Synthesis of polymers and copolymers of *c*-(N^{ϵ}-AcrLys–Gly) and interactions with metal ions in solution

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A vinyl compound carrying a cyclic dipeptide in the side chain, *c*-(N^{ϵ}-AcrLys–Gly), has been synthesized and polymerized using radical initiators. The homopolymer was soluble only in water, Me_2SO, H_2O/Me_2SO and $H_2O/HCONMe_2$. In the latter solvent, an initially clear solution became turbid upon standing. Copolymers with styrene were prepared and a styrene-rich fraction was found to be soluble in a variety of organic solvents. However, the reduced viscosity of a $HCONMe_2$ solution of the copolymer increased with dilution. CH_2Cl_2-soluble copolymers extracted barium picrate and $NaBPh_4$ from aqueous solution, and copolymer gels swollen in dioxane bound $NaBPh_4$ effectively. Metal salt binding should have resulted from the ion–dipole interaction between Ba^{2+} or Na^+ and cyclic dipeptides. However, the polymer effect due to the intramolecular cooperation of cyclic dipeptides upon ion binding was not quantitatively estimated.

INTRODUCTION

Carrier proteins play an essential role in the selective binding and transport of metal ions, amino acids, and saccharides *in vivo*; some of these are cyclic oligomers. Thus, crown ethers[1], cryptands[2], and cyclic peptides[3], are interesting as their model compounds. Recently, these cyclic ligands have been bound to the side chain of polymers, and the enhancement of the efficiency and specificity as a result of the intramolecular cooperation of side-chain cyclic ligands has been attempted. This attempt has been particularly popular with crown ethers[4], but no such attempt has been made with cyclic peptides.

The combination of a functional oligomer and a polymer results in an enhanced functionality as follows. First, the increased stability of the complex and the improved selectivity of complexation as a result of the intramolecular cooperation of side chains are attained. Second, the solubility of the complex may be regulated according to the hydrophobicity and hydrophilicity of the main chain of the polymer and the neighbouring side chains. Third, the combination of the excellent mechanical properties of the polymer with the functionalities of the oligomer develop functional materials.

We have investigated the complexation of many synthetic cyclic peptides with metal ions[5]. In particular, we have reported the instantaneous formation of insoluble complexes of *c*-$(Sar)_2$ with alkali and alkaline-earth metal ions in organic solvents such as ethyl acetate[6]. With *c*-$(Sar)_2$-$LiClO_4$ (2:1) complex the crystalline structure was determined to be a network in which a central lithium ion is surrounded by the carbonyl groups of four molecules of *c*-$(Sar)_2$[7]. These experimental results suggest a large enhancement of metal ion binding by cyclic peptides by incorporation into the polymer side-chain. As a first example of such polymers, we synthesized polymers and copolymers of *cyclo*-(N^{ϵ}-acryloyl-L-lysylglycyl), [*c*-(N^{ϵ}-AcrLys-Gly)], and the formation of metal–ion complexes in organic solvents was investigated.

EXPERIMENTAL

Synthesis

c-(N^{ϵ}-AcrLys–Gly) was synthesized by the method described in *Scheme 1*. Each procedure was that usually employed in liquid-phase peptide synthesis, and Z and DCC represent a carbobenzyloxy group to protect an amino group and dicyclohexyl carbodiimide (a condensation reagent), respectively. The yield of reaction intermediate is shown in each reaction step: m.p. 204°–206°C. Elemental analysis, calculated for $C_{11}H_{17}N_3O_3$: C, 55.22%; H, 7.16%; N, 17.56%. Found: C, 55.32%; H, 7.04%; N, 17.47%. The infra-red spectrum of *c*-(N^{ϵ}-AcrLys–Gly) is shown in *Figure 1*.

c-(N^{ϵ}-AcrLys–Gly) is a crystalline material, and it is soluble in dimethylsulphoxide (Me_2SO), H_2O/Me_2SO mixed solvent and H_2O/dimethylformamide ($HCONMe_2$), and slightly soluble in water and $HCONMe_2$.

Radical polymerizations of *c*-(N^{ϵ}-AcrLys–Gly) were carried out in $K_2S_2O_8/H_2O$, $H_2O_2 + FeCl_2/H_2O$, and azobisisobutyronitrile (AIBN)/H_2O–$HCONMe_2$ (4:1 v/v) systems. $K_2S_2O_8$ and AIBN were recrystallized from MeOH twice. Commercial H_2O_2 and $FeCl_2$ were used without further purification. Ion-exchanged water was distilled and used as the polymerization solvent. $HCONMe_2$ was distilled *in vacuo* over $CaCl_2$ twice. Polymerizations were carried out under a nitrogen stream or in a tube sealed under a vacuum. After a requisite time, the solution was poured into a large amount of MeOH.

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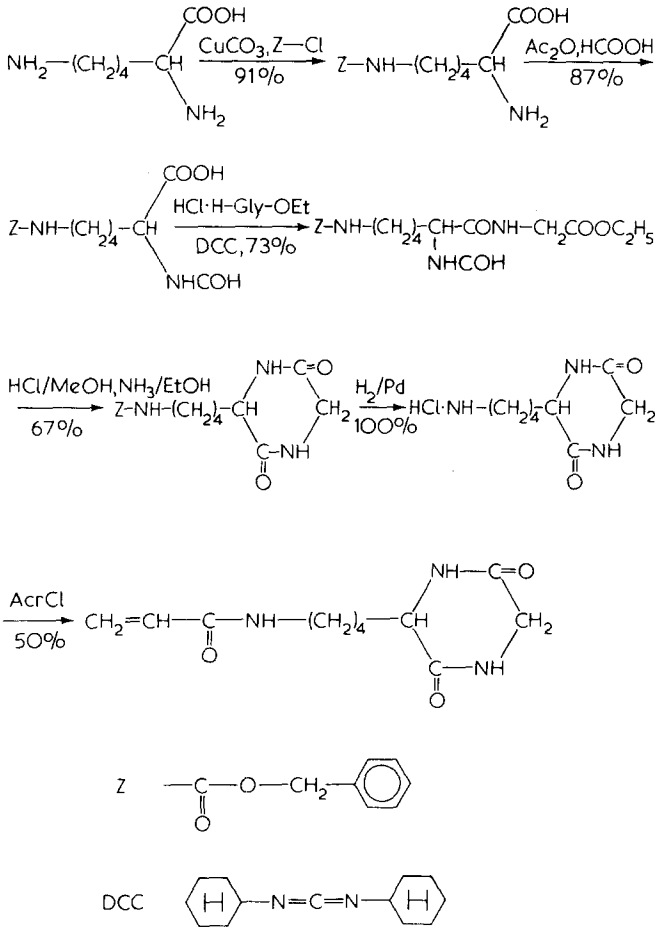

Scheme 1 Synthesis of c-(N$^{\epsilon}$-AcrLys–Gly)

The precipitation recovered was reprecipitated several times with H_2O/MeOH, and dried under vacuum. The polymers were dissolved in H_2O/$HCONMe_2$ (4:1 v/v) and the viscosity measured at 30°C.

Radical copolymerization of c-(N$^{\epsilon}$-AcrLys–Gly) with styrene was carried out in Me_2SO with AIBN as initiator. Styrene was purified as usual, and Me_2SO was twice distilled under vacuum and over $CaCl_2$. The copolymerization was carried out in a nitrogen atmosphere, and the copolymer was recovered by precipitation with water. It was extracted with dioxane. The dioxane-insoluble fraction was washed with water and dried *in vacuo*. The dioxane-soluble fraction was extracted with CH_2Cl_2 again. Each fraction was reprecipitated with dioxane/*n*-hexane several times, and dried *in vacuo*. The copolymer was thus fractionated into three fractions. The content of the c-(N$^{\epsilon}$-AcrLys–Gly) unit in each fraction was determined by elemental analysis. The viscosity of the copolymer was measured in $HCONMe_2$/CF_3COOH solution (9:1 v/v) at 25°C.

Metal–salt complexation

Interactions of polymers and copolymers of c-(N$^{\epsilon}$-AcrLys–Gly) with metal picrates or metal thiocyanates in a homogeneous solution were investigated by ultra-violet spectroscopy. Commercial sodium picrate (NaPi) was used without further purification. Commercial sodium thiocyanate was recrystallized from MeOH. Ion-exchanged water was distilled and used as a solvent for spectroscopic measurement. Me_2SO was distilled under vacuum over $CaCl_2$. The shift of the absorption maximum of the picrate ion pair due to the dissociation is particularly useful in measuring the extent of counterion solvation by the polymer ligand. It has been reported that potassium picrate ion-pair absorbs at 357 nm when it is tightly ion-paired, but absorbs at 380 nm when it is dissociated into free ions[8].

Interaction of c-(N$^{\epsilon}$-AcrLys–Gly)–styrene copolymer with metal picrates or sodium tetraphenyl borate ($NaBPh_4$) were investigated by the partition equilibrium method. An aqueous solution (5 ml) containing picric acid and NaOH or $Ba(OH)_2$ was brought into contact with CH_2Cl_2 solution (5 ml) containing the CH_2Cl_2-soluble fraction of copolymer. The mixture was stirred for 3 min and left overnight at room temperature. The CH_2Cl_2 phase was separated and the residual concentration of copolymer was determined by ultraviolet spectroscopy. Commercial picric acid and $Ba(OH)_2$ were used without further purification. Commercial CH_2Cl_2 (guaranteed reagent) was distilled over $CaCl_2$. The reference experiment was carried out using polystyrene which was synthesized by the same method as that used in copolymerization.

An aqueous solution (5 ml) of $NaBPh_4$ and a CH_2Cl_2 solution (5 ml) of the copolymer were stirred for a requisite time at room temperature. After standing for 5 min, the aqueous phase was separated and shaken with cyclohexane (5 ml) to remove trace amounts of CH_2Cl_2 contaminant. After standing for 5 min the aqueous solution was separated and the residual concentration of $NaBPh_4$ was determined by the absorption at 240 nm. Commercial $NaBPh_4$ was used in this experiment without further purification.

The adsorption of metal salt by the copolymer gel was investigated. To 1.46×10^{-2} M dioxane solution (10 ml) of $NaBPh_4$, various amounts of a dioxane-insoluble fraction of c-(N$^{\epsilon}$-AcrLys–Gly)–styrene copolymer were added. The copolymer was swollen by standing overnight at room temperature. After stirring for 90 min at room temperature, the copolymer was filtered and washed twice with 5 ml dioxane (previously distilled over sodium). The combined filtrate and washings were evaporated. After vacuum drying, the amount of $NaBPh_4$ was determined. The gel adsorption experiment was also carried out with crosslinked polystyrene as a reference, which was synthesized according to the procedure described in the literature[9].

The interaction of NaPi with the copolymer was also investigated by dialysis. A cellulose acetate tube was used

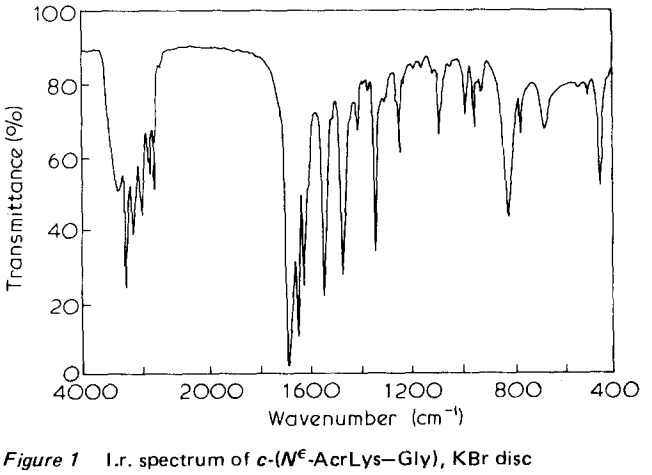

Figure 1 I.r. spectrum of c-(N$^{\epsilon}$-AcrLys–Gly), KBr disc

Table 1 Radical polymerization of *c*-(*N*$^{\epsilon}$-AcrLys–Gly)

No.	Solvent	[M] (wt %)	Initiator	[I] (wt %)	Condition	Yield (%)	$[\eta]^a$ (100 ml g^{-1})
1	H_2O	2.5	$K_2S_2O_8$	1	N_2 atmosphere, 60°C, 24h Room temperature, 44h	60	0.11
2	H_2O	2.5	$K_2S_2O_8$	1	N_2 atmosphere, 80°C, 50h	78	0.19
3	H_2O	2.5	$H_2O_2/FeCl_2$	1	Vacuum, 50°C, 15h	58	0.064
4	$H_2O/HCONMe_2$ 4 : 1	5	AIBN	3	Vacuum, 60°C, 46h	52	–

[a] 30°C, $H_2O/HCONMe_2$ (4 : 1 v/v)

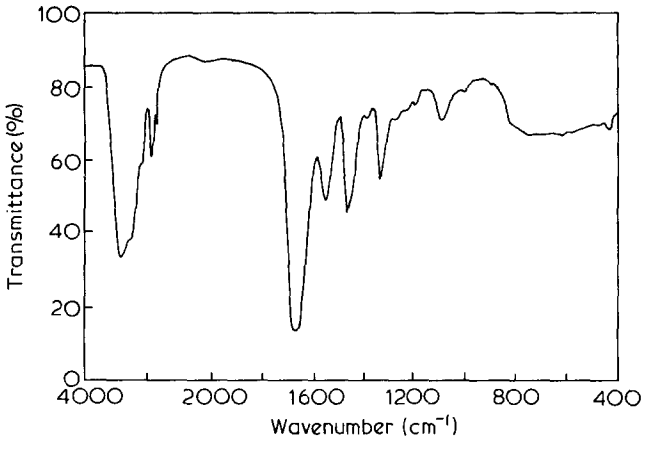

Figure 2 I.r. spectrum of poly [*c*-(*N*$^{\epsilon}$-AcrLys–Gly)], KBr disc

Table 2 Fractionation of *c*-(*N*$^{\epsilon}$-AcrLys–Gly)–styrene copolymer

No.	Solubility	Weight fraction (%)	Mol % of cyclic peptide unit	$[\eta]^a$ (100 ml g^{-1})
1	Dioxane-insoluble	55	31.6	0.093
2	Dioxane-soluble CH_2Cl_2-insoluble	35	20.4	0.085
3	CH_2Cl_2-soluble	10	16.0	0.066

[a] 25°C, $HCONMe_2/CF_3COOH$ (9 : 1 v/v)

as dialysis membrane (Nakarai Chem. Co. VT-351-type, maximum molecular weight for permeation 3500, diameter 11 mm). Prior to the dialysis, the cellulose tube was immersed in dioxane/MeOH (19:1 v/v) mixed solvent overnight. Copolymer solutions of given concentration (4 ml) were placed in the cellulose acetate tube and dialysed against NaPi solution (12 ml) of various concentrations. After stirring the NaPi solution for a requisite time at room temperature, the concentration of NaPi solution was determined by ultra-violet spectroscopy.

RESULTS AND DISCUSSION

Polymerization of c-(N$^{\epsilon}$-*AcrLys–Gly) and characterization of its polymers*

Experimental results obtained in the radical polymerizations of *c*-(*N*$^{\epsilon}$-AcrLys–Gly) are shown in *Table 1*. In runs 1 to 3 polymers having yellow to pale orange colour were obtained, possibly contaminated by initiator metal salts bound to the cyclic peptide side chain. In the polymerization run 4 a white polymer was produced.

The infra-red spectrum of poly[*c*-(*N*$^{\epsilon}$-AcrLys–Gly)] is shown in *Figure 2*. Absorptions at 970 and 1640 cm^{-1} appearing in the spectrum of *c*-(*N*$^{\epsilon}$-AcrLys–Gly) (*Figure 1*), which are assigned to a double bond, do not appear in the spectrum of the polymerization product, indicating the formation of a polymer.

As shown subsequently (see *Table 3*), the solubility of poly[*c*-(*N*$^{\epsilon}$-AcrLys–Gly)] is quite low. It is soluble only in water, H_2O/Me_2SO mixed solvent and $H_2O/HCONMe_2$ mixed solvent, and slightly soluble in Me_2SO. Intrinsic viscosities of poly[*c*-(*N*$^{\epsilon}$-AcrLys–Gly)] were measured in $H_2O/HCONMe_2$ (4:1 v/v) mixed solvent. The dissolution of the polymer resulted in a clear solution, but it sometimes became turbid upon standing. The strongly hydrogen-bonding property of cyclic peptide side chains may have a bearing on this phenomenon. Thus, the intrinsic viscosities reported in *Table 1* are not totally reliable. The polymers do not appear to have high molecular weights, possibly up to 10000.

c-(*N*$^{\epsilon}$AcrLys–Gly) 10 wt%–styrene 4.2 wt% mixture (an equimolar mixture) were dissolved in Me_2SO, and the copolymerization was carried out with AIBN (3 wt% against monomers) at 80°C for 84 h under a nitrogen atmosphere. The yield of copolymer was 55.3%. The product was fractionated and the results are given in *Table 2*. It is clear that fractionation took place with different molecular weights as well as with different compositions of the product. The higher the styrene content, the lower the intrinsic viscosity becomes and the higher the solubility of the product.

Infra-red spectra of the three fractions of copolymer were investigated, and found to be very similar to each other, apart from the relative intensities of the bands. The infra-red spectrum of the CH_2Cl_2-soluble fraction is shown in *Figure 3*. The absorption at 1670 cm^{-1} is assigned to an amide group which is characteristic of the *c*-(*N*$^{\epsilon}$-AcrLys–Gly) unit. The absorptions at 700 and 3000 cm^{-1} are assigned to a phenyl group characteristic of styrene unit. These features of the infra-red spectrum as well as the solubility behaviour indicate the formation of a true copolymer. All copolymer fractions contained more styrene units than *c*-(*N*$^{\epsilon}$-AcrLys–Gly) units. Since the copolymer was produced from an equimolar mixture of two monomers, styrene is more reactive than *c*-(*N*$^{\epsilon}$-AcrLys–Gly) in radical copolymerization.

The solubility of the copolymer (fraction no. 3) was examined and is shown in *Table 3* in comparison with that of homopolymer. The solubility of poly *c*-(*N*$^{\epsilon}$AcrLys–Gly)] was greatly increased by the incorporation of styrene units. However, the solubility behaviour of the copolymer is still unusual. When the intrinsic viscosities of three copolymer fractions were measured in $HCONMe_2$, polyelectrolyte-like behaviour was observed; that is, the

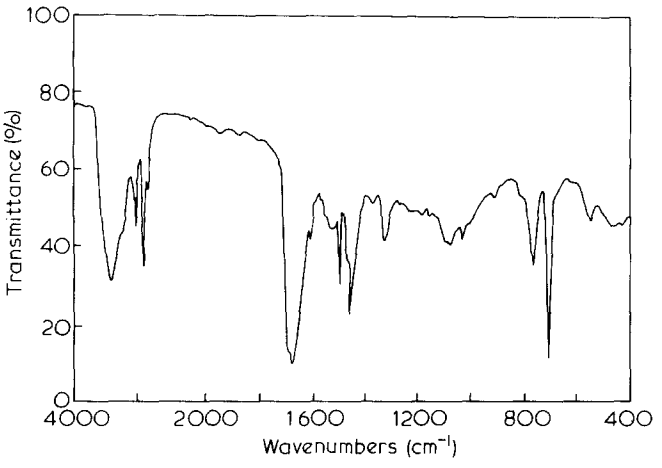

Figure 3 I.r. spectrum of *c*-(*N*$^{\epsilon}$-AcrLys–Gly)–styrene copolymer, CH_2Cl_2-soluble fraction, KBr disc

Table 3 Solubility of polymer and copolymer with styrene of *c*-(*N*$^{\epsilon}$-AcrLys–Gly)

	Homopolymer	Copolymer
H_2O	Soluble	Insoluble
MeOH	Insoluble	Soluble
EtOH	Insoluble	Partly soluble
$HCONMe_2$	Insoluble	Highly soluble
Me_2SO	Soluble	Highly soluble
Dioxane	Insoluble	Highly soluble
THF	Insoluble	Highly soluble
CH_2Cl_2	Insoluble	Highly soluble
$CHCl_3$	Insoluble	Highly soluble
Et_2O	Insoluble	Insoluble

reduced viscosity $[\eta]$ increased with dilution. To determine $[\eta]$ it was necessary to add a small amount of CF_3COOH. The presence of *c*-(*N*$^{\epsilon}$-AcrLys–Gly) units, which are strongly hydrogen bonded, may be responsible for this phenomenon. It is interesting that an unfractionated copolymer containing 81.1 mol % styrene units behaved normally in dichloroethane, giving $[\eta] = 0.083_5$ at 25°C (in $HCONMe_2/CF_3COOH$ 9:1 v/v mixed solvent, $[\eta] = 0.084$). By the application of the $[\eta]$–molecular weight relationship reported for polystyrene[10], the molecular weight of the styrene-rich copolymer was estimated to be 8680. Therefore, the molecular weight of the copolymer listed in *Table 2* should be in the range of up to ten thousand. The CH_2Cl_2-soluble fraction was used in the following metal-salt binding experiments.

Metal-salt binding by poly[c-(M$^{\epsilon}$-*AcrLys–Gly*)]*—ultra-violet study*

To a 2×10^{-3} wt % aqueous solution of poly[*c*-(*N*$^{\epsilon}$-AcrLys–Gly)] various amounts of 2×10^{-3} wt % aqueous solution of NaSCN were added, and ultra-violet spectra were recorded. Unfortunately, thiocyanate group and amide group absorb at neighbouring frequencies and the spectral change was therefore impossible.

To a 2×10^{-3} wt % aqueous solution of NaPi, various volumes of an aqueous solution of poly[*c*-(*N*$^{\epsilon}$-AcrLys–Gly)] were added up to a cyclic dipeptide–NaPi molar ratio of 128, but no shift of the absorption maximum of the picrate ion was observed. In Me_2SO, the picrate absorption maximum remained unchanged at 380 nm by the addition of poly[*c*-(*N*$^{\epsilon}$-AcrLys–Gly)]. In H_2O/Me_2SO 1:1 v/v mixed solvent the situation was the same. In these highly polar media, the ion(picrate)–dipole(cyclic peptide) interaction may have been destroyed.

Interactions between c-(N$^{\epsilon}$-*AcrLys–Gly*)*–styrene copolymer and metal picrate—partition equilibrium study*

An aqueous solution containing 9.5×10^{-5} M picric acid and 0.01 M NaOH was brought into contact with CH_2Cl_2 solution containing 3.2×10^{-5}–1.02×10^{-3} M copolymer (fraction no. 3). The mixture was stirred and left to stand; an insoluble material came out at the interface of the two solutions. The same observation was made in the experiment using $Ba(OH)_2$ instead of NaOH. Without metal picrate or with polystyrene instead of the copolymer, the insoluble material did not appear. Therefore, it must be a complex formed between the copolymer which is insoluble in water and metal picrate which is insoluble in CH_2Cl_2.

The extent of complexation was estimated from the residual concentration of copolymer in CH_2Cl_2 solution after it was partition-equilibrated with aqueous metal picrate solution. With 9×10^{-5} picric acid and 0.01 M $Ba(OH)_2$ in aqueous solution, the concentration of copolymer in CH_2Cl_2 solution was changed. The experimental results are shown in *Table 4*. When a higher initial concentration of copolymer in CH_2Cl_2 solution was used, a larger decrease after the partition equilibrium was observed. Next, with 5×10^{-4} M copolymer in CH_2Cl_2 solution and 9×10^{-5} M picric acid in aqueous solution, the concentration of $Ba(OH)_2$ in aqueous solution was changed. The experimental results are shown in *Table 5*. With increasing initial concentration of barium picrate ($BaPi_2$) in aqueous solution, the decrease in copolymer concentration in CH_2Cl_2 solution after the

Table 4 Effect of initial concentration of *c*-(*N*$^{\epsilon}$-AcrLys–Gly)–styrene copolymer in CH_2Cl_2 on the residual concentration of copolymer after partition equilibrium with aqueous $Ba(OH)_2$ solution[a]

Copolymer concentration[b] before equilibrium	Copolymer concentration[b] after equilibrium
3.6×10^{-4} M	3.3×10^{-4} M
7.2×10^{-4} M	6.1×10^{-4} M
14.4×10^{-4} M	10.9×10^{-4} M

[a] Initial $Ba(OH)_2$ concentration in aqueous phase, 0.01 M; 9×10^{-4} M picric acid added;
[b] Represented in terms of the concentration of the cyclic peptide unit

Table 5 Effect of initial concentration of metal salt in aqueous phase on the residual concentration of *c*-(*N*$^{\epsilon}$-AcrLys–Gly)–styrene copolymer in CH_2Cl_2 after partition equilibrium[a]

Initial concentration of $Ba(OH)_2$ in water	Residual concentration[b] of copolymer in CH_2Cl_2
1×10^{-1} M	4.1×10^{-4} M
1×10^{-2} M	4.3×10^{-4} M
5×10^{-3} M	4.5×10^{-4} M
1×10^{-3} M	4.9×10^{-4} M

[a] Initial copolymer concentration in CH_2Cl_2, 5×10^{-4} M; picric acid, 9×10^{-4} M, was added to aqueous phase;
[b] Represented in terms of the concentration of the cyclic peptide unit

Table 6 Effect of initial concentration of *c*-(*N*$^{\epsilon}$-AcrLys–Gly)–styrene copolymer gel swollen in dioxane on the concentration of free $NaBPh_4$ in CH_2Cl_2 after gel adsorption equilibrium[a]

[*c*-Peptide]/[$NaBPh_4$]$_0$	[$NaBPh_4$] x 10^3 (M)
0.0	1.4
0.5	1.0
1.0	0.9
2.0	0.7
4.0	0.2

[a] [$NaBPh_4$]$_0$ = 1.4 x 10^{-3} M

partition equilibrium was larger. These experimental results indicate that the vinyl polymer carrying a cyclic dipeptide side chain binds $BaPi_2$ at the water–CH_2Cl_2 interface. Because of the insoluble complex a quantitative analysis of the complexation was impossible. The low solubility of metal picrate in organic solvents must be responsible for the insolubility of the complex. Therefore, we used $NaBPh_4$ in subsequent experiments, which is soluble either in water or in CH_2Cl_2.

Interaction between c-(N$^{\epsilon}$-*AcrLys–Gly*)*–styrene copolymer and* $NaBPh_4$*—partition equilibrium study*

10^{-4} M aqeuous $NaBPh_4$ solution and 2.5–40 x 10^{-5} M copolymer solution in CH_2Cl_2 were mixed and stirred. After standing, the CH_2Cl_2 solution was found to be turbid, but no precipitation took place. When $NaBPh_4$ was not present in aqueous solution, turbidity was not observed. When a CH_2Cl_2 solution of polystyrene was used instead of a copolymer solution, turbidity was not observed. It was therefore plausible that $NaBPh_4$ in aqueous solution was partitioned into a CH_2Cl_2 phase and that partitioned $NaBPh_4$ formed a complex with the copolymer, which was not completely soluble in CH_2Cl_2. The course of the partitioning of $NaBPh_4$ between aqueous and CH_2Cl_2 phases and the complexation were followed.

In *Figure 4a* the residual $NaBPh_4$ concentration in the aqueous phase is shown at suitable time intervals after a 10^{-4} M aqueous $NaBPh_4$ solution and a 2×10^{-4} M CH_2Cl_2 solution of copolymer were mixed and stirred. Curve A shows the spectrum of aqueous $NaBPh_4$ solution after 1.5 h stirring with a CH_2Cl_2 solution of polystyrene. The absorption is stronger than in curves B and C taken after stirring for a short time with a CH_2Cl_2 solution of copolymer. This means that the solubility of $NaBPh_4$ in CH_2Cl_2 was lowered by the addition of the copolymer. However, absorption decreased after prolonged stirring with a CH_2Cl_2 solution of copolymer and reached a final value after 90 min stirring. This observation suggests that the partition of $NaBPh_4$ between aqueous solution and CH_2Cl_2 solution and the subsequent complexation with copolymer proceeds slowly, but that an equilibrium is established after 90 min stirring. In *Figure 4b* is shown the absorption spectrum of an aqueous solution after 10^{-4} M aqueous $NaBPh_4$ solution and CH_2Cl_2 solution of copolymer of various concentrations were stirred for 90 min. The effect of the copolymer concentration in CH_2Cl_2 solution on the residual $NaBPh_4$ concentration in the aqueous phase seems to be complex because it brings $NaBPh_4$ into the CH_2Cl_2 phase by complexation and it expels $NaBPh_4$ from the CH_2Cl_2 phase by 'reverse salting-out' (see *Figure 4a*). Therefore comparison was made by comparing the absorption intensity at 240 nm, as shown in *Figure 5*. With increasing copolymer concentration in CH_2Cl_2 solution, the residual $NaBPh_4$ concentration in the aqueous phase fell but not in a straightforward manner. The binding of metal salt by the cyclic peptide-carrying polymer was again apparent.

Interactions between c-(N$^{\epsilon}$-*AcrLys–Gly*)*–styrene copolymer and metal salts—gel adsorption and dialysis studies*

The adsorption of $NaBPh_4$ by the copolymer swollen in dioxane was investigated. The effect of the initial amount of copolymer gel on the concentration of $NaBPh_4$ left unbound in dioxane solution is shown in *Figure 6*. With increasing amounts of the gel, the $NaBPh_4$ concentration decreased. This phenomenon was not observed when

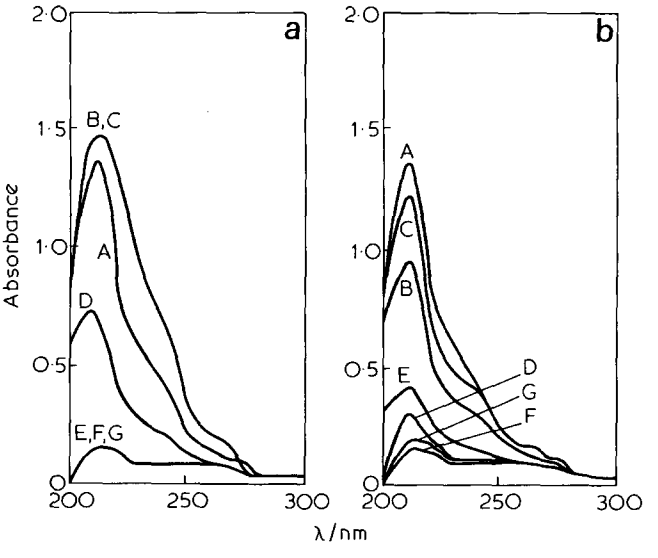

Figure 4 (a) Effect of stirring time on the complexation between *c*-(*N*$^{\epsilon}$-AcrLys–Gly)–styrene copolymer and $NaBPh_4$. Initial concentration of $NaBPh_4$ in water, 10^{-4} M; copolymer in CH_2Cl_2, 2 x 10^{-4} M. A, Blank (polystyrene added); B, 5 min; C, 10 min; D, 30 min; E, 90 min; F, 120 min; G, 180 min. (b) Effect of $NaBPh_4$/*c*-peptide unit molar ratio on the complexation between $NaBPh_4$ and *c*-(*N*$^{\epsilon}$-AcrLys–Gly)–styrene copolymer. Initial concentration of $NaBPh_4$ in water, 10^{-4} M; solutions stirred for 90 min. A, Blank (polystyrene added); B, 8; C, 4; D, 2; E, 1; F, 0.5; G, 0.25

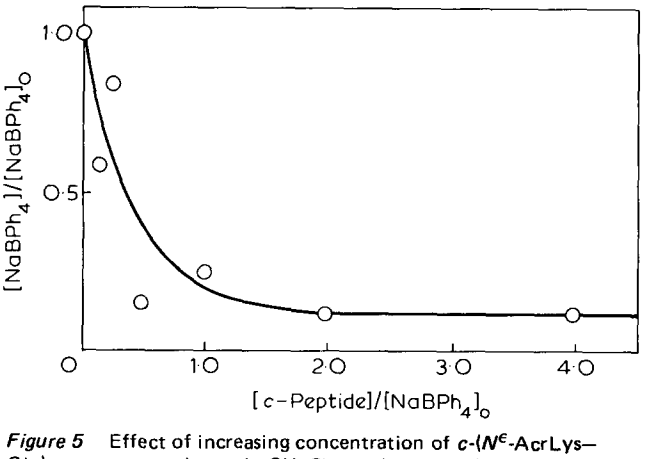

Figure 5 Effect of increasing concentration of *c*-(*N*$^{\epsilon}$-AcrLys–Gly)–styrene copolymer in CH_2Cl_2 on the residual concentration of $NaBPh_4$ in water after equilibrium. Initial concentration of $NaBPh_4$ in CH_2Cl_2, 10^{-4} M; solutions stirred for 90 min

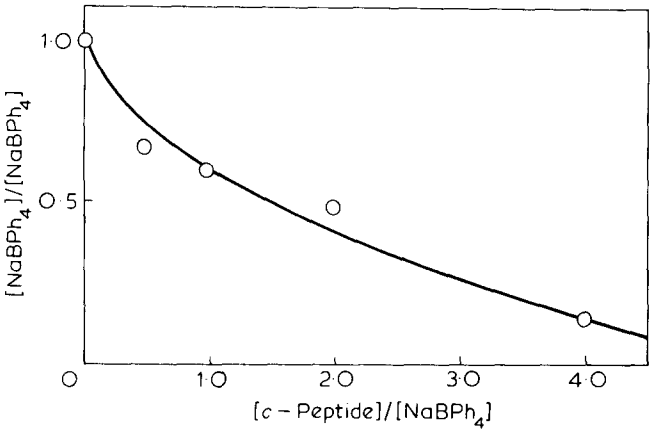

Figure 6 Effect of increasing amount of *c*-(*N*$^{\epsilon}$-AcrLys–Gly)–styrene copolymer on the concentration of free $NaBPh_4$ in dioxane after gel adsorption experiment. Initial concentration of $NaBPh_4$ in dioxane, 1.46×10^{-3} M

crosslinked polystyrene swollen in dioxane was used instead of the dioxane-insoluble copolymer. Clearly the copolymer gel carrying cyclic peptide side chains is effective in binding $NaBPh_4$ by ion–dipole interaction.

A dialysis experiment was carried out as described in the experimental section. The concentration of NaPi in the dialysed solution was not affected by the addition of the copolymer. This result may be due to the stronger interaction of NaPi with the membrane material (cellulose acetate) than with the copolymer.

CONCLUSIONS

A vinyl polymer carrying cyclic peptides in its side chains, *c*-(*N*$^{\epsilon}$-AcrLys–Gly)–styrene copolymer, was found to form a complex with $NaBPh_4$ or $BaPi_2$ in low polarity solvents such as CH_2Cl_2 and dioxane. The complex was insoluble or slightly soluble in CH_2Cl_2 when formed in the partition equilibrium between water and CH_2Cl_2. Since the mixture of the copolymer and $NaBPh_4$ in CH_2Cl_2 is homogeneous, network formation by intermolecular hydrogen bonding involving water molecules might be responsible for the insoluble complex in the partition equilibrium.

Since *c*-(*N*$^{\epsilon}$-AcrLys–Gly) is not soluble in low polarity solvents such as CH_2Cl_2, comparison of metal ion affinities was not undertaken between polymeric and monomeric ligands.

Metal ion binding by *c*-(*N*$^{\epsilon}$-AcrLys–Gly)–styrene copolymer did not seem to be very efficient. This should be because styrene units incorporated to increase the solubility work as 'diluent' and lower the intramolecular cooperation of cyclic peptide side chains for metal ion binding.

To overcome these problems, a homopolymer carrying a cyclic peptide in its side chains which is soluble in low polarity solvents should be synthesized.

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