Direct Observation of Internal Structure in Poly(N-isopropylacrylamide) Gel

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Abstract: Direct observation of internal structure in gels was for the first time carried out by means of laser scanning confocal microscopy. Opaque poly(N-isopropylacrylamide) gel was found to have the bicontinuous structure consisting of dense and sparse polymer network domains in length scale of μm.

Introduction

Polymer gels are three-dimensionally cross-linked polymer network containing fluid. The characteristics of gels, such as optical, mechanical and thermal properties, are considered to be related to their internal structures and recently there have been an increasing number of reports which imply significance of inhomogeneities of the network structure in polymer gels.[1-14] The origin of the inhomogeneities of gels is mainly classified into the two causes.[11] One is frozen inhomogeneities which are produced during the gelation process: the critical concentration fluctuations or the domains formed due to the microphase separation are cross-linked, yielding static (or frozen) inhomogeneities. Another is the dynamic inhomogeneities, *i.e.*, thermal concentration fluctuations of the network polymer, amplitude and relaxation time of which depend on the position in the phase diagram of the gel.

The mechanical and the thermal properties of poly(N-isopropylacrylamide) isochore gel were investigated as a function of temperature and the formation of a "macro network" was deduced.[12] Although it is not yet clarified whether or not the inhomogeneities occurring at the high temperatures are static or dynamical objects, the inhomogeneities are certainly relaxed upon lowering temperature. Thus the inhomogeneities are not frozen in this case. On the other hand, as for the frozen inhomogeneities, there are few reports concerning the real space analysis. Recently, an atomic force microscopy was applied to observe the polymer gel surface and found spongelike domains with submicrometer scale.[14] But the internal inhomogeneities of the gel are still unknown.

In the present paper, the direct observation of internal structure of opaque poly(N-isopropylacrylamide) (PNIPAAm) gel was for the first time carried out by means of laser scanning confocal microscope (LSCM). LSCM has an advantage that the image coming only from the focal plane is detected. A series of optically sliced images observed at different depths makes it possible to construct the three-dimensional picture by the aid of computational stacking.[15]

Experiments

Preparation of Gels. Pregel solution was prepared by dissolving of N-isopropylacrylamide (NIPAAm) (0.7524g), N,N'-Methylene-bis(acrylamide) (Bis) (7.71mg) and N,N,N',N'-tetramethylethylenediamine (12.0μl) into deionized water (4.5ml). After addition of 0.4% ammonium peroxodisulfate aqueous solution (0.5ml), the pregel solution was quickly transferred into the cell which was assembled with two round glass plates (22mmϕ, 0.17mm thickness), a ring spacer (0.5mm thickness) and a holder. After the pregel solution was loaded into the cells, they were immersed into the temperature controlled water bath for 1hr. The disk shape gels thus obtained were used for the microscope observation.

Confocal Laser Scanning Microscope. The observation of the internal structures of the gels was performed at room temperature by laser scanning confocal microscope (LSCM) (Carl-Zeiss LSM410) equipped with Ar (488nm) and UV (364nm) lasers. The objective lens used was water-immersed lens (Carl-Zeiss Apochromat x40/1.20 W). Ar laser (488nm) was used for observation under a reflection mode (a simple LSCM method) and UV laser (365nm) under fluorescence LSCM. The images obtained with the former and latter methods are referred to as the "reflection LSCM images" and "fluorescence LSCM images",respectively. To observe the fluorescence images, the fluorescent probe, that is, 8-Anilino-1-naphthalene sulfonic acid ammonium salt (ANSA), was introduced into the gel by immersion in the aqueous solution of ANSA (70mM) for 3 days. ANSA has a maximum absorption at 365nm and a maximum fluorescent emission at 482nm in methanol.

Results

Appearance of the Gels. PNIPAAm gels were prepared at various temperatures. The gel prepared at 20°C was transparent. On the other hand, the gels prepared at temperatures higher than 25°C showed opacity. As the preparation temperature increased, the degree of the opacity increased and the gels prepared at 32 and 38°C were completely opaque. The observed opacity of the gels did not disappear even when the temperature was lowered, which may indicate that the inhomogeneity was permanently frozen in the gel by the cross-linking.

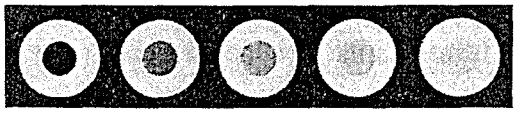

Figure 1. Appearance of poly(NIPAAm) gels. Preparation Temperatures; (a):20°C, (b):25°C, (c):27°C, (d):32°C, (e):38°C, Swelling ratio; 1.0. Other preparation conditions: [NIPAAm] = 1.33M, [Bis] = 10.0mM.

LSCM Observation and Identification of the Images. LSCM was applied to observe internal structure of the gels. The transparent gel prepared at 20°C showed no meaningful reflective LSCM image. However, the opaque gels obtained at 25, 27, 32 and 38°C exhibited the clear reflection images by the irradiation of Ar laser (488nm). Figure 2 shows the representative reflection LSCM images of the gel prepared at 27°C. The images show a pattern in which bright spots are regularly arranged in the dark background.

In order to identify origin of the contrast in the LSCM reflection images, fluorescence LSCM was applied to the gel by use of ANSA. ANSA emits fluorescent light, only when the molecules locate in hydrophobic area.[16] The more hydrophobic environment the probe has, the stronger intensity of the fluorescent light the probe emits. Figure 3 shows the comparison of the fluorescence (a) and the reflection images (c) obtained in the same area. The bright areas in Figure 3 (a) are those illuminated by fluorescent light from ANSA molecules. On the basis of the characteristics of ANSA molecules, the bright areas in the fluorescent LSCM image is considered to be a relatively more hydrophobic region compared with the dark areas. Since the network polymer has hydrophobic isopropyl groups as side chains, it is concluded that the bright areas in the fluorescence LSCM image were identified as the dense polymer network domains and dark areas as the sparse polymer network domains.

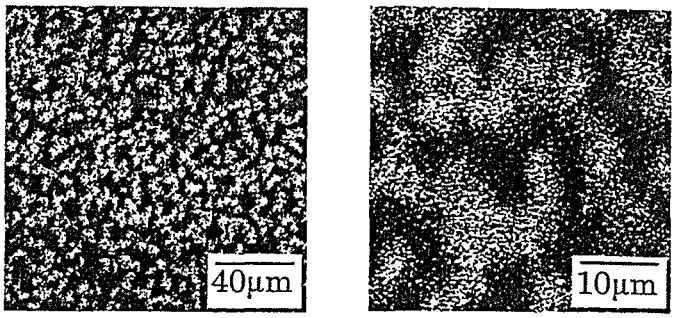

Figure 2. CLSM images of Poly(N-isopropylacrylamide) gel at different magnifications.
Images were taken at 17.5μm deep from the gel surface.
Preparation conditions: [NIPAAm] = 1.33M, [Bis] = 10.0mM, 27°C.

The pattern of the bright areas in the reflection image is close to that in the fluorescence image, and the intensity profiles along the line A in those two images are rather similar to each other, except for the broadness of the peak in the fluorescence one. Therefore, taking into account of the pattern being static (that is, not time-dependent), we can conclude that the LSCM reflection image would reflect the static inhomogeneities, that is, internal structures of the gel due to the frozen concentration fluctuations in polymer network.

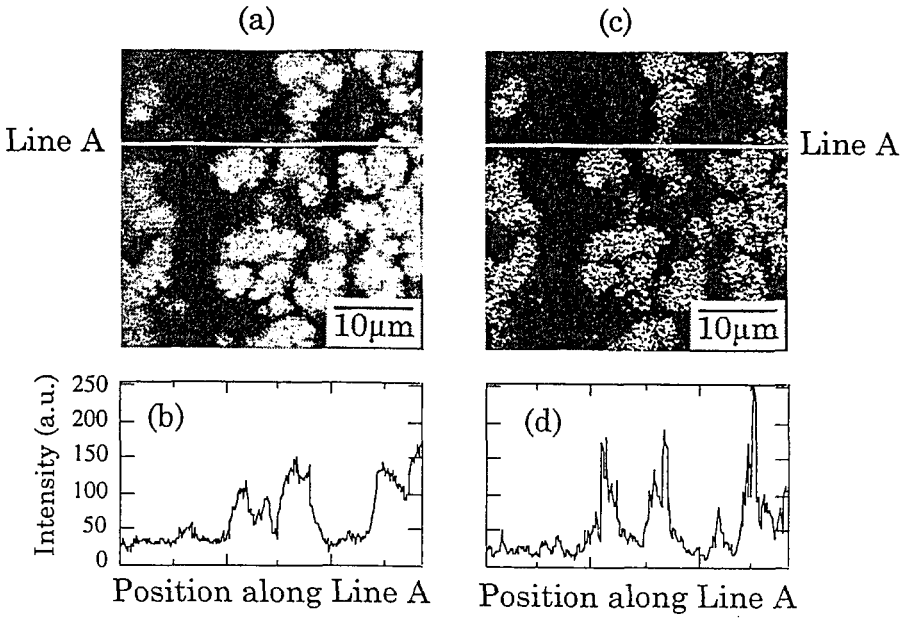

Figure 3 Comparison of a fluorescence CLSM image with a reflection CLSM image observed for the same area in the NIPAAm gel doped with ANSA (Swelling ratio: 4.1). (a):Fluorescent CLSM image. (b): Intensity profile along the line A in the CLSM image of (a). (c): Reflection CLSM image. (d): Intensity profile along the line A in reflection CLSM image of (c). Preparation conditions: [NIPAAm] = 0.126M, [Bis] = 4.74mM, 38°C.

Three-Dimensional Internal Structures of the Gel. It is interesting to elucidate the three-dimensional (3-D) internal structures in gel. The 3-D internal structures of the gel was captured by the aid of computational stacking of a series of the reflection LSCM sliced images. Figure 4 shows the 3-D picture of the gel prepared at 27°C. Both the bright region representing the dense polymer network domain in Figure 4(a) and dark region representing the sparse polymer network domain in Figure 4(c) clearly demonstrate a bicontinuous domain structure. The interface between these two domains was traced in Figure 4(b). It was found for the first time that the macroscopically homogeneous gel had the bicontinuous domain structure consisting of dense and sparse polymer network domains at mesoscopic scale.

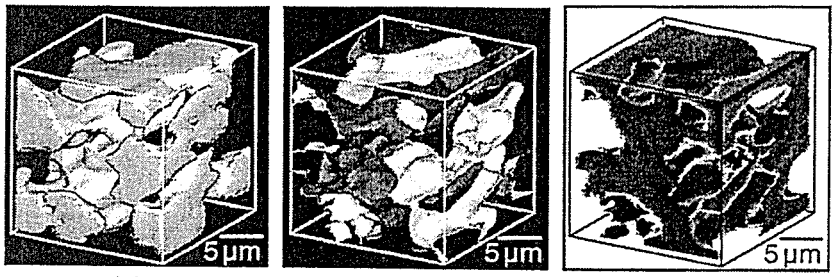

Figure 4 3-D images constructed for the NIPAAm gel, showing a bicontinuous domain structure of the networks. (a): Bright solid part represents the dense polymer network domain. (b): Interface between dense and sparse polymer network domains. (c): Dark solid part represents sparse polymer network domain. Preparation conditions: [NIPAAm] = 1.33M, [Bis] = 10.0mM, 27°C.

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