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The Adsorption of Polypeptides on Polystyrene Latices and Their Effects on Colloid Stability

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The adsorption behavior of poly(L-lysine) and poly(L-glutamic acid) on negatively and positively charged latices was examined in combination with their stabilizing effects on the latex dispersion. It was realized that the polypeptide adsorption was, to a large extent, determined by the coulombic interaction between the polypeptide and the charged surface, and by the conformational change (helix-coil transition) in the polypeptide in the solution. The flocculation behavior of the dispersion in the presence of polypeptide was also explained reasonably by taking account of the results of electrophoretic measurements of the polypeptide-coated particles.

The stability of a colloid dispersion can be widely modified by adding polymer molecules. Especially, a partially ionic polymer is important as a flocculant in various practical fields.[1] As for the mechanism of destabilization to the dispersion by a polymer, two principal ideas have been proposed. One is the bridging effect,[2] which is built up between the particles containing an adsorbed polymer layer. Under this mechanism, loops and tails in the adsorption layer play an important role. Another mechanism is based on the charge neutralization of the particles by the adsorption of oppositely charged polyelectrolytes.[3] The problem is that these two mechanisms for destabilization are not independent; rather, the colloid stability is practically determined by the combined action of these two effects.[4]

Poly(L-lysine)(PLL) and poly(L-glutamic acid)(PLG) are typical cationic and anionic polypeptides, and the study of molecular conformation, *i.e.*, the helix-coil transition in solution, has been examined extensively. According to Chou *et al.*,[5] the helix-coil transition of PLL is brought about by the change in pH from around pH 11, where the polymer is helix, to $\mathrm{pH}<8$, where the helix content is zero and where all the molecules take an extended form. On the other hand, the PLG takes the helical conformation in the $\mathrm{pH}<\approx 5.5$ range and is changed to a random or a stretched structure in the $\mathrm{pH}>6$ range.

In this work, PLL and PLG were used as adsorbates and the adsorption behavior was examined by combining their flocculation effects on the latex dispersion. As the colloidal dispersion, several polystyrene latices of different polarities were used to suppress the effects of electrostatic interaction. The main object in this work is to clarify the following problems:

a) What is the principal mechanism of destabilization (or stabilization) in the present system?

b) How does the helix-coil transition influence the adsorption phenomena?

c) How does the destabilization (or stabilization) appear in the different conformations of the polymers?

Working with well-defined latices of different characters as adsorbents will assist in gaining a better understanding of these ends.

Experimental

Materials. To emphasize the polarity of the substrate, three kinds of polystyrene latices were employed. Latex-A was prepared by the usual Kotera-Furusawa-Takeda method.[6] Latex-B was made by incorporating a small amount of an ionic comonomer, sodium *p*-vinylbenzeulsulfonate, into a polystyrene chain according to Juang *et al.*[7] The charge density of Latex-B is much higher than that of Latex-A. Latex-C consists of amphoteric lat prepared by the method described by Homola *et al.*[8] The characterization data for these samples are shown in Table 1. All the latices were prepared by the use of an emulsifier-free system to reduce the spurious effects of surface-active substance. Also, the use of buffers has been avoided in order to obtain a clean system.

Table 1. Characterization data of polystyrene lattices

Sample	Diameter (2a)	Surface charge σ_0	ζ-Potential/mV (25 °C, 10^{-3} M KCl)	
	A	μC cm^{-2}	pH 4.5	pH 10
Later-A	5260	3.1	−39	−42
Latex-B	1600	9.0	−67	−70
Latex-C	1950	—	+38	−17

The hydrobromide of PLL was purchased from the Sigma Chemical Co. Ltd. (U. S. A.) and had the reported molecular weights of 4000, 32500, and 57500 respectively. The hydrobromide form was converted to the hydrochloride form by dissolving the PLL in distilled water and by dialyzing them exhaustively against 0.01 M HCl (1 M = 1 mol dm^{-3}) and then against distilled water to remove the excess chloride ions.

The sodium salt of PLG was obtained from the Ajinomoto Co., Ltd. (Yokohama, Japan); the molecular weight was determined by the supplier to be 42000 from viscosity measurements.

The other reagents were commercially available and of an analytical grade. All the solutions of these materials were made with deionized and distilled water, using an all-Pyrex apparatus.

Electrophoresis. The electrophoretic mobilities of the bare latex particles were determined at various pH values in an aqueous solution of 1×10^{-3} M KCl at 25 °C. The measurements were performed in a Rank Brothers Microelectrophoresis apparatus (MK-2) using a rectangular glass cell. The ζ-potential was derived following the method of Wiersema *et al.*[9] Polymer-coated particles for electrophoretic measurements were prepared as follows: A dilute latex suspension (*ca.* 0.01 wt%) controlled at a definite pH in 10^{-3} M KCl was mixed immediately with an equal volume of the polypeptide solution ($C_p=0.2$ mg/dm^{-6}) which gave enough plateau adsorption. After the mixture had been left standing for 2 h at room temperature, the electrophoretic mobilities of the polymer-coated particles were determined by using the same technique with bare particles. The results for Latex-A

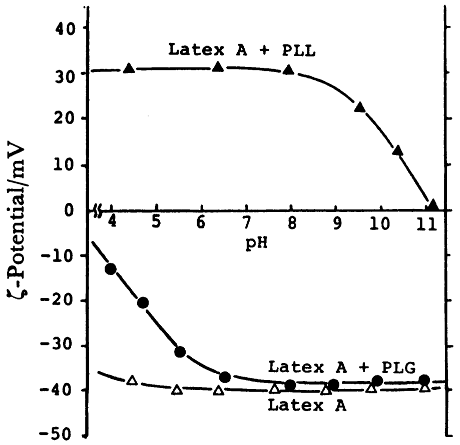

Fig. 1. ζ-Potentials of Latex-A (—△—), PLL-covered Latex-A(—▲—), and PLG-covered Latex-A(—●—) ($M_{PLL}=57500$, $M_{PLG}=42000$, 10^{-3} M KCl, 25 °C).

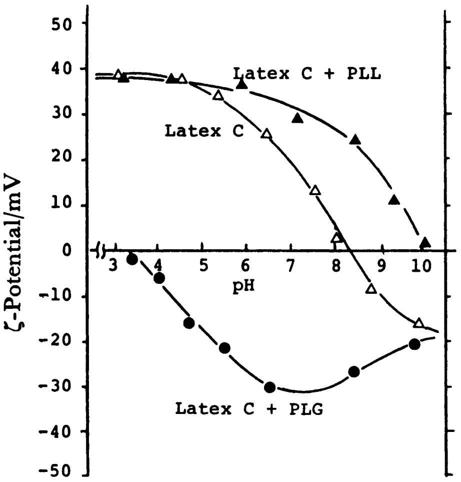

Fig. 2. ζ-Potentials of Latex-C (—△—), PLL-covered Latex-C (—▲—), and PLG-covered Latex-C (—●—) ($M_{PLL}=57500$, $M_{PLG}=42000$, 10^{-3} M KCl, 25 °C).

and -C covered by PLL and PLG are shown in Figs. 1 and 2 respectively, along with the data for the respective bare particles.

Adsorption Experiments. In glass-stoppered vials (5 ml), 2 ml of a latex suspension was added to 2 ml of the polypeptide solution. Prior to mixing, both the solutions were brought to the same pH and KCl concentration without using any buffers. The vials were rotated end-over-end for 5 h. The suspension was then centrifuged for 30 min at 25000 g. The concentration of polypeptide in the supernatant as well as in the initial solution was determined by analytical methods, *i.e.*, by the Ninhydrin method for L-lysine and PLL and by the Microbiuret method for PLG. From the difference in the concentrations before and after the adsorption process, the amounts of adsorption per unit of surface area (A_p) was calculated. All the measurements were made at a constant temperature of 25 °C.

Flocculation Experiments. In the flocculation experiments using polyelectrolyte as a flocculant, careful attention must be paid to the method of mixing the polymer solution and the colloid dispersion, because the adsorption of polyelectrolyte on a charged surface is usually very rapid and irreversible.[10] Here, the mixing was performed as follows: Five-ml portions of KCl solutions of various concentrations were added carefully on top of a latex suspension ($C_{solid}=0.4$ wt%, 2 ml) in such a way that a sharp boundary between the dispersion and the salt solution was formed. Thereafter, on top of these, 2-ml portions of polymer solutions of different contents were added using a pipet. After rotating end-over-end (10 min^{-1}) for 2 h, the system was left to stand for 20 h in order to allow the flocculated particles to settle out. Then, the extinction of supernatant was measured using a Jasco Digital Spectrophotometer (Uvidec-410) at a wavelength of 550 nm. The critical flocculation concentration (CFC) was defined as the salt concentration at which the absorbance of the supernatant was reduced to 50% of the original. The ionic strength was varied using KCl, while all the experiments were carried out at a constant temperature of 25 °C.

Direct Observation of Flocculating States. The flocculating states of latex particles were examined visually by means of a direct-observation technique.[11] The optical microscope used was a lateral-type metallurgical microscope with a magnification of 1500, Type-Mg, Olympus Optical Co., Ltd. (Tokyo, Japan). Some amounts of the latex sample prepared by the same procedure as were used in the flocculation experiments were charged into the observation cell, made of a Pyrex glass tube, 100 mm high and 15 mm in diameter, with a thin glass window at its lower end. The sample was observed through the window by means of the oil-immersion method.

Results and Discussion

Adsorption Behavior. Figure 3 shows the adsorption isotherms of L-lysine on Latex-A, -B, and -C at different pH values in a 10^{-3} M KCl solution. In all the systems, the isotherms have low affinities, with small plateaus. In the acidic medium, Latex-A and -B have electrical signs opposite that of the adsorbate, but Latex-C has the same sign. As may be seen in the figure, the experimental results show the same behavior as would be anticipated from the estimation of the elec-

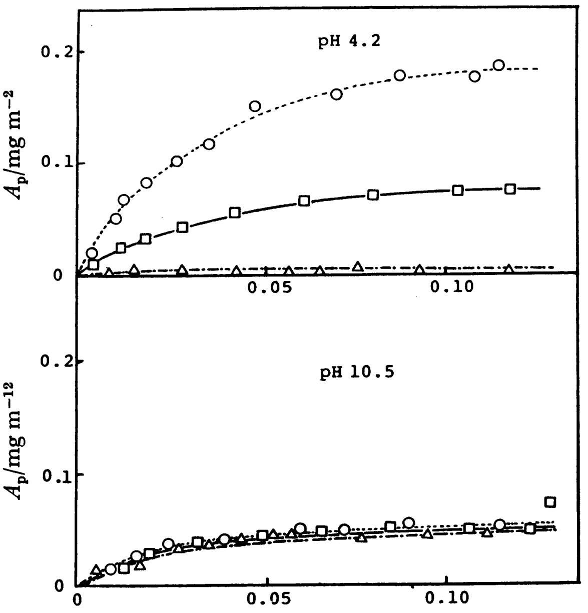

Fig. 3. Adsorption isotherms of L-lysine on Latex-A (—□—), Latex-B (—○—), and Latex-C (—△—) (10^{-3} M KCl, 25 °C).

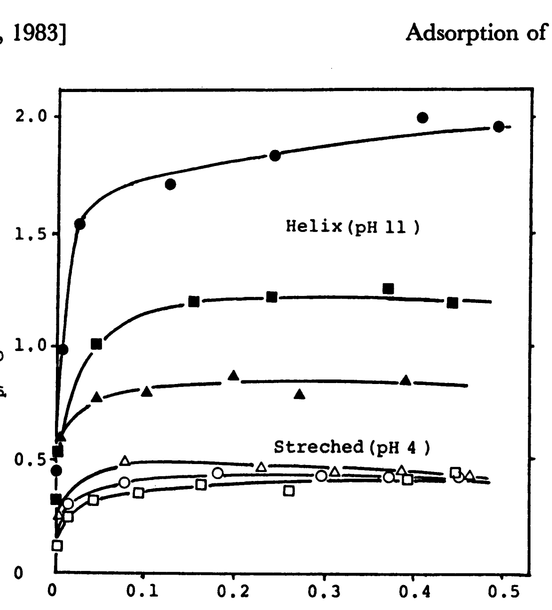

Fig. 4. Adsorption isotherms of PLL on Latex-B. Open symbols; pH 4, filled symbols; pH 11, —○—; M_{PLL}=57500, —□—; M_{PLL}=32500, —△—; M_{PLL}=4000 (10^{-3} M KCl, 25 °C).

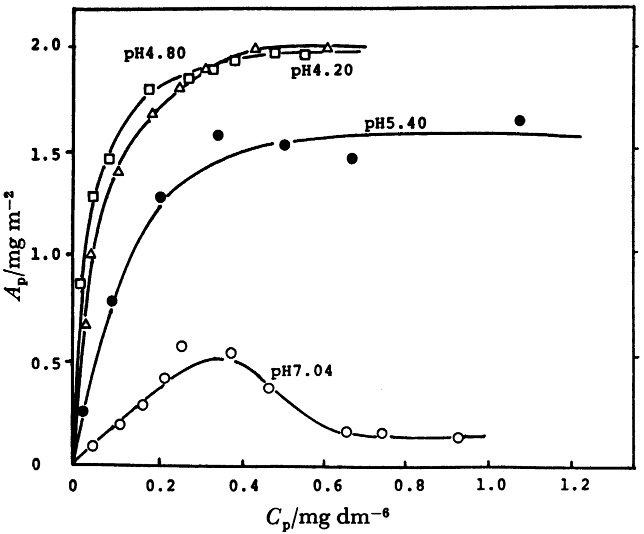

Fig. 5. Adsorption isotherms of PLG on Latex-C (M_{PLG}=42000, 10^{-3} M KCl 25 °C).

trostatic interaction: *i.e.*, Latex-C gives no adsorption, and -A and -B, 0.06_8 mg/m^2 and 0.18 mg/m^2 respectively, values which accord with the calculated values (0.06_4 mg/m^2, 0.18_6 mg/m^2), assuming an equimolar reaction between $-OSO_3^-$ on the latex surface and NH_3^+ in the peptide. In the basic condition (pH 10.5), however, all the latices have a nearly constant plateau (*ca.* 0.05 mg/m^2) which would seem to be based on the deionization of the lysine molecule in the alkaline solution.

As may be seen in Fig. 4, the adsorption isotherms of PLL have a high affinity, with a well-defined plateau. In the acidic solution, the plateau is independent of the molecular weight, which indicates the adsorption in a flat conformation, *i.e.*, with trains only. According to a theoretical study of the molecular model of polypeptide,[12] it may be possible for PLL to take two kinds of conformation in extreme cases: *i.e.*, A fully extended

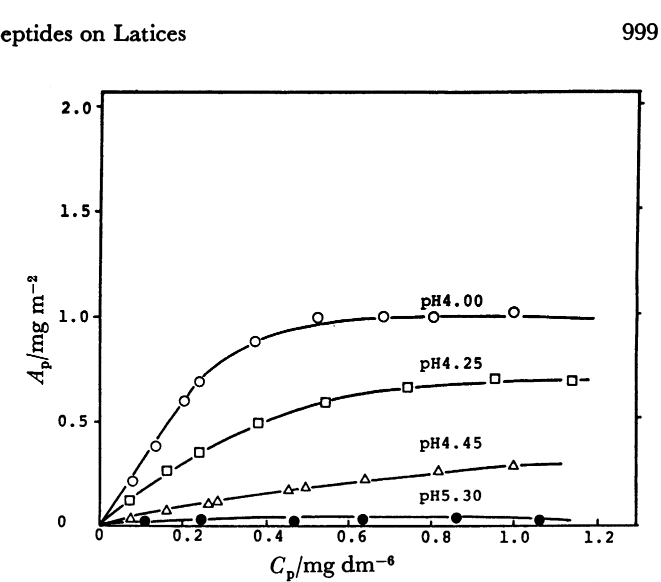

Fig. 6. Adsorption isotherms of PLG on Latex-B (M_{PLG}=42000, 10^{-3} M KCl 25 °C).

conformation, and a compact one. Using these molecular dimensions, the maximum monolayer coverages for these two configurations would amount to 0.41 mg/m^2 and 0.61 mg/m^2 respectively. The plateau value (0.48 mg/m^2) obtained by the present experiments in the acidic medium suggests that PLL molecules would be adsorbed by an intermediate form of these extreme conformations. According to the electrophoretic measurements of the PLL-coated particles (which can be estimated from Fig. 1 for Latex-A), a charge reversal of the original latex particles has occurred at the saturated PLL adsorption (the same result has been reported by Corry[13]). It is indicated that a more positive charge due to PLL than the negative charge on the latices is accumulated on the surface. Though such a superequivalent adsorption, which indicates that the chain effect of the adsorbed polymer overcomes the electrostatic instability, has been observed frequently in polyelectrolyte-colloid systems, the occurrence in the present PLL adsorption with trains only is very interesting as showing a new singularity of polymer adsorption.

The PLL adsorption in the basic medium, however, shows a specific nature. According to the double-layer capacitance measurements on charged silver iodide surfaces in the presence of various oligo- and polypeptide molecules, the helical form in the solution of PLL persists in the adsorbed state.[12] Therefore, the increased plateau values at pH 11 in Fig. 4 may be recognized to be based on the stiff structure of the helical conformation of PLL. When stiff rods of a helical form adsorb on a solid surface, they can either lie flat on the surface or stand on end. The resulting molecular-weight dependence of the plateau adsorption, as may be seen in Fig. 4, suggests that they are adsorbed vertically on the ends of the rods. This is surprising: A detailed analysis of this point will be discussed in the next paper of this series.

Figures 5 and 6 show examples of the adsorption isotherm of PLG on Latex-B and -C. In Fig. 5, in which adsorbate and adsorbent have opposite electric charges at pH values lower than 8.5, the isotherms again exhibit a high plateau with a steep initial slope, but, as a special feature, an additional reduction of A_p is found at pH 7.04. This reduction is probably due to a partial flocculation of the latex particles: *i.e.*, Under these conditions, the latex particles used as adsorbates become unstable and lose their apparent adsorbable areas. In contradistinction to Fig. 5, the isotherms of Fig. 6 (PLG–Latex-B system) are of a low-affinity type, and the plateaus are much influenced by the pH value. Such behavior may be explained as a consequence of coulombic interaction and the helix-coil transition of PLG. The importance of the coulombic interaction and the helix-coil transition in the polypeptide adsorption can be illustrated by plotting the plateau adsorption (A_{pm}) as a function of the pH value in the medium. Figure 7 shows the A_{pm}(pH) curves for four systems, *i.e.*, PLL–Latex-B, PLL–Latex-C, PLG–Latex-B, and PLG–Latex-C. We can realize that the A_{pm}(pH) curves for PLL–Latex-B and PLG–Latex-C are related symmetrically to the vertical line at pH 7.5. The same trend is also found in the A_{pm}(pH) curves for PLL-Latex-C and PLG–Latex-B. These two trends show that A_{pm} is, to a large extent, determined by the coulombic interaction between the polypeptide and the charged surface. Especially, the first trend indicates that the helical structures of both the polypeptides exert the same influence on the adsorption behavior under the relevant conditions.

Flocculation Behavior. The stability of polymer-coated particles and the conformation of the adsorbed polymer are closely related. By this sense, the study of the stability of polymer-coated particles is instructive with regard to the conformation of the adsorbed polymer. In Fig. 8, the dependence of the critical flocculation concentration (CFC) on the PLL dosage is given for two medium conditions, *i.e.*, the streched-induced conditions (pH 4) and the helix-induced conditions (pH 11). In pH 4, a sharp decrease in the CFC is observed, after which the CFC increases slowly again. Thereafter, the CFC is kept at a constant value (*ca.* 200 mmol/dm^3 of KCl), which is rather lower than the CFC for the bare particles of Latex-B. It is indicative that at pH 4, the adsorption layer of PLL does not confer any steric stabilization on the particles, but brings about the charge reversal in the diffuse double-layer which is shown in Fig. 1. Furthermore, it is known that the narrow flocculation range seen in Fig. 8 does not correspond to the polymer concentration for making an effective interparticle bridging, but does correspond to the charge neutralization performed by the adsorption of PLL with the opposite electrical charge. On the other hand, the CFC in the helix-induced pH stays at a low value for a long period after decreasing slowly from the CFC of bare particles. This phenomena seems to be due to the adsorption of uncharged helix molecules, while the broad range of polymer dosage for flocculation corresponds to the high capacity of polymer adsorption under those conditions (see Fig. 4). Also, the appearance of strong stabilization in the high-polymer dosage may be explained as a result of the thicker adsorption layer. The helical rods of PLL are adsorbed vertically on the latex surface, and their denser (and thicker) adsorption layer throughly resist direct contact with the latex particles.

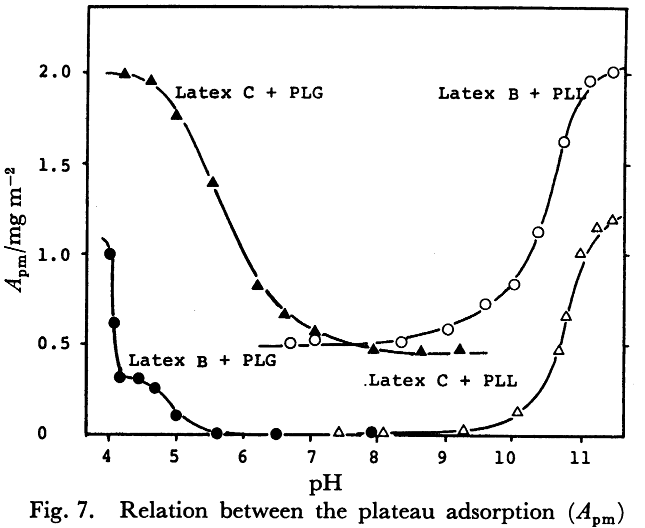

Fig. 7. Relation between the plateau adsorption (A_{pm}) and the medium pH (M_{PLL}=57500, M_{PLG}=42000, 10^{-3} M KCl, 25 °C).

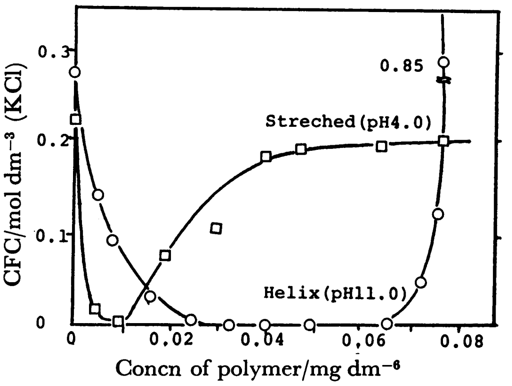

Fig. 8. Dependence of the CFC on the polymer concentration (Polymer; M_{PLL}=57500, Colloid; Latex-B, 25 °C).

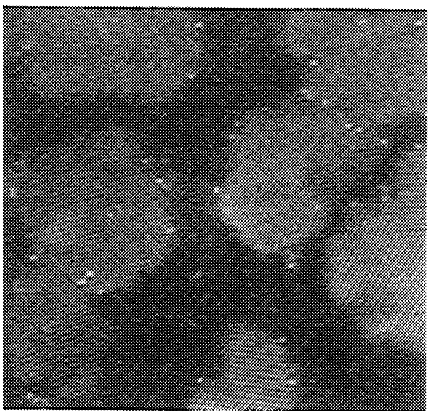

Fig. 9. Microphotograph showing latex aggregates with ordered structure (Polymer; M_{PLL}=57500, Colloid; Latex-A).

Formation of Aggregates With an Ordered Structure. Usual latex sediments appear milky white and clusters with an irregular structure are visible under an optical microscope. Under some conditions, however, especially in concentrated PLL solutions, some latex sediment with a brilliant iridescence is formed. It has been shown that such latex sediments are built up by the large aggregates with an ordered structure.[14)] As may be seen in Fig. 9, the particles in the aggregates are arranged in a hexagonal array and seem to be in face-to-face contact. On the formation of such aggregates in the PLL–Latex-A system, the following special features are found:

a) Ordered aggregates are formed only at pH values lower than 8, which are the stretched or random coil-induced conditions for the PLL.

b) In a 10^{-3} M KCl solution, the aggregates were formed in the concentration range of PLL from 0.1—0.6 mg/dm^{-6}. In order to produce the same aggregates in the more concentrated solution of KCl, we must prepare the aggregates in more concentrated PLL solutions.

c) The ordered aggregates were also observed in the system of PLG–Latex-C. In this combination, however, the aggregating capacity was very limitted.

Though, from these results, only a very little can be said about the mechanism of this phenomena, the appearance of such characteristic aggregates suggests that some attractive interaction (perhaps some bridging attraction) is working between the particles covered by the flat adsorption layer of polypeptide.

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