CHEMISTRY LETTERS, pp. 431-434, 1993.

Inter-vesicular Communications by a Synthetic Amphiphilic Polypeptide

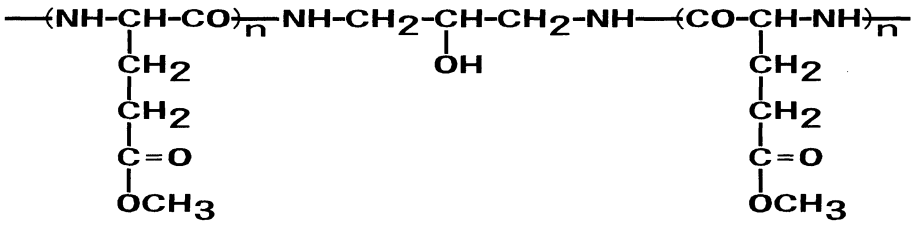
Takatoshi KINOSHITA, Masahiro HIGUCHI, Akira TAKIZAWA, and Yoshiharu TSUJITA
Department of Materials Sicence and Engineering, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya 466

The direct vesicle-to-vesicle communication can be made by an amphiphilic polypeptide composed of two amphiphilic helices jointed with a hydrophilic spacer between them prepared by "Monolayer reaction method".

Intercellular communication is mediated in many tissues by a major intrinsic protein called gap junction. It has been suggested, from analysis of the sequence of the gap junction protein,[1] that it traverses the lipid bilayer via a transmembrane amphiphilic helix, which is essential for the formation of an intercellular aqueous channel.

We have already reported a novel and simple technique, "Monolayer reaction method",[2-4] for the preparation of an amphiphilic helix. In the present study, we applied this method to the preparation of the polypeptide composed of two amphiphilic helices joined with a hydrophilic spacer between them, and examined its activity for the formation of an inter-vesicular aqueous channel as a gap junction model.

The starting material, two poly(γ-methyl L-glutamate)s anchored on a 2-propanol, [Poly(Glu(OMe))]$_2$PrOH, was obtained by polymerization of the N-carboxy anhydride of L-glutamic acid γ-methyl ester (0.016 mol) with 1,3-diamino-2-propanol (5.43×10^{-4} mol) as an initiator in 1,2-dichloroethane (100 mL). In this manner the initiator could be feasibly introduced between poly(γ-methyl L-glutamate) and Poly(Glu(OMe)) as a flexible spacer.



[Poly(Glu(OMe))]$_2$PrOH

DP of the both Poly(Glu(OMe))s was estimated to be 46 from the intrinsic viscosity measurements of a dichloroacetic acid solution of [Poly(Glu(OMe))]$_2$PrOH by the equation $[\eta]=2.24 \times 10^{-3} M^{0.58}$.[5] And then [Poly(Glu(OMe))]$_2$PrOH was saponified by using the monolayer reaction method, which is described in detail elsewhere,[2] to give the amphiphilic nature. A known amount of [Poly(Glu(OMe))]$_2$PrOH dissolved in chloroform was placed at the air-water interface in a hydrophobic vessel with a syringe. The area occupied by the monolayer was decreased by moving a hydrophobic barrier to form the solid condensed state of the monolayer. When the area per the monomer residue reached 15 Å^2, 40 mL of an aqueous solution of potassium hydroxide (2.5 mol·dm^{-3}) was injected into the aqueous phase (1000 mL; 0.1 mol·dm^{-3}) beneath the solid condensed monolayer to saponify [Poly(Glu(OMe))]$_2$PrOH. After ca. 10 min, excess hydrochloric acid was added to convert the polypeptide to its acidic form.

NMR analysis showed that the resulting copolypeptide contained 35 mol% of L-glutamic acid residue, am[Poly(Glu/Glu(OMe))]$_2$PrOH. Furthermore, the sequence of the polypeptide components, determined by the gel filtration chromatographic analysis[1] (Wakopak WB-G-30 column) of the oligopeptide fraction yielded by the hydrolysis of am[Poly(Glu/Glu(OMe))]$_2$PrOH by Staphylococcus aureus,[6] Strain V8 Protease, could be written as

$$-(\mathrm{Glu}\cdot\mathrm{Glu}\cdot\mathrm{Glu(OMe)}\cdot\mathrm{Glu(OMe)}\cdot\mathrm{Glu}\cdot\mathrm{Glu(OMe)}\cdot\mathrm{Glu(OMe)}\cdot\mathrm{Glu(OMe)}\cdot\mathrm{Glu}\cdot\mathrm{Glu(OMe)}\cdot \mathrm{Glu(OMe)}\cdot\mathrm{Glu}\cdot\mathrm{Glu(OMe)}\cdot\mathrm{Glu(OMe)}\cdot\mathrm{Glu(OMe)}\cdot\mathrm{Glu}\cdot\mathrm{Glu(OMe)}\cdot\mathrm{Glu(OMe)})_n- \quad (1)$$

where Glu(OMe) and Glu denote γ-methyl L-glutamate and L-glutamic acid residues, respectively, thus confirming the amphiphilic α-helical structure.

Figure 1 shows the rate of inter-vesicular transport of D_2O at 20 ℃ using dipalmitoylphosphatidylcholine (DPPC; SIGMA) as a vesicle forming component. At first a thin film of DPPC (2.5 mg) was formed in the inner surface of a glass flask. 50 mL of a buffer solution (pH 6.8 Tris-HEPES/H_2O) containing 0.5 mmol·dm^{-3} $TbCl_3$ was added to this flask, and it was sonicated at 0 ℃ under a stream of nitrogen by using a Bionic model 7250B ultrasonic processor for 10 min to prepare the vesicles. The clear aqueous solutions obtained were subjected to gel filtration at room temperature (ca. 20 ℃) using a Biogel P-6DG column (10 x 400 mm) and Tris-HEPES/H_2O buffer solution (pH 6.8) as an eluting solvent to remove the external $TbCl_3$. The vesicle appears at fraction Nos. 16 - 20. An electron microscopic examination of the vesicles stained negatively by uranyl acetate showed that almost all vesicles with diameter of ca. 40 nm seems to be single-walled. DPPC vesicles with D_2O in the vesicular interior were prepared in a similar manner as above using Tris-HEPES/D_2O buffer solution. And then, the DPPC vesicles

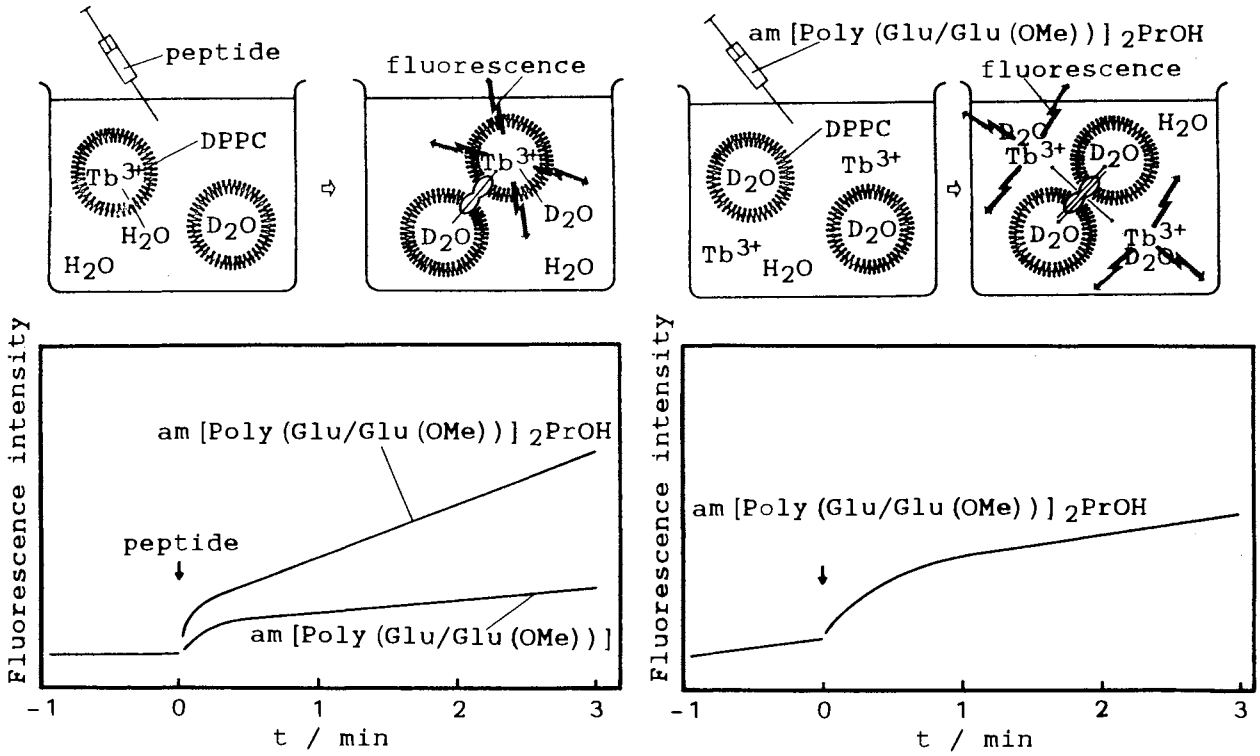


Fig. 1. Effects of the addition of polypeptides on the fluorescence intensity of Tb^{3+} due to the complexation in the vesicles with D_2O transported from the different vesicular interior at 20 ℃.

Fig. 2. Effects of the addition of am [Poly (Glu/Glu (OMe))] $_2$PrOH on the fluorescence intensity of Tb^{3+} due to the complexation in the external aqueous solution with D_2O released from the vesicular interior at 20 ℃.

containing $TbCl_3$-H_2O and D_2O, respectively, were mixed each other in a buffer solution (pH6.8 Tris-HEPES/H_2O; 50 mL). λ_{ex} and λ_{em} of Tb^{3+} were 370 and 545 nm, respectively. The arrow marks the time at which the polypeptides were added to the solution. The fluorescence intensity of Tb^{3+} is known to be weak in H_2O, however, the replacement of aqueous phase from H_2O to D_2O effectively increases the fluorescence intensity.[7] Therefore, the increase in the fluorescence intensity in the vesicle after the addition of am [Poly (Glu/Glu (OMe))] $_2$PrOH confirms the evidence of transport of D_2O from the original vesicle to the vesicle containing Tb^{3+}. It is noted, here, that inter-vesicular transport of D_2O can be enhanced more effectively by am [Poly (Glu/Glu (OMe))] $_2$PrOH containing two amphiphilic helix rods compared with the single amphiphilic α-helical polypeptide,[2] am [Poly (Glu/Glu (OMe))] (DP = 62), whose sequence is as same as Eq. 1. Moreover, the effect of am [Poly (Glu/Glu (OMe))] $_2$PrOH on the acceleration of D_2O leakage from the vesicular interior to the external aqueous phase could be examined by the similar fluorescence study using D_2O in vesicle - Tb^{3+} in aqueous solution

system (Fig. 2). It is apparent that the rate of D_2O leakage in the steady state after am[Poly(Glu/Glu(OMe))]$_2$PrOH addition is comparable with that before the polypeptide addition corresponding to the natural leakage of D_2O. A quantitative comparison between D_2O transfer in Figs. 1 and 2 shows that the degree of D_2O release (Fig. 2) is within 5% of the direct vesicle-to-vesicle D_2O transport (Fig. 1) induced by am[Poly(Glu/Glu(OMe))]$_2$PrOH.

The gel filtration analysis was also carried out for the vesicle solution after the D_2O transport measurements in Fig. 1. The trace obtained for the DPPC vesicle - am[Poly(Glu/Glu(OMe))]$_2$ PrOH system showed a new broad peak centered at the elution volume of 8.9 mL, in addition to the peak at the elution volume of 10.7 mL associated with the pure DPPC vesicle, indicating an increase in size of the DPPC vesicles. This implies that DPPC vesicle adhesion may be induced by am[Poly(Glu/Glu(OMe))]$_2$PrOH added. Moreover, CD spectra of the polypeptide in trimethylphosphate show a typical α-helix pattern, however, in DPPC vesicle solution they were converted to a distorted α-helix pattern, typical of those produced by aggregation of polypeptide molecules,[8] indicating that am[Poly(Glu/Glu(OMe))]$_2$PrOH can interact with the bilayer membrane of the vesicles and then associates each other in the membrane phase.

From these results, the effective micro-injection of D_2O in Fig. 1 may be explained in terms of the simultaneous incorporation of am[Poly(Glu/Glu(OMe))]$_2$PrOH into the bilayer membranes of the different vesicles and the induced vesicular adhesion accompanying the polypeptide association in the membrane phase.

Further studies, the direct observations of the vesicle structure with the polypeptide using a transmission electron microscope, are in progress.

The authors gratefully acknowledge the support of the Japanese Ministry of Education, Science and Culture through a Grant-in-Aid for Developmental Science Research (No.03650721).

References

1) M.B. Gorin, S.B. Yancey, J. Cline, J.P. Revel, and J. Horwitz, Cell, 39, 49 (1984).
2) M. Higuchi, A. Takizawa, T. Kinoshita, Y. Tsujita, and K. Okouchi, Macromolecules, 23, 361 (1990).
3) M. Higuchi, T. Kinoshita, A. Takizawa, Y. Tsujita, and K. Okouchi, Bull. Chem. Soc. Jpn., 63, 1916 (1990).
4) M. Higuchi, T. Kinoshita, A. Takizawa, Y. Tsujita, K. Okouchi, and N. Hattori, Polym. J., 23, 15 (1991).
5) A. Nakajima and S. Tanaka, Polymer Preprints, Japan, 17, 473 (1968).
6) G.R.Drapeau, Y.Boily, and J.Houmard, J. Biol. Chem., 247, 6720 (1972).
7) A. Heller, J. Am. Chem. Soc., 88, 2058 (1966).
8) H. Maeda, H. Hato, and S. Ikeda, Biopolymers, 23, 1333 (1984).

(Received November 30, 1992)