molecular weight. For star-branched polymers, values of Ψ increase with the number of branches.[2] Also, in combs values of Ψ are substantially higher than for linear polymers.[3] The values of Ψ for the H-polymers are given in Table II. As expected from the branch structure, the average value, 0.43, is somewhat smaller than 0.53 observed in four-arm polystyrenes.[2]

The intrinsic viscosities of the H-polystyrenes are compared with those of linear polystyrenes in Figure 5. The ratio of the intrinsic viscosity of an H-polymer to that of the linear homologue, $g' = [\eta]_{br}/[\eta]_{lin}$, is 0.80 in cyclohexane and 0.73 in toluene, based on $[\eta]_{lin} = 8.3 \times 10^{-4} M_w^{0.5}$ for polystyrene in cyclohexane and $[\eta]_{lin} = 1.02 \times 10^{-4} M_w^{0.73}$ in toluene.[10]

In a relation between g' and g_{th} of the form

$$g' = g_{th}^{m}$$

$m = 0.65$ and 0.92 for the Θ-solvent and toluene data, respectively. These values of m for the H polystyrenes are different from the proposed approximation $m = {}^1/_2$.[19] It was observed that for star polymers in a Θ solvent $m = 0.58$ and this seems also the lower limit for starlike comb polymers with many long branches.[3] The values of m for the H-shaped polymers fall between that of the regular star polymers and that of heterogeneous combs with many branches, for which $m \approx 1$ at $g_{th} = 0.712$.[3] In general, m depends on the structure of the branch polymer as well as on the value of its g_{th}. Notice also that $g'_{Tol} < g'_{\Theta}$ observed for the H-polystyrenes was also found in star polystyrenes.[10,16]

In Figure 6 the molecular weight is plotted logarithmically against the maximum in the elution volume (V_e). Based on the universal calibration concept $([\eta]M_w)_{V_e}$ = constant[9]

$$[(M_w)_{lin}/(M_w)_{br}]_{V_e}^{1+a} = g'_{GPC}$$

Since THF is an equally good solvent for polystyrene as toluene, $a = 0.73$ is taken.[20] In the case of the H-polystyrenes $g'_{GPC} = 0.88 \pm 0.02$. This *apparent* ratio of intrinsic viscosities is quite different from that obtained in the good solvent toluene. Similar $g'_{GPC} > g'$ have also been observed for star-branched polymers when toluene was the eluting solvent,[11] and it was found not to be caused by different V_e–concentration dependences for linear and lightly branched polymers. Caution seems therefore required when deducing the polymer branch structure from GPC elution volumes with the aid of the universal calibration principle.

In conclusion, it has been been shown that H-shaped polystyrenes of good quality can be prepared. Their dilute-solution properties resemble those of star-branched polymers. We hope to report on the rheological properties of the H-polystyrenes in another paper.

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Salt Linkage Formation of Poly(diallyldimethylammonium chloride) with Acidic Groups in the Polyion Complex between Human Carboxyhemoglobin and Potassium Poly(vinyl alcohol) Sulfate

Etsuo Kokufuta,* Hidetoshi Shimizu, and Isei Nakamura

Institute of Applied Biochemistry, The University of Tsukuba, Sakura-mura, Niihari-gun, Ibaraki 305, Japan. *Received February 2, 1981*

ABSTRACT: The salt linkage formation of poly(diallyldimethylammonium chloride) (PDDA) with the acidic groups (carboxyl, mercapto, and phenolic OH groups) in the polyion complex formed stoichiometrically from human carboxyhemoglobin (Hb) and potassium poly(vinyl alcohol) sulfate (KPVS) was investigated by means of colloid titration. The carboxyl group stoichiometrically forms a salt linkage with PDDA ion in the neighborhood of pH 7.7. In the pH range above 12, the PDDA ion binds not only to all of the acidic groups but also to the $^-OSO_3$ group which results from the cleavage of the salt linkage between the basic groups and KPVS ions in the KPVS–Hb complex.

Colloid titration is a useful method for obtaining information about the stoichiometry of the salt linkage formation between the ionizable groups in oppositely charged polyelectrolytes.[1,2] Previously we studied the colloid titration of human carboxyhemoglobin (Hb) with potassium poly(vinyl alcohol) sulfate (KPVS) and reported that three kinds of basic groups in Hb, i.e., amino, imidazolyl, and guanidyl groups, stoichiometrically form salt

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linkages with KPVS ion.[3] After the complexation of Hb with KPVS, the acidic groups (carboxyl, phenolic OH, and mercapto groups) in the Hb component still remain in the complex. Thus it is of interest to investigate the salt linkage formations of three acidic groups with polybase in connection with the preparation of three-component polyion complexes of protein with acidic and basic polyelectrolytes.

In the present study, the salt linkage formation of poly(diallyldimethylammonium chloride) (PDDA) with the acidic groups in the KPVS-Hb complex was investigated at different pHs by means of colloid titration.

Experimental Section

Materials. Hb, KPVS, and PDDA were the same samples as used previously.[2-6] The equivalent weights for KPVS[4] and PDDA[5,6] were 166 and 158, respectively. The KPVS-Hb complex was prepared at pH 2.8, where the complexation follows a stoichiometric relationship,[3] washed with a 7:3 methanol-0.1 N HCl mixture, and dried at 50 °C for 1 week under reduced pressure. The contents of sulfur and iron in the complex are 1.38 mmol/g and 52.6 μmol/g, respectively. These values are in agreement with those (S, 1.43 mmol/g; Fe, 51.9 μmol/g) calculated by assuming a stoichiometric complexation between KPVS and Hb.

Colloid Titration. The titration was carried out at 25 ± 0.1 °C in a nitrogen atmosphere, using a Hirama automatic recording titrator. The KPVS-Hb complex (2-10 mg) was dissolved in 50 mL of 0.01 N NaOH, and the pH of the solution was adjusted by slow addition of 0.1-1 N HCl. The sample was titrated with 0.005 13 N PDDA solution adjusted to the pH of the sample solution.

The titration with KPVS solution was carried out for KPVS-Hb-PDDA complex to identify the basic groups which are isolated from the KPVS-Hb complex during the complexation with PDDA ion. The KPVS-Hb-PDDA complex was prepared by the titration of the KPVS-Hb complex with PDDA at pH 12.3, and the resulting suspension was acidified with 1 N HCl to dissolve the complex. The sample solution was titrated with 0.002 49 N KPVS solution at pH 3.1 and 6.1. The end point of the titration was always indicated by the measurement of turbidity at 720 nm.

Results and Discussion

A precipitate or turbidity developed when PDDA was mixed with the alkaline solution of KPVS-Hb complex. This indicates the formation of a three-component complex caused by the salt linkages of the residual acidic groups in the KPVS-Hb complex with PDDA ion, although Hb-PDDA complex was not formed even if the complexation was carried out in the basic region, where the acidic groups in Hb are dissociated completely.[3] Salt linkage formation was supported by the fact that the IR absorption at 1720 cm^{-1}, assigned to the carboxyl group in the KPVS-Hb complex, is not observed in the spectra of the KPVS-Hb-PDDA complexes prepared by titrating the KPVS-Hb complex with PDDA until the end point at pH 7.7 and 12.5, separating by centrifugation, and washing with a 7:3 mixture of methanol and 0.1 N HCl.[7]

In Figure 1, the relationship between the PDDA volume at the end point of the titration and the weight of KPVS-Hb complex is shown as function of pH. It is found that plots of the PDDA volume against the complex weight are straight lines passing through the origin. This shows that KPVS-Hb complex and PDDA form quantitatively three-component polyion complex.

Accurate information on the electrochemical properties of PDDA ion is required to calculate the mole number (M_s) of the quaternary ammonium ion which is bound by the salt linkages to the acidic groups in 1 g of KPVS-Hb complex. Electrophoretic studies[5,6] showed that the dissociation of PDDA ion is kept constant at pHs below 5 and above 8 but varies slightly in the neutral region. This

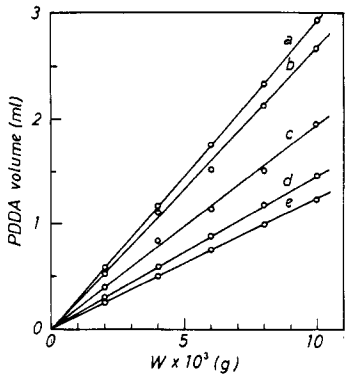

Figure 1. Linear relationship between the volume of 0.005 13 N PDDA and the weight (W) of KPVS-Hb complex at different pHs: (a) pH 13.0; (b) pH 10.5; (c) pH 8.9; (d) pH 7.1; (e) pH 5.5. W is in grams of the complex in 50 mL of sample solution.

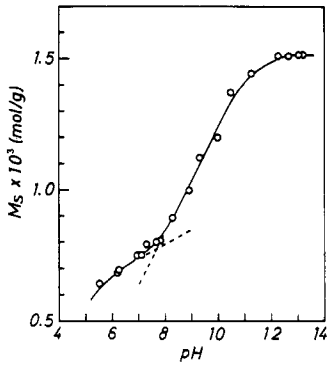

Figure 2. Colloid titration curve of KPVS-Hb complex with 0.005 13 N PDDA solution.

dissociational change can be attributed to incomplete quaternization of the nitrogen in PDDA.[5,6] However, this amount is less than 1-2% of the total nitrogen, as estimated by conductometric titration. Therefore, the pH change in the molarity of the PDDA solution is negligible within experimental error. Figure 2 shows the colloid titration curve obtained by plotting M_s against pH. The M_s value was estimated by means of the slope of the straight line in Figure 1. The titration curve shows an inflection at pH 7.7, a rapid increase in the pH range 8-11, and a constant value in the pH range above 12.

The amino acid sequence of human hemoglobin is available in the literature.[8,9] Therefore the number of acidic groups in the hemoglobin can be obtained from the amino acid composition: 58 for carboxyl groups in aspartyl, glutamyl, and C-terminal residues, 12 for phenolic OH groups in tyrosyl residues, and 6 for mercapto groups in cysteinyl residues. The contents of the acidic groups in 1 g of the stoichiometric KPVS-Hb complex are further calculated from the number of acidic groups in Hb and the composition[10] of the complex: 0.75 mmol for carboxyl groups, 0.16 mmol for phenolic OH groups, and 0.08 mmol for mercapto groups. From the titration curve in Figure 2, it is found that the M_s value (about 0.78 mmol/g) at the inflection point is comparable to the carboxyl group content. Taking into account the results of IR analyses mentioned above, this finding indicates that the carboxyl group in the KPVS-Hb complex stoichiometrically forms a salt linkage with PDDA ion in the neighborhood of pH 7.7. This result can be supported by the fact that the contents of sulfur (1.40 mmol/g) and iron (46.9 μmol/g) in KPVS-Hb-PDDA complex agree approximately with those (S, 1.31 mmol/g; Fe, 47.6 μmol/g) calculated by assuming a stoichiometric salt linkage between the car-

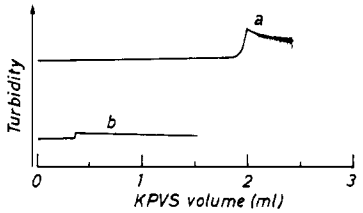

Figure 3. Turbidimetric titration data of KPVS–Hb–PDDA complex with 0.002 49 N KPVS at (a) pH 3.1 and (b) pH 6.1. The sample solution (52.63 mL) contains the three-component complex prepared by the titration of 8.74 mg of KPVS–Hb complex with 0.005 13 N PDDA solution (2.63 mL) at pH 12.3. The titration curve was not represented by absolute turbidity because the change in turbidity was measured with an automatic recording titrator.

boxyl group and PDDA ion.

The increase of M_s in the pH range above the inflection point can be related to the salt linkage of PDDA ion with the phenolic OH and mercapto groups which are ionized in the basic region. However, the M_s value (1.51 mmol/g) observed at pHs above 12 is larger than the total contents (0.99 mmol/g) of the acidic groups in the KPVS–Hb complex. This contradiction might be avoided if we assume that the salt linkage between the $^-OSO_3$ and basic groups in the KPVS–Hb complex is severed during the course of the complexation with PDDA ion in the basic region and that the isolated $^-OSO_3$ group in the KPVS component forms a new salt linkage with PDDA ion. In order to confirm this assumption, the colloid titration with KPVS titrant was carried out for the KPVS–Hb–PDDA complex prepared at pH 12.3. From the turbidimetric titration curve shown in Figure 3, it is observed that the titrant volume at pH 3.1 is larger than that at pH 6.1. This could indicate the existence of a free basic group[11] in the KPVS–Hb–PDDA complex which results from the cleavage of the salt linkage between the KPVS and Hb components during the complexation with PDDA ion.

On the basis of the results obtained here and reported previously,[3] it is found that a three-component polyion complex can be prepared by the salt linkage formations of the acidic and basic groups in Hb with KPVS and PDDA ions if the complexation is carried at an appropriate pH. In the basic region, however, the cleavage of the salt linkage between the $^-OSO_3$ and basic groups in the KPVS–Hb complex is observed in the process of the complexation with PDDA ions. Thus the salt linkage in the KPVS–Hb (or KPVS–Hb–PDDA) complex can be regarded as relatively loose. This could be due to the fact that the Hb component is polyampholite and also the ionizable groups are irregularly located in the α- and β-globin chains.

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(11) The amount of basic groups isolated from 1 g of KPVS–Hb complex is 0.57 and 0.52 mmol, as estimated by KPVS volume at pH 3.1 (Figure 3) and from the difference between the M_s value at pH >12 and the total acidic group content of KPVS–Hb complex (Figure 2), respectively. These results seem to agree approximately with the content (0.49 mmol) of imidazolyl group in 1 g of KPVS–Hb complex.

Mechanistic Aspects of Selective Formation of 10-, 20-, and 25-Membered Macrocyclic Oligoesters in the Cationic Polymerization of 6,8-Dioxabicyclo[3.2.1]octan-7-one

Ichiro Tajima, Masahiko Okada,* and Hiroshi Sumitomo

Faculty of Agriculture, Nagoya University, Chikusa, Nagoya 464, Japan.
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ABSTRACT: Mechanisms of the selective formation of 10-, 20-, and 25-membered macrocyclic oligoesters (cyclic dimer, tetramer, and pentamer, respectively) from 6,8-dioxabicyclo[3.2.1]octan-7-one (**1**) are discussed on the basis of product distributions in the oligomerization of racemic monomer **1**, optically active (+)-(1*R*,5*R*)-6,8-dioxabicyclo[3.2.1]octan-7-one (**1R**), and enantiomerically unbalanced monomer mixtures. Optically active cyclic tetramer (**4R**) and cyclic pentamer (**5R**) of **1R** are predominantly formed by a tail-biting reaction of a growing oligomer chain, while racemic cyclic tetramer (**4**) and cyclic pentamer (**5**) are mainly produced by a back-biting reaction of an initially formed polymer of **1**. Cyclic dimer (**2**) is formed primarily by intramolecular reaction of unsymmetrical oligomers which are formed from **4** and **5** by the reaction with monomer. All these macrocyclic oligomers are formed via an S_N2-type mechanism involving the exclusive alkyl-oxygen fission of **1**. The selective formation of **2**, **4**, **5**, **4R**, and **5R** is remarkably dependent upon the reaction conditions, especially temperature, time, solvent, and optical purity of the monomer. Solubility and molecular symmetry of cyclic oligomers, interactions between a cyclic oligomer and its opposite enantiomer or a solvent molecule, and conformation of a growing chain are important factors controlling the selective formation of the cyclic oligomers of specific ring sizes.

It is not an unusual but rather common phenomenon that cyclic oligomers of various ring sizes are formed in the cationic ring-opening polymerization of a variety of cyclic monomers.[1] These cyclic oligomers are often in equilib-

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