Control of Helix–Helix Association induced by Alkali Metal Ions in α-Helical Polypeptide having a Terminal Crown Ether

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Head-to-head type interconnection of an α-helical polypeptide **1** having a terminal benzo-15-crown-5 is regulated by sodium or potassium ions.

Monomer and/or subunit association of proteins is a basic event in the regulation of biological activities. For example, the changes in the helix–helix association state of membrane proteins such as gramicidin,[1] alamethicin,[2] and melittin[3] induced by external stimuli are known to be correlated with their ion-channel character. In artificial molecular systems, photoregulation of association or dissociation of molecular species has recently been achieved with modified cyclodextrin.[4] However, helix–helix association of α-helical polypeptides induced by alkali metal ions has not been reported.

Crown ethers form metal ion complexes,[5] *e.g.* benzo-15-crown-5 forms a 1 : 1 (crown : Na^+) complex with a Na^+ ion.[6] The same crown ether forms a 2 : 1 (crown : K^+ complex) with the larger K^+ ion (a sandwich-type structure).[6] In this study, we attempted to use such complexes as connectors of helix-rods. The ion-binding behaviour of the crown ethers attached to polypeptides was similar to that of the small molecule analogue. Interconversion between the helix monomer and the helix dimer of α-helical polypeptide with a terminal crown ether can be regulated by addition of Na^+ or K^+ ions. Fig. 1 shows the association state of helix rods schematically. K^+ ions couple two helix-rods head-to-head, while associated Na^+ ions prevent coupling.

Poly(γ-benzyl-L-glutamate) **1** having a benzo-15-crown-5 at the end of the main chain was prepared by using aminobenzo-15-crown-5 as the initiator and the polymerization technique[7] of γ-benzyl-L-glutamate *N*-carboxy anhydride. The ratio of the γ-benzyl-L-glutamate-NCA to the initiator was 50. The polymerization reaction was carried out in dioxane at room temperature.

The structure of the polypeptide **1** was confirmed by comparing the FTIR spectra of this product with those of the analogous poly(γ-benzyl-L-glutamate) and benzo-15-crown-5. The circular dichroism (CD) spectrum of the polypeptide **1** in 1,2-dichloroethane solution showed a minimum at 222 nm, suggesting the existence of α-helical structure.[8]

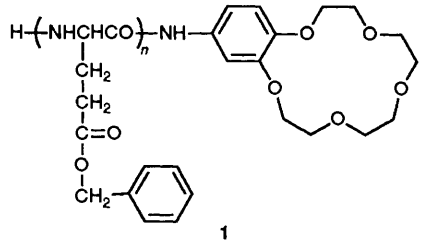
The molecular mass of the polypeptide **1** was measured by two methods. The number-average molecular mass M_n of the polypeptide **1** measured by vapour-pressure osmometry (type 117, Corona Co. Ltd, Japan) was $(2.48 \pm 0.17) \times 10^4$. In this measurement benzene was used as the solvent. The viscosity-average molecular mass M_v, obtained from a dichloroacetic acid solution of the polypeptide **1**, was $(3.31 \pm 0.19) \times 10^4$, from eqn. (1),[9] where $[\eta]$ is intrinsic viscosity. However, this

$$[\eta] = 2.78 \times 10^{-5} M_v^{0.78} \quad (1)$$

equation is for poly(γ-benzyl-L-glutamate). In this case, the polypeptide **1** has a bulky terminal crown ether that may influence the result. Nevertheless, agreement between the two techniques for estimating the molecular mass was fair. (When M_v/M_n is unity, $M_n = M_v$; for these polymers, $M_v/M_n < 1.1$.)

To introduce the metal ions, solid picrates were added to a 1,2-dichloroethane solution of the polypeptide (4 mg 5 ml^{-1}) in a slight excess of their solubility limit. After filtration, the concentration of the picrate in the 1,2-dichloroethane was determined spectrophotometrically (λ_{max} = 359 nm in Na^+ complex, λ_{max} = 374 nm in K^+ complex). Mol ratios of the dissolved sodium and potassium picrates to the polypeptide **1** were 1.30 ± 0.13 and 0.60 ± 0.06, respectively, when the number-average molecular mass of the polypeptide **1** was used for the calculation. When the viscosity-average molecular mass was used, the mol ratios were 1.73 ± 0.17 and 0.80 ± 0.08, respectively. Thus, Na^+ ions formed a complex with the polypeptide **1** according to a 1 : 1 stoichiometry and K^+ ions formed a sandwich-type 2 : 1 complex (2.2 ± 0.4) within the experimental error.

If two helix-rods are coupled by K^+ ion, the apparent molecular mass should increase. A 1,2-dichloroethane solu-



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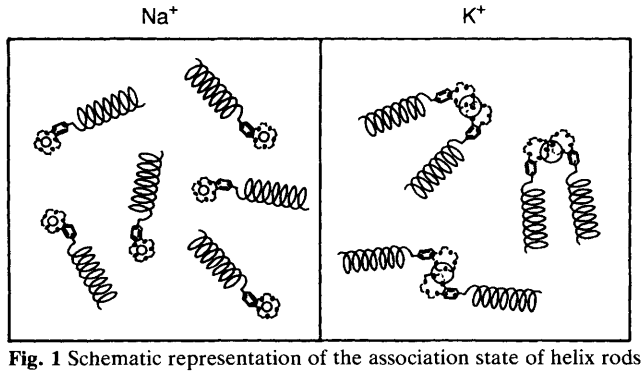

Fig. 1 Schematic representation of the association state of helix rods

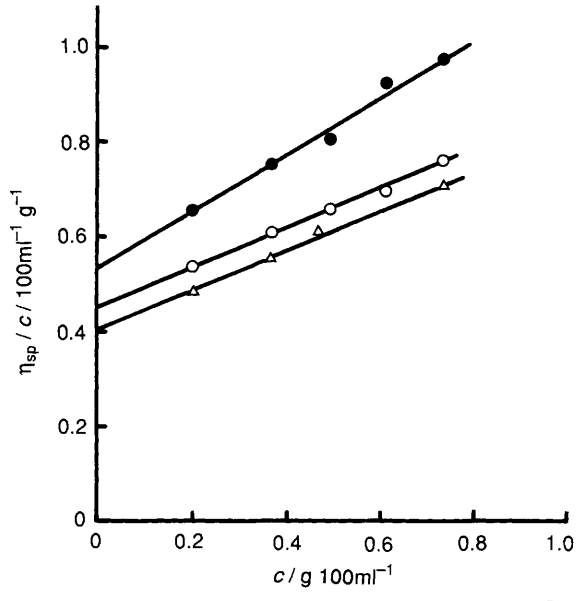

Fig. 2 Concentration dependence of specific viscosity; ●: K^+ complex, ○: Na^+ complex, △: polypeptide without ions

tion, containing the complexes, was analysed with a Toso TSKgel column (type G4000HxL) for gel permeation chromatographic analysis (GPC) with a spectrophotometric detector (UV-8010, Toso Co Ltd, Japan). The column was calibrated using 1,2-dichloroethane solutions of different molecular mass of polystyrene. Since the column was calibrated with a polymer of dramatically different chemical nature and structure, results from GPC can only be compared qualitatively. (The polypeptide **1** in 1,2-dichloroethane is rod-shaped, whereas the polystyrene is spherical.) The ratios of the apparent molecular masses of K^+ complex and Na^+ complex to the apparent molecular mass of the polypeptide **1** without ions were estimated to be 1.16 ± 0.03 and 1.01 ± 0.03, respectively, and the ratios of weight-average molecular mass to number-average molecular mass (M_w/M_n) of K^+ complex, Na^+ complex, and the polypeptide **1** without ions were 1.05, 1.06 and 1.05, respectively. Although the apparent molecular mass of the K^+ complex was significantly larger (16%) than that of the polypeptide **1** without ions, the difference was not as large as the expected. The reason for this result is unclear. The linked polypeptides are probably not all straight and may have a distribution of effective lengths.

The viscosity measurements gave similar results. Fig. 2 shows the concentration dependence of specific viscosity measured at 25 °C by using Ubbelohde-type viscometer. At a given concentration, the viscosity of the 1,2-dichloroethane solution of K^+ complex was higher than that of Na^+ complex.

The alkali metal cations complexing with the crown ether part of the polypeptide **1** can be exchanged. When an excess amount of potasium picrate was added to a 1,2-dichloroethane solution of the Na^+ complex, the specific viscosity increased. Inversely, when an excess amount of sodium picrate was added to a 1,2-dichloroethane solution of the K^+ complex, the specific viscosity decreased. The formation of the helix-helix associates was reversible.

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