Gel Chromatography of Chlorophyllin (Cu–Na Salt) in Water and in Aqueous Poly(*N*-vinylpyrrolidone) Solution

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Synopsis. Gel chromatography of chlorophyllin (Cu–Na salt) in water was compared with that in aqueous poly(*N*-vinylpyrrolidone) (PVP) solution. A shoulder at 430 nm in the absorption spectra, which was observed in all fractionated PVP solution system, is due to the monomer of chlorophyllin (Cu–Na salt). PVP tends to suppress aggregation of chlorophyllin (Cu–Na salt).

G. Oster et al.[1,2] have studied the interaction between chlorophyllin a and poly(*N*-vinylpyrrolidone) (PVP). K. Enmanji[3] has investigated by electronic spectroscopy the interaction between chlorophyllin (Cu–Na salt) and PVP. The binding mechanism of chlorophyllin (Cu–Na salt) with PVP was analyzed by use of Scatchard plot to confirm the existence of cooperative binding.

In order to more clarify the interaction between chlorophyllin (Cu–Na salt) and PVP, we assayed gel chromatography.

Experimental

Materials. Chlorophyllin (Cu–Na salt) was purchased from Nacalai Tesque, Inc. PVP (molecular weight 40000) was from Kishida Chemical Co., Ltd. and was purified by reprecipitation in water–acetone.

Preparation of Aqueous Chlorophyllin (Cu–Na Salt)/PVP Solution. Chlorophyllin (Cu–Na salt) (2 mg) and PVP (0.9 g) were dissolved in water to give 13.2 ml of an aqueous solution (pH 4.8). The pH of the solution was adjusted with NaOH. Aqueous chlorophyllin (Cu–Na salt) solutions were prepared in a similar way.

Gel Chromatography. Gel chromatography of aqueous chlorophyllin (Cu–Na salt) and chlorophyllin (Cu–Na salt)/PVP solutions was performed as follows. Two milliliters of the sample (pH 10) was applied onto a column (0.8×40 cm) of Toyopearl HW-55 equilibrated with water (pH 10). Then, it was eluted with water (pH 10) at flow rate of 0.13 ml min^{-1}. Concentrations of samples applied to the column were 1.51×10^{-4} g ml^{-1} for chlorophyllin (Cu–Na salt), and 6.8% wt/vol-solution for PVP. The content of chlorophyllin (Cu–Na salt) in the eluted fraction (4 ml) was monitered by measuring the maximum absorbance in a wavelength range of 300 to 800 nm. The amount of PVP was monitered by weighing the fraction after evaporation, which usually contained a negligible content of chlorophyllin (Cu–Na salt).

Analytical Method. Absorption spectra at room temperature were measured with a double beam spectrophotometer UVIDEC-510 (Japan Spectroscopic Co., Ltd.).

Results and Discussion

Figure 1 shows the elution patterns of gel chromatography. Each plot in this figure gives the amount of chlorophyllin (Cu–Na salt) or PVP in each fraction at pH 10. As can be seen in Fig. 1 A and B, the elution pattern of chlorophyllin (Cu–Na salt) in water at pH 10 is similar to that in aqueous PVP solution at pH 10. It is noted, however, that the amounts of chlorophyllin (Cu–Na salt) (from Fr 7 to Fr 14) eluted in aqueous PVP solution at pH 10 are larger than those in water at pH 10. This difference is due to chlorophyllin-solubilization effects of PVP.

Figure 2 shows the absorption spectra of chlorophyllin (Cu–Na salt) in unfractionated (sample applied onto the column) and fractionated solution. In Fig. 2

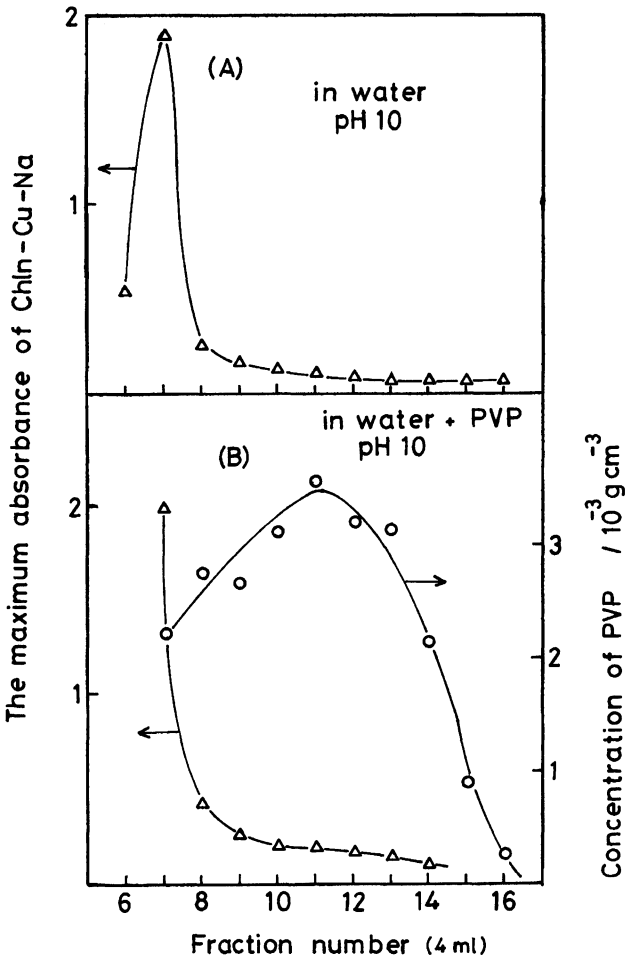

Fig. 1. Elution Patterns of gel chromatography. (A) chlorophyllin (Cu–Na salt) in water at pH 10, (B) chlorophyllin (Cu–Na salt) in aqueous PVP solution at pH 10.

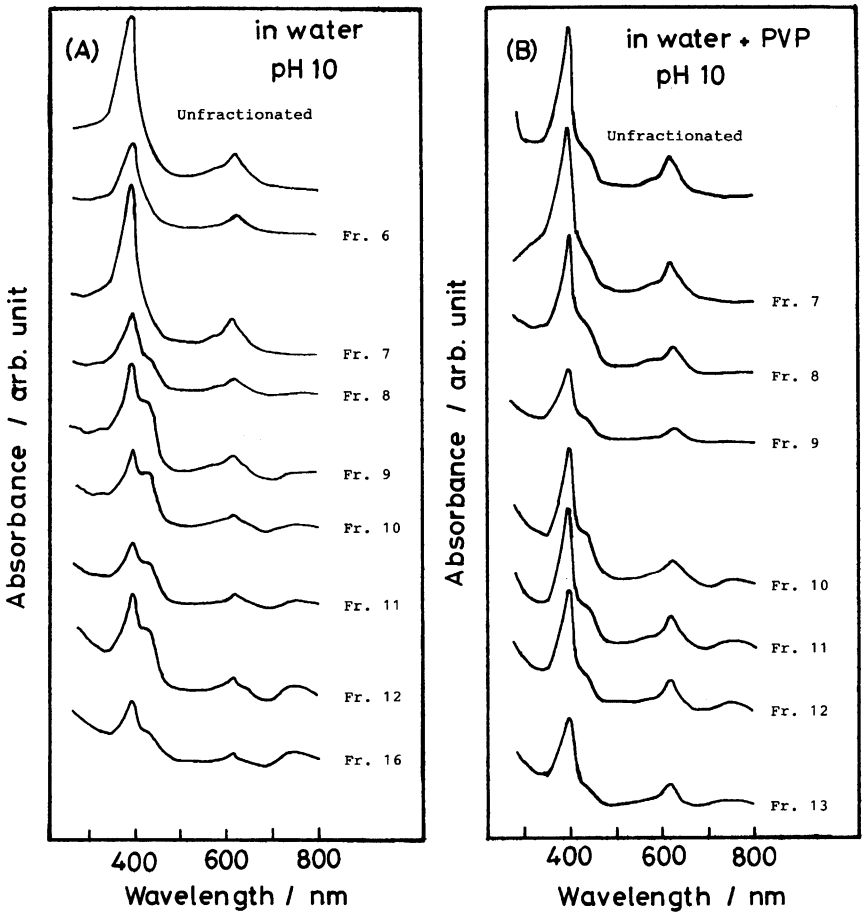

Fig. 2. Absorption spectra of chlorophyllin (Cu–Na salt) in unfractionated and fractionated solutions in gel chromatography. (A) chlorophyllin (Cu–Na salt) in water at pH 10, (B) chlorophyllin (Cu–Na salt) in aqueous PVP solution at pH 10. The absorption spectra of fractions 13—15 for sample (A) are not shown, because they changed successively as the fraction number grew.

A, chlorophyllin (Cu–Na salt) in water at pH 10 did not show a shoulder at 430 nm in unfractionated, Fr 6 and Fr 7 solutions. From Fr 8 to Fr 16, however, a shoulder at 430 nm is observed. In Fig. 2 B, chlorophyllin (Cu–Na salt) in aqueous PVP solution at pH 10 shows a shoulder at 430 nm in unfractionated solution and in all fractionated solutions. And each spectral feature of Fig. 2 B corresponds to that of chlorophyllin (Cu–Na salt) in aqueous Triton X-100 solution (the data are not shown). A column chromatography with talc of chlorophyllin (Cu–Na salt) has given no species having a shoulder at 430 nm.[4)]

In gel chromatography, it is expected that, as the fraction number increases, the molecular weight is getting lower. Since the shoulder at 430 nm tends to appear in later fractions, it seems to be due to the small species. This species is inferred to be a monomer of chlorophyllin (Cu–Na salt).[3)] A monomer of chlorophyllin (Cu–Na salt) is bound in a random coil of PVP chain. On the other hand, the species without the shoulder at 430 nm is ascribed to an aggregate of chlorophyllin (Cu–Na salt). From Fig. 2 A and B, PVP tends to suppress aggregation of chlorophyllin (Cu–Na salt).

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