase A have been extensively studied in buffer solutions previously.[14] There are several structurally distinguishable species in the unfolded state, which make different folding phases with different time constants (ms to min) upon the change of the solution conditions. These phases have been characterized in terms of cis–trans isomerization of the four Xaa–Pro peptide bonds present along the peptide chain:[14d] RNase A has two trans (Pro42 and Pro117) and two cis (Pro93 and Pro114) Xaa–Pro peptide bonds in the native folded state but they are in cis–trans equilibriums in the completely unfolded state. Isomerization of these peptide bonds to the native configurations is essential to fold into the native structure. However, the isomerization may be accompanied by large conformational changes and therefore would be significantly restricted in the limited space in the wet porous silica gel.

The thermally denatured state of RNase A in the gel showed a similar CD spectrum (Figure 3) to that observed in the solution, indicating that the secondary structures were completely broken in the gel upon heating. On the other hand, the UV spectrum observed for the denatured species in the gel (Figure 2) was slightly different from that in the solution. Although collapse of the native structure was confirmed by the enzymatic assay using the gel heated at 75 °C for 4 h (vide supra), attenuation of the absorption change at 287 nm in the gel suggested that the denatured state in the gel has more compact structures than that in the solution. In addition, the spectral change of CD started at a lower temperature than that of UV upon heating. The different behaviors of the UV and CD spectra observed in the gel are indicative of the possibility that transient unfolding intermediates, which are difficult to detect in the solution, will be characterized by using a more sophisticated porous gel method.

The unfolded species in the gel could refold back to the native state in good yield (ca. 80%) in the temperature-jump experiments, but the native structure did not form after prolonged heating. This suggests that a slow conformational transition takes place in the porous gel at high temperatures (ca. 75 °C). We propose that this transition couples to the cis–trans isomerization of the Xaa–Pro peptide bonds. In the gel, the unfolded species formed just after the temperature increase to 75 °C would have a relatively compact structure with the four

Xaa–Pro peptide bonds still in the native configurations and can refold back to the native state upon cooling. However, the initially formed unfolded species would gradually transform to the second unfolded species upon prolonged heating, presumably accompanied by cis–trans isomerization of the four Xaa–Pro bonds. The decrease in the ratio of the recovered native state (Table 1) is consistent with this consideration. A distinct difference of the UV spectrum observed for the denatured states obtained by the gradual heating and temperature-jump experiments (Figures 2 and 4, respectively) also supports the structural transition in the gel. Once the second unfolded species is formed, it would not be feasible to fold back to the native state probably due to the strong interaction with the surface of the pores.

The refolded state of RNase A formed in the gel after the rapid temperature decrease from 75 to 40 °C showed almost the same features in the UV spectrum as native RNase A in solution, suggesting the recovery of the native structure in the gel. This was indeed confirmed by measurement of the enzymatic activity of the refolded RNase A by using c-CMP as the substrate (Figure 6). Thus, reversible heat denaturation of RNase A was evidently demonstrated in the porous gel. It is also important to note that unfolding is not obviously decelerated in the gel compared with that in the solution while folding is remarkably decelerated. This indicates that the folding is more sensitive to the porous environment than the unfolding, suggesting that the initially formed unfolded state has stronger interaction with the porous environment than the native state. It may also be possible that the transition-state structure during unfolding is different from that during folding: the unfolding transition state would have a more compact structure than the folding transition state. Similar deceleration of the folding in porous gel was reported previously in the case of carbonmonoxymyoglobin.[12]

Utilities of the Porous Silica Gel as a Functionalized Material. The RNase A encapsulated in the porous silica gel exhibited enzymatic activity. This clearly showed that the c-CPM substrate and its hydrolyzed species can go through the holes of the gel to reach the active site of the protein. Similar phenomena have been reported for oxygen,[7,8] guanidine,[10,11] and glycerol.[12] c-CMP employed in the present study is a rather large molecule, compared to the previous cases, with a molecular weight of ca. 300. The holes of the porous silica gel should be larger than the size of c-CPM and smaller than the size of RNase A. The present study further suggests the possibility that the porous gel encapsulating an enzyme in its pores can be used as a catalyst for selective molecular transformation or as a functionalized soft material. Indeed, the catalytic function of RNase A persisted at least for one week in the gel: the gel soaked in a buffer solution for one week still exhibited almost the same catalytic activity as it had shown toward c-CMP, indicating that RNase does not undergo degradation inside the pores.

Conclusion

Transparent porous silica gel encapsulating RNase A in the nanoscale pores was synthesized. The changes of the UV and CD spectra shown in Figures 2 and 3 clearly demonstrated that RNase A unfolds in the pores of wet silica gel upon heating. However, the unfolding was irreversible with prolonged treatment at high temperatures due probably to the structural transition from the initially formed unfolded state to the second unfolded state, which would have strong interaction with the surface of the pores. Cis–trans Xaa–Pro isomerization was suggested to be relevant for the structural transition.

On the other hand, RNase A repeatedly unfolded and refolded in the porous silica gel in the temperature-jump experiments. The unfolding was not obviously decelerated more than the dead thermal equilibration time (ca. 6 min) but the refolding remarkably slowed in the porous gel. Moreover, the refolded protein exhibited the distinct enzymatic activity toward c-CMP in the gel, indicating that small molecules, such as c-CMP and its hydrolyzed species, can go through the holes of the silica gel.

In conclusion, several novel features of the porous silica gel system have been indicated. First, the gel is useful not only for denaturant- or pH-induced unfolding and refolding studies of a protein[7–12] but also for the heat denaturation study. However, to further utilize the porous-gel encapsulation method for thermal denaturation study of proteins, the strategies to control the proline isomerization and to suppress the strong interaction between the unfolded species and the pore surface must be explored. Second, in the porous gel the structural folding of RNase A is more decelerated than the unfolding, probably reflecting a stronger interaction of the initially formed unfolded state than the native folded state with the porous environment or a more compact transition-state structure for the unfolding than the folding. Third, the gel encapsulating a protein can be applied as a functionalized soft material with enzymatic activity as it has been shown for the case of RNase A. These features will be informative for future studies of applications of wet nanoporous gels to material science as well as biological chemistry fields.

Experimental

General. Bovine pancreatic ribonuclease A (RNase A Type I-A) was purchased from Sigma-Aldrich Japan. Tetramethyl orthosilicate ($Si(OCH_3)_4$; TMOS) was purchased from Tokyo Chemical Industry Co., Ltd. Other chemicals were obtained from general commercial sources and were used without further purification. Ultraviolet (UV) and circular dichroism (CD) spectra were recorded on a Shimadzu UV-1700 spectrophotometer and a Jasco J-820 spectropolarimeter, respectively, by using a quartz cell (10 × 10 mm) equipped with a screw cap. The temperature of the cell holder was regulated within an error of ±0.1 °C by using a circulating water-bath system.

Preparation of Porous Silica Gel. Mono-layered transparent porous silica gel encapsulating RNase A was prepared according to a procedure previously reported by Shibayama et al.[7] A mixture of TMOS (0.740 mL), H_2O (0.168 mL), and 0.04 M HCl (0.011 mL) was sonicated at 0 °C for 30 min. A portion of the obtained stock sol solution (0.160 mL) was mixed in a quartz cell with a 100 mM phosphate buffer solution at pH 7.0 (0.240 mL) containing RNase A (3–6 mg) so that thin-layered transparent silica gel was formed inside on the wall. The gel was maintained in the quartz cell at 30 °C for at least 1 day with a screw cap. Thickness of the gel was 0.62 ± 0.14 mm.

Triple-layered transparent silica gel was prepared by a similar procedure to that employed for preparation of mono-layered gel, but the gel was applied three times inside of the quartz cell at intervals of 1 h. The first and third layers were synthesized by using a mixture of the sol solution (0.100 mL) and a 100 mM phosphate buffer solution at pH 7.0 (0.150 mL) without RNase A. The second layer was synthesized by using a mixture of the sol solution (0.100 mL) and the phosphate buffer solution (0.150 mL) containing RNase A (2–3 mg). Thus, the layer with the protein was sandwiched between the layers of empty porous silica gels.

The mono- and triple-layered silica gels thus prepared were washed with H_2O (3 mL × 2) and a 100 mM phosphate buffer solution at pH 7.0 (3 mL × 2) before UV and CD spectral measurements and temperature-jump experiments.

Heat Denaturation. The mono-layered transparent silica gel formed on the wall of a quartz cell was soaked in 3 mL of a 100 mM phosphate buffer solution at pH 7.0. The cell was set in a cell holder so that the light passes through the gel (light path I). The UV and CD spectra were measured at temperatures from 40 to 76 °C in increments of 1 or 2 °C, respectively. The gel was kept at a constant temperature for 5 min before the spectrum was recorded. The conditions for the UV spectral measurement were 240–300 nm for the wavelength range, 30 nm min^{-1} for the scan speed, and 0.1 nm for the band width. Those for the CD spectral measurement were 260–200 nm for the wavelength range, 50 nm min^{-1} for the scan speed, 1 nm for the band width, and 1 s for the response.

Temperature-Jump Experiments. Two circulating water baths, the temperatures of which were set at 40 and 75 °C, respectively, were used for the temperature-jump experiments. The water bath at 40 °C was first connected to the cell holder, in which the quartz cell with a triple-layered porous gel was inserted. After 20 min, the UV spectrum was measured through light paths I and II under the conditions of 240–300 nm for the wavelength range, 300 nm min^{-1} for the scan speed, and 0.1 nm for the band width. The temperature of the cell holder was then rapidly changed to 75 °C by disconnecting the cell holder from the water bath at 40 °C and reconnecting it the other water bath at 75 °C. After 30 min, the temperature was rapidly changed back to 40 °C by connecting the cell holder to the water bath at 40 °C, and the temperature was kept at 40 °C for 30 min. This pre-heating treatment was repeated two times in order to get rid of a slight leak of RNase A from the porous gel: we observed the leak in the UV spectrum that was measured through light path II (<1%) after the first pre-heating, but it was not detected at all after the second treatment as it was confirmed by the enzymatic assay (vide infra) for the soaking solution that was taken away from the quartz cell. After the pre-heating treatments, the phosphate buffer solution was exchanged with new phosphate buffer solution at pH 7.0, and the UV spectrum was measured through light path I. The temperature of the cell holder was subsequently changed to 75 °C. The UV spectrum was recorded seven times at intervals of 1.5 min. After 10.5 min, the temperature was rapidly decreased to 40 °C, and the UV spectrum was recorded twenty times at intervals of 1.5 min. After 30 min, the temperature was raised again to 75 °C. These temperature increases were repeated several times. The dead time required to attain thermal equilibration was found to be ca. 6 min.

Enzymatic Activity. By using cytidine 2′,3′-cyclic monophosphate monosodium salt (c-CMP) as a substrate,[16] the enzymatic activity of RNase A encapsulated in porous silica gel was assayed. The temperature of the triple-layered gel soaked in 3 mL of a 100 mM phosphate buffer solution at pH 7.0 was raised twice to 75 °C for 30 min in a similar manner as the pre-heating in the temperature-jump experiments. After the pre-heating, the phosphate buffer solution was exchanged with new phosphate buffer solution at pH 7.0, and several repetitions of the experiments (vide supra) were carried out. The gel was then cooled at room temperature (25 °C). The phosphate buffer solution was exchanged with a 100 mM tris(hydroxymethyl)aminomethane (Tris) buffer solution at pH 7.0, and the UV spectrum was measured through light path II. To the Tris buffer solution was added 3.0 μL of the Tris buffer solution containing c-CMP (6.0 mg/0.100 mL). The UV absorbance at 286 nm was monitored until the reaction time of 180 min. Similarly, the enzymatic activity of the phosphate buffer solution that was taken away from the gel was assayed. To obtain the reference data, the enzymatic assay was also carried out for RNase A (0.29 μM) in a Tris buffer solution at pH 7.0 as well as for that totally unfolded in the porous gel after prolonged heating at 75 °C for 4 h.

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Supporting Information

Differential UV and CD spectra of RNase A encapsulated in mono-layered porous silica gel (Figures S1 and S2) and UV absorption changes at 287 nm for RNase A in solution by temperature-jump experiments (Figure S3). This material is available free of charge on the Web at http://www.csj.jp/journals/bcsj/.

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