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Stoichiometric Formation of Poly-ion Complexes Between Human Carboxyhemoglobin and Potassium Poly(Vinyl Alcohol) Sulfate

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Summary

Colloid titrations of human carboxyhemoglobin were carried out at different pH. The total number of basic groups in the hemoglobin was 95, as evaluated by the titration with potassium poly(vinyl alcohol) sulfate (KPVS). The value estimated was comparable with that obtained from the amino acid sequence of human hemoglobin. This finding indicates the stoichiometric salt-linkage formation between the basic groups in the hemoglobin and the $KOSO_3$-groups in KPVS.

Introduction

Colloid titration, which is based on the formation reaction of poly-ion complexes between polyacidic and polybasic ions, is an analytical method for the determination of ionizable groups in polyelectrolytes. We recently demonstrated that the colloid titration curve gives information about the stoichiometry of the salt-linkage formation between the ionizable groups in polyacids and polybases (KOKUFUTA 1979; KOKUFUTA and IWAI 1977). It is now of interest to investigate the colloid titration behavior of protein in order to obtain information about the salt-linkage formation between ionizable groups in the protein and in synthetic polyelectrolytes. For that purpose human hemoglobin was chosen in view of the available information on the amino acid sequence (HILL et al. 1962; BRAUNITZER et al. 1961; KONIGSBERG et al. 1961) and the structure (MUIRHEAD and PERUTZ 1963).

Materials and Methods

The human carboxyhemoglobin was obtained from ICN pharmaceuticals, Inc. The sample was purified in the same manner as described in the literature (HILL et al. 1962). The purity was confirmed by the analyses of the iron content and the amino acid composition. Potassium poly(vinyl alcohol) sulfate (KPVS), trimethylammonium glycol chitosan iodide (TGCI) and poly(diallyl-dimethyl-

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ammonium chloride)(PDDA) were used as the standard titrant of colloid titration. The physical properties of these polyelectrolytes were already characterized by viscometric measurements and electrophoresis (KOKUFUTA et al. 1976 and 1975).

The colloid titration was carried out at 25 ± 0.1 °C under nitrogen using a Hirama Automatic Recording Titrator. The human carboxyhemoglobin (2-10 mg) was dissolved in 50 ml of distilled water, and then the pH of the solution was adjusted with 0.1-1 N HCl or NaOH. The sample was titrated with standard titrant adjusted to the pH of the sample solution. The end point was determined by the measurements of turbidity at 720 nm and of conductivity.

Results and Discussion

Typical examples of turbidimetric and conductometric titrations with KPVS are represented in Fig. 1. As the titration proceeds, the sample solution begins to become turbid. At the end point of titration, the turbidimetric and conductometric curves show a pronounced inflection, indicating the formation of the poly-ion complex. In the cases of the titration with TGCI and PDDA, however, neither the turbidimetric nor the conductometric curves show the characteristic pattern as exhibited in Fig. 1. From these reslts, it is found that TGCI and PDDA do not form the poly-ion complex with hemoglobin.

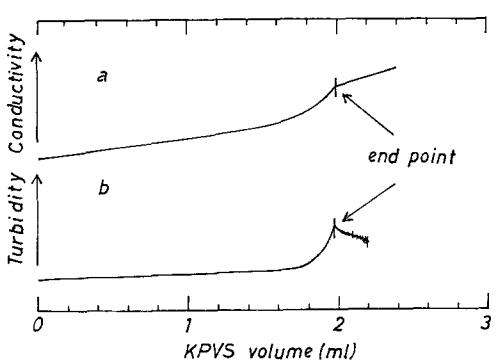

Fig. 1. Titration curves of human carboxyhemoglobin with KPVS titrant. Fifty milliliters of the sample solution containing 3.95 mg of the hemoglobin was titrated with 0.002495 N KPVS. The pH of the titration system was adjusted to 3.48. The end point was determined by the methods of conductivity (a) and turbidity (b).

The formation of the poly-ion complex between KPVS and human carboxyhemoglobin was also investigated by the measurement of the infrared spectrum. The infrared spectrum of human carboxyhemoglobin in KBr disk shows absorptions at 1640 and 1420 cm^{-1} which are assigned to carboxylate ions, because of inter and/or intra-molecular salt-linkage between carboxy groups and basic (amino, imidazolyl and guanidyl) groups in the hemoglobin. On the other hand, the complex, which was separated from the solution titrated until the end point with KPVS, has an absorption at 1720 cm^{-1} assigned to carboxy groups. The appearance of carboxy groups in the complex indicates that the complex formation is due to the salt-linkage formation between basic groups in the hemoglobin and $KOSO_3$-groups in KPVS.

In order to obtain information about the stoichiometry of the salt-linkage formation mentioned above, the number of basic groups in human carboxyhemoglobin was investigated by colloid titration with KPVS. The dependence of the KPVS volume on the hemoglobin weight was obtained at various pH (Fig. 2). It is observed that the plots of the KPVS volume against the hemoglobin weight are expressed by straight lines passing through the origin. This shows that the hemoglobin and KPVS form quantitatively the poly-ion complex. The equivalent weight (Ew), which is represented by the weight of human carboxyhemoglobin per one mole of basic groups in the hemoglobin, can be estimated by means of the slope of the straight line as shown in Fig. 2. From the electrophoretic study of KPVS in the precious paper (KOKUFUTA et al. 1975), it was confirmed that the dissociation of protons from $^{\ominus}OSO_3$-groups in KPVS is independent of pH. Therefore, the reciprocal of Ew represents the number of basic groups per one gram of human carboxyhemoglobin, and the change of 1/Ew with pH is related to the change in dissociation of basic groups in the hemoglobin. To determine the pH region where basic groups in the hemoglobin are protonated completely, the value of 1/Ew was expressed as a function of pH (Fig. 3). From the result shown in Fig. 3, it is found that the basic groups in human carboxyhemoglobin are protonated in the pH region from 6 to 3, and the protonation is complete below pH 2.5. This dissociation behavior is similar to that of horse carboxyhemoglobin (COHN et al. 1937) evaluated by potentiometric titration. The number of basic groups in human carboxyhemoglobin can be estimated to be 95, as calculated by dividing the molecular weight (64,503) of the hemoglobin by the Ew value (679) observed in the pH region of 2-2.5. Here, the molecular weight was obtained from the amino acid sequence of human hemoglobin (HILL et al. 1962; BRAUNITZER et al. 1961; KONIGSBERG et al. 1961). The number of basic groups in human hemoglobin can also be obtained from the amino

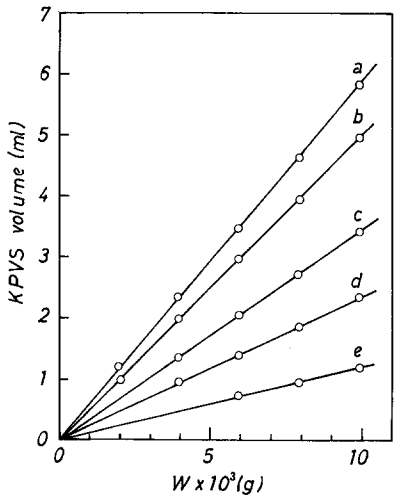

Fig. 2. Linear relationship between the KPVS volume and the hemoglobin weight (W) at different pH (a, 2.12; b, 3.48; c, 4.12 d, 4.71 and e, 5.79). W was represented by grams of human carboxyhemoglobin in 50 ml of sample solution.

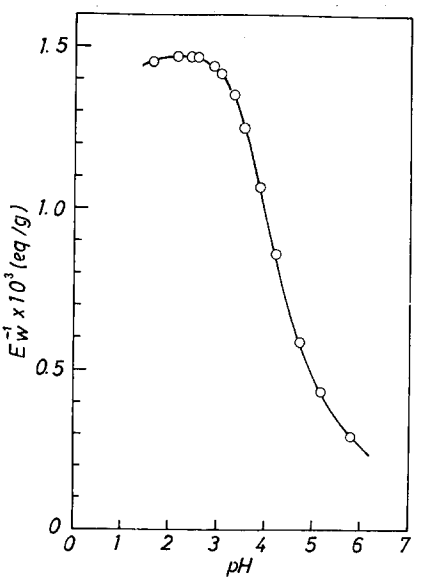

Fig. 3. The curve of 1/Ew vs. pH. The value of 1/Ew represents the mole number of basic groups salt-linked with $KOSO_3$-groups in KPVS per 1 g of human carboxyhemoglobin.

acid composition: 44 for amino groups (lysyl residues), 38 for imodazolyl groups (histydyl residues), 12 for guanidyl groups (arginyl residues), and 4 for N-terminal amino groups. The total number (98) of these basic groups approximately agrees with the number (95) obtained by colloid titration. A slight difference might be avoided if we assume that the imidazolyl nitrogen, which is coordinated to iron in proto-porphyrin (MUIRHEAD and PERUTZ 1963), is not salt-linked completely with $KOSO_3$-groups in KPVS.

To confirm this assumption the measurement of the visible spectrum was carried out. The sample solution containing human carboxyhemoglobin was titrated until the end point at different pH, and then the resulting poly-ion complex was removed by centrifugation. The supernatant solution has no absorption band in the wavelength range (380-700 nm) where the human carboxy-hemoglobin shows a characteristic spectrum. From spectrophotometric studies (LEWIS 1957) of hemoglobin, it has been found that the heme in the hemoglobin was split off and extracted with acetone when the pH of the solution was adjusted to pH 3-4 with HCl or H_2SO_4. In contrast to the acid cleavage of hemoglobin, the results obtained here suggest that no cleavage of hemoglobin has occurred in the poly-ion complex formation. This could support the assumption mentioned above.

On the basis of the results of the colloid titration, it is may be concluded that the salt-linkage formation between the three kinds of basic groups in the hemoglobin and the $KOSO_3$-groups in KPVS follows a stoichiometric relationship, although each basic group is irregularly located in α- and β-globin chains of the hemoglobin.

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