Poly(ethylene glycol)-induced shrinkage of Sephadex gel

A model system for quantitative analysis of osmoelastic coupling

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ABSTRACT Shrinkage of Sephadex gels caused by addition of a high-molecular weight molecule, poly(ethylene glycol) (PEG) was studied. A quantitative analysis based on the cross-linked network theory by Flory and Tanaka (Tanaka, T. 1978. *Phys. Rev. Lett.* 40:820–823) showed that the shrinkage is due to a mechanochemical coupling between the elasticity of the network and the osmotic stress arising from preferential exclusion of PEG. These results may provide good evidence for "osmoelastic coupling", the coupling between elasticity of macromolecular structures and osmotic stress, which has been predicted in some biological systems such as phospholipid bilayer membranes (Ito, T., M. Yamazaki, and S. Ohnishi. 1989. *Biochemistry.* 28:5626–5630; Yamazaki, M., S. Ohnishi, and T. Ito. 1989. *Biochemistry.* 28:3710–3715) or actin filaments (Ito, T., K. S. Zaner, and T. P. Stossel. *Biophys. J.* 51:745–753; Suzuki, A., M. Yamazaki, and T. Ito. 1989. *Biochemistry.* In press).

INTRODUCTION

Recently, "osmoelastic coupling," a mechanochemical coupling between osmotic stress and elasticity of macromolecular structures, has been suggested in several systems.

Elastic compression of actin filaments caused by an osmotic stress has been predicted by Ito et al. (1987) from analysis of volume flow of actin filament solution. In this case, the actin filaments should be compressed by the osmotic stress caused by dialysis of an actin filament solution against a solution of higher osmolarity. As quantitatively analyzed in that paper, the free energy increase due to the compression should be proportional to the second power of the osmotic stress.

A comprehensive thermodynamic analysis of osmotic response of phospholipid vesicles done by Ito et al. (1989) predicted the possibility that phospholipid bilayer membranes should be strained elastically by osmotic stress arising from preferential exclusion of a high-molecular weight molecule from the membrane surface. Consequently, the vesicles subject to the osmotic stress should increase the free energy in the dispersed state, and above a critical intensity of the osmotic stress, they should aggregate tightly to each other to annul the osmotic stress. Based on this analysis, the mechanisms of poly(ethylene glycol) (PEG)–induced aggregation and fusion have been analyzed quantitatively (Yamazaki et al., 1989).

In these studies, however, the participation of "osmoelastic coupling" in the osmotic responses has been predicted indirectly from the results of thermodynamic analysis. To show a direct evidence for "osmoelastic coupling," we make a quantitative analysis of shrinkage of Sephadex gel caused by addition of PEG in this report. As schematically represented in Fig. 1, Sephadex gels are known to exclude macromolecules preferentially from the inside. The degree of exclusion is dependent on the size of the molecules as well as the cross-linking density of the network. The exclusion of macromolecules results in an imbalance of the osmolarity between the inside and outside of the gel, which exerts an osmotic stress on the network structure. Therefore, the gel shrinkage caused by macromolecule, which was reported in gel chromatographic experiments (Hellsing, 1968), should be a well-defined phenomenon for the analysis of "osmoelastic coupling."

MATERIALS AND METHODS

PEG 200, 1,500, and 20,000 were purchased from Nakarai Chemicals, Kyoto, Japan. The average molecular weights of these PEGs are 200, 1,500, and 20,000, respectively, and the molarities were calculated from the average molecular weights.

Sephadex gels were obtained from Pharmacia Chemical Co., Piscataway, NJ. 300 mg of Sephadex G 50 or G 75, or 100 mg of G 200 was equilibrated in the presence of various concentrations of PEG in phosphate-buffered saline (137 mM NaCl, 3 mM KCl, 8.1 mM

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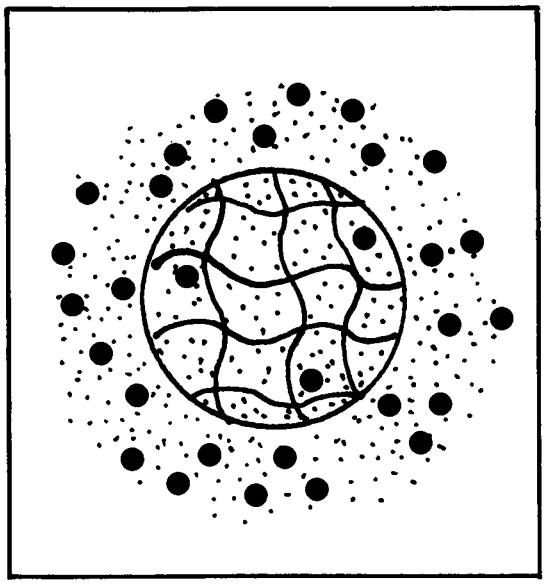

FIGURE 1 Preferential exclusion of PEG molecules by gel particle. Water and PEG molecules are represented by small dots and black circles, respectively. The flexuous lines represent the network of the gel particle.

Na_2HPO_4, 1.5 mM KH_2PO_4, 2 mM $MgCl_2$, pH 7.0) and the volume of packed gel particles were measured at room temperature. In the absence of PEG, the equilibrated volumes were 3.3 ml for G 50, 4.15 ml for G 75, and 3.48 ml for G 200, respectively.

RESULTS

PEG-induced shrinkage of Sephadex gels

Experiments

As shown in Fig. 2, addition of high molecular weight PEG caused shrinkage of Sephadex gels extensively. The shrinkage was evident at weight concentrations of PEG as low as 1% (wt/wt). Higher molecular weight PEGs were more effective. For example, the volume change at 5% (wt/wt) of PEG 20,000 was about eight times larger than that at the same concentration of PEG 200. Ethylene glycol at concentrations up to 50% (wt/wt) did not cause any shrinkage. The shrinkage also depended on the type of Sephadex gel, the gel with higher cross-linking density shrunk less (Fig. 2).

In Fig. 3, the volume changes of Sephadex gels after equilibration are plotted against various concentrations of PEGs. The experimental data can be well fitted by the following empirical equation:

$$\Delta V/V_0 = A[1/(KC + C_0) - 1/C_0] = -(A/C_0)[KC/(KC + C_0)], \quad (1)$$

where C is the molar concentration of PEG and A, C_0, and K are the empirical parameters to be fitted. Data for PEG 20,000 were analyzed by putting $K = 1$ and adjusting the values for A and C_0. The best fit parameter values are given in Table 1, and the simulated curves are shown in Fig. 3. The A and C_0 values were dependent on the Sephadex gel type, larger for gels with higher cross-linking density. However, the ratio A/C_0 was nearly unity, independent of the Sephadex type. The K value for PEG of lower molecular weight was determined using the A and C_0 values obtained for PEG 20,000 (Table 1). It was smaller than unity, smaller for gels with lower cross-linking density, and smaller for PEGs with lower molecular weight.

PEG-induced shrinkage of Sephadex gels

Theory

Gel particles take an equilibrium volume in aqueous medium. This should be determined by the equilibrium condition that chemical potential of water should be equal between the inside and outside of the gel. Two factors should contribute to the chemical potential of water inside the gel; one is mixing of the network segments with solvent, and the other is elastic strain of the network structures (Flory, 1953).

To analyze the equilibrium volume of Sephadex gel quantitatively, we shall define at first the osmotic pressure inside the gel π^i as

$$\pi^i = (-1/\bar{V}_1)[\mu_1^i - \mu_1^\circ(0)],$$

where μ_1^i and $\mu_1^\circ(0)$ are the chemical potentials of water in the gel and the PEG-free bulk solution, respectively, and $\bar{V}_1$ is the molar volume of water. Then we shall assume the rubber elasticity for Sephadex gels. Using Flory's formula modified by Tanaka (1978) for gels with rubber elasticity, we can create the following relation:

$$\pi^i = -RT\{1/\bar{V}_1)[\ln(1 - v_2) + v_2 + \chi v_2^2] + \nu_0[(v_2/v_0)^{1/3} - \tfrac{1}{2}(v_2/v_0)]\}, \quad (2)$$

where R is the gas constant, T is the absolute temperature, v_2 is the volume fraction of the network in the gel, v_0 is the volume fraction of the network at the condition the constituent polymer chains have random-walk configurations, χ is the chain segment-solvent interaction parameter and ν_0 is the number of constituent chains per unit volume at $v_2 = v_0$. Because the conditions that $v_2 \ll 1$ and $v_2/v_0 \ll 1$ hold in the swollen gels used in the present experiments, Eq. 2 can be approximated as:

$$\pi^i = -RT[(1/\bar{V}_1)(\chi - \tfrac{1}{2})v_2^2 + \nu_0(v_2/v_0)^{1/3}]. \quad (3)$$

The first term in larger parentheses in Eq. 3 is due to the mixing free energy and is represented as C_m and the

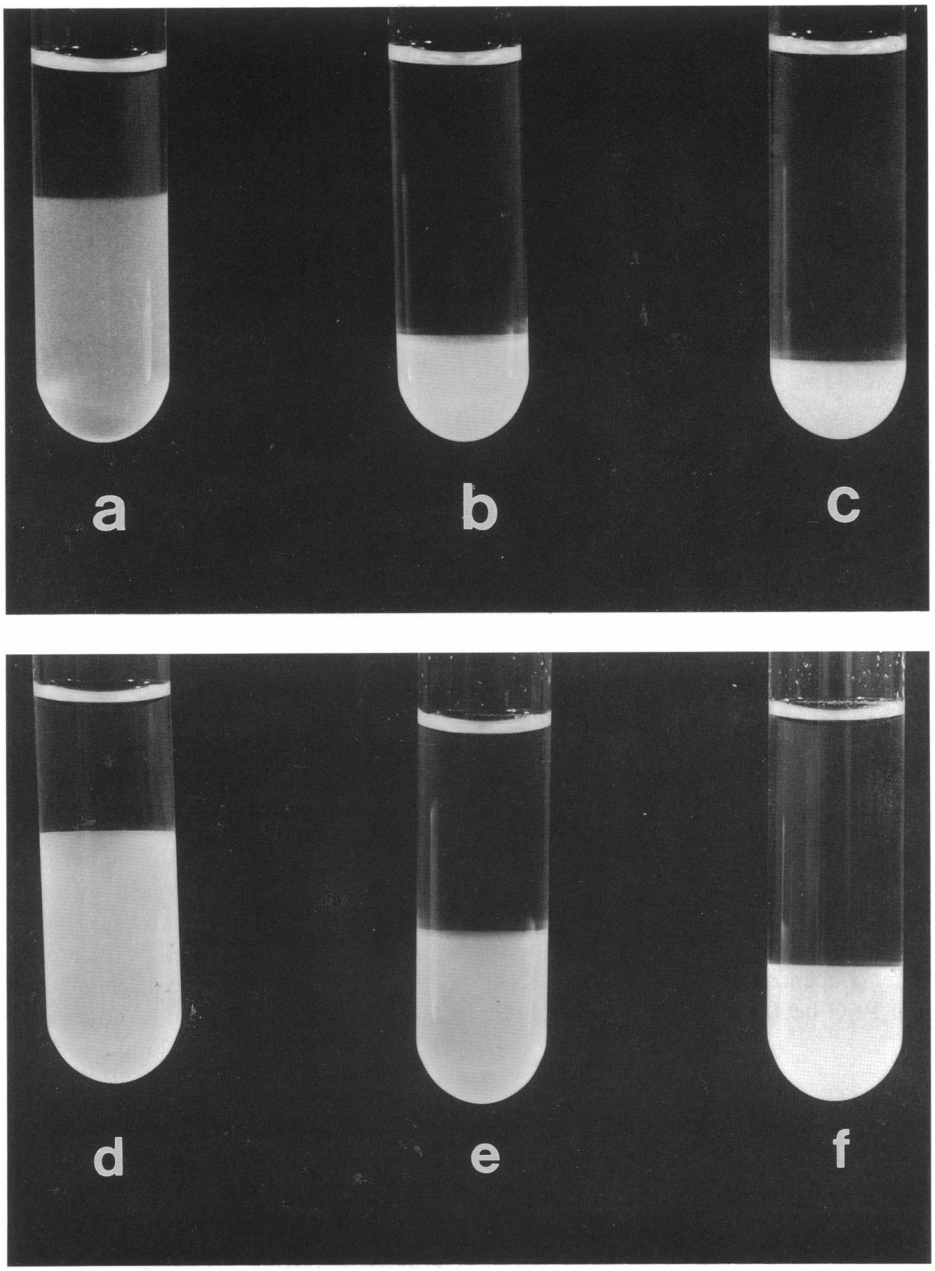

FIGURE 2 Equilibrium volumes of Sephadex G200 and G50 in the presence of PEG 20,000. The photographs represent equilibrium volumes of Sephadex G200 (*a–c*) and G50 (*d–f*) in the phosphate-buffered salines containing 0% (wt/wt) (*a,d*), 5% (wt/wt) (*b,e*), and 10% (wt/wt) (*c,f*) of PEG 20,000, respectively. The dry weights of those gel particles were 100 mg for G200 and 300 mg for G50.

second term due to the elastic contribution and represented as $-C_{el}$, hereafter.

In the absence of PEG, the osmotic pressure inside the gel π_0^i is equal to zero at equilibrium:

$$\pi_0^i = RT(C_{m,0} - C_{el,0}) = 0, \quad (4)$$

where $C_{m,0}$ and $C_{el,0}$ are the values in the absence of PEG.

Addition of high molecular weight PEG to the aqueous medium creates an imbalance of osmolarity due to preferential exclusion of the PEG from the gel inside, as represented in Fig. 1. In this case, a new equilibrium is attained when the imbalance of osmolarity is counterbalanced by the osmotic pressure of the gel π^i. Here, we shall define the imbalance of osmolarity as osmotic stress π^e:

$$\pi^e = \pi^i = RT(C_m - C_{el}). \quad (5)$$

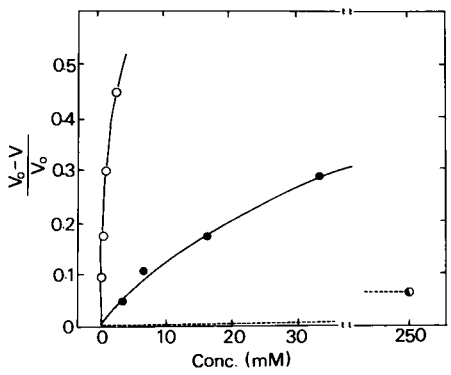

FIGURE 3 Shrinkage of Sephadex G75 caused by PEGs. Measurements were made in the phosphate-buffered saline containing various concentrations of PEG. Values of the volume change of Sephadex gels $\Delta V/V_0$ are plotted against molar concentrations of PEGs. The full lines are simulated curves computed from Eq. 1. See text for details. (○) PEG 20,000; (●) PEG 1,500; (◐) PEG 200.

From Eqs. 4 and 5,

$$\pi^e = RT(\Delta C_m - \Delta C_{el}), \tag{6}$$

where $\Delta C_m = C_m - C_{m,0}$ and $\Delta C_{el} = C_{el} - C_{el,0}$.

π^e can be written as

$$\pi^e = RTKC_{osm}, \tag{7}$$

where C_{osm} is the osmolarity of PEG. If the PEG is so dilute as to behave as osmotically ideal, C_{osm} can be approximated by the concentration of PEG, C. K is related to partition of PEG between inside and outside of the gel:

$$K = (C^e - C^i)/C,$$

where superscripts i and e denote the inside and outside of the gel. Combining Eqs. 7 and 6,

$$KC = \Delta C_m - \Delta C_{el}. \tag{8}$$

TABLE 1 Best fitted values of the empirical parameters in Eq. 1

Type	PEG 20,000			PEG 1,500	PEG 200
	A	C_0	A/C_0	K	K
	mM	*mM*			
G 50	6.4	6.0	1.1	0.050	ND
G 75	1.4	1.8	0.80	0.036	0.0006
G 200	0.98	1.1	0.92	0.018	ND

Best fit parameter values A and C_0 for PEG 20,000 were obtained by assuming $K = 1$. Data for PEG 1,500 and PEG 200 were fitted using the A and C_0 values and K as the adjustable parameter.

The volume change of the network $\Delta V = V - V_0$, where V and V_0 are the volumes of the gel particle before and after the addition of PEG, can be related to the volume fraction of the network v_2 and its change $\Delta v_2 = v_2 - v_{2,0}$ by

$$\Delta V/V_0 = -(\Delta v_2/v_2). \tag{9}$$

Neglecting more than second order of $\Delta v_2/v_{2,0}$, we can derive the following relations from Eq. 3,

$$\Delta C_m = -2/\bar{V}_1 \cdot (\chi - 1/2) \cdot (\Delta v_2 \cdot v_{2,0}) \tag{10}$$

$$\Delta C_{el} = (\nu_0/3v_0^{1/3}) \cdot (\Delta v_2/v_{2,0}^{2/3}). \tag{11}$$

Finally, the relationship between the volume change of the gel and the concentration of PEG is obtained from Eqs. 8–10 and 11:

$$\Delta V/V_0 = -KC/[KC + (5/3)C_{m,0}]. \tag{12}$$

Eq. 12 derived from the theoretical analysis agrees with the empirical Eq. 1 determined by the present experiments, because the relation that $A/C_0 = 1$ was indicated in Eq. 1 experimentally (Table 1). This agreement provides a strong support for the present analysis based on the osmoelastic coupling. C_0 should be equal to $(5/3)C_{m,0}$, or $(5/3)C_{el,0}$, which corresponds to the water chemical potential contributed by the intrinsic elastic strain in the absence of PEG. It is reasonable to see that C_0 value increased for gels with higher cross-linking density because $C_{el,0}$ should increase with the cross-linking density (Flory, 1953). The observed dependence of K on the Sephadex gel type as well as the molecular weight of PEG can also be reasonably explained because smaller molecular weight PEGs should be more easily partitioned into gels with lower cross-linking density. The assumption of complete exclusion of PEG 20,000 from the inside of gels ($K = 1$) may not rigorously hold. If the K value were not unity, then the values for other PEGs would be smaller, but the relative values of K should be unchanged.

DISCUSSION

A quantitative analysis of shrinkage of Sephadex gels induced by the addition of PEG has been done, based on Flory and Tanaka's theory on the swelling of rubberlike gel. The molecular event underlying the phenomenon can be summarized as follows. The preferential exclusion of large PEG molecules by the gel results in osmotic imbalance between the inside and outside of the gel, which exerts osmotic stress on the gel. Osmotic stress, in turn, shrinks the gel to be counterbalanced by elastic pressure consequential to the elastic strain of the network structure. Both the results of the theoretical analysis and the experiments have agreed quite well with each other. This

should be a direct example of "osmoelastic coupling", a kind of mechanochemical coupling in which osmotic stress is coupled with elastic strain of polymer structures.

The salt-induced gradual shrinkage or discrete transition in equilibrium volume of a partially ionized acrylamide gel, demonstrated by Ohmine and Tanaka (1982), may be due to a similar mechanism. The negative charges fixed on the gel set up a Donnan potential across the gel-solvent boundary, which brings about an unequal distribution of ions in and out of the gel, thereby producing an osmotic pressure π_{ion} inside the gel. When the salt concentration becomes comparable with that of the negative charge fixed on the gel, π_{ion} decreases with a rise of the salt concentration. The decrease in π_{ion} is theoretically equivalent to putting an osmotic stress on the gel, and the intensity of the osmotic stress should be in proportion to the extent of the decrease in π_{ion}. The osmotic stress decreases the equilibrium volume gradually (osmoelastic coupling) or discretely (phase transition), depending upon the solvent condition.

"Osmoelastic coupling" has been recently predicted in phospholipid membranes or actin filaments (Ito et al., 1987; Ito et al., 1989; Yamazaki et al., 1989; Suzuki et al., 1989). Based on it, the mechanisms of PEG-induced aggregation and fusion of phospholipid vesicles or macromolecule-induced bundle formation of actin filaments have been analyzed quantitatively in those papers. The possibility that osmoelastic coupling between actin filaments in cytoplasm and osmotic stress across cell membrane may play important roles in cell response to osmotic fluctuation without changing the volume has been also suggested (Ito et al., 1987). Probably, "osmoelastic coupling" may occur in other biological structures such as membrane structures of cells and may affect their physiological functions.

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