Effect of Temperature on Polyacrylamide Gel Electrophoresis and Chromatography of Histones and Protamines

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SUMMARY

Polyacrylamide gel electrophoresis or chromatography of histones (H1, H5, H2A, H2B, H3 and H4) and protamines (salmine and clupeine) in 0.9 M acetic acid or 0.01 M HCl, respectively, was carried out at various temperatures. A retardation of either electrophoretic migration or chromatographic elution of the proteins was observed at lower temperatures. H1 and H5 histones exhibited the least extent of retardation. The retardation with lowering temperature was also observed in the presence of 0.02–0.05 M NaCl but not in the presence of 5–6.25 M urea. The extent of retardation in polyacrylamide gel chromatography with lowering temperature increased with proteins of higher arginine contents. The fact was explained as that ionic interactions between guanido groups of arginine residues in the proteins and carboxyl groups of polyacrylamide are strengthened at lower gel temperatures.

INTRODUCTION

Histones are a family of basic proteins found in eukaryotic chromatin. Protamines are basic proteins rich in arginine and are found specifically in sperm chromatin of higher eukaryotes. Molecular weights of five histones of calf thymus,
H5 histone of chicken erythrocyte and fish protamines are as follows, in order of decreasing molecular weight, H1(21,000-22,000), H5(20,580), H3(15,324), H2A(14,002), H2B(13,775), H4(11,282), salmine (protamine of salmon sperm, 4,358) and clupeine (protamine of herring sperm, 4,106).

The polyacrylamide gel electrophoresis in 0.9 M acetic acid is useful for the characterization and identification of histones and protamines, and has been carried out usually at room temperature (1,2). The migration of H3 and H4 histones in the gel electrophoresis was much more retarded than that of other histones by cooling the gel (3,4). On the other hand, the gel chromatography in 0.01 M HCl on polyacrylamide gel (Bio-Gel P) column has so far been used for the fractionation of the six histone molecular species and protamines (5-12). It has been suggested that interactions between histones and the gel retard the elution of some histones, in addition to the molecular sieving effect on the basis of the molecular dimensions of individual histones (7). The anomalous elution of H3 histone which is not related to its molecular weight has been reported with polyacrylamide gel chromatography operated at room temperature (5-7,13). In addition, a significant retardation of the elutions of H3 and H4 histones was observed when the gel chromatography was carried out at low temperatures (4,0). Thus the effect of the gel temperature on the resolutions of histones has been noticed in some studies.

In the present report the electrophoretic and chromatographic behaviors of six histones, salmine and clupeine in polyacrylamide gels at various gel temperatures were studied in relation to the extent of adsorption of the proteins to the gel. The effect of temperature in the presence of urea or NaCl in gels was also examined.
EXPERIMENTAL PROCEDURE

Whole histones of calf thymus, chicken erythrocyte and Tetrahymena were isolated by the methods described previously (6,13). The whole histone fraction of Tetrahymena contains LG-1 protein, a high mobility group (HMG) protein (14). H1, H2A and H3 histones of calf thymus were fractionated and purified by CM-cellulose chromatography and polyacrylamide gel chromatography (6,15).

Prior to polyacrylamide gel electrophoresis, whole histone (20 µg) or a mixture of salmine (Wako Pure Chemical) (10 µg) and H1 histone (5 µg) was incubated in 0.1 M mercaptoethanol/8 M urea to reduce and disaggregate the samples. Conditions used for gel electrophoresis are given in the legends to each figure. Electrophoresis in gel columns (0.5 x 7.5 cm) was performed in a temperature controled Mitsumi electrophoresis chamber, model SJ-1060-DC. The gels stained with Amido Black 10B were scanned with a Canalco densitometer, model G.

Gel chromatography was carried out on columns (1 x 150 cm) of a series of porous polyacrylamide beads, Bio-Gel P-4, P-10, P-60 or P-200 (100-200 mesh) (Bio-Rad) in various eluents shown in the legends to each figure. The reduced and disaggregated whole histone (4 mg), a mixture of clupeine (Wako Pure Chemical)(2 mg) and H1 histone (1 mg), or a mixture of H2A (1 mg) and H3 histone (1 mg) were separately loaded onto gel columns. Flow rates were 3.5 ml/hr in the absence of urea and 1.5 ml/hr in the presence of 5 M urea. Columns with water jacket were used. The protein concentration in the effluents was determined by the absorbance at 230 nm or the turbidity monitored at 360 nm in 1.1 M trichloroacetic acid (13).

RESULTS

Gel electrophoresis
Figure 1 shows polyacrylamide gel (15%T-0.66%C) electrophoresis of whole histones of calf thymus, chicken erythrocyte and *Tetrahymena* in 0.9 M acetic acid at different temperatures. It is apparent that the resolution of bands of whole histones is affected by temperature, and in some cases (CE and T) the order of the band positions among H2A, H2B and H3 histones is changed with temperature. The mobilities of all histones calculated as mobility(U)=migrated distance/(voltage gradient x time)(cm².volt⁻¹.sec⁻¹) at constant voltage gradient decreased by cooling the gels (16). But the extent of retardation of the mobilities by cooling differed with individual histone species. H1 histone exhibited the least extent of retardation. Furthermore, whereas the relative mobilities to H1 histone of H2A, H2B, H3 and H4 histones decrease by cooling those of H5 histone of chicken erythrocyte and LG-1 protein of *Tetrahymena* remain unchanged by cooling. A decrease in the mobility relative to H1 by cooling is also observed for salmon, as shown in Fig.2. In a highly cross-linked gel (15%T-5.28%C) calf thymus H3 histone exhibits a marked decrease in mobility, compared with other calf thymus histones, by lowering temperature of the gel (Fig.3).

Fig.4 presents effect of temperature on the migrations of calf thymus histones in polyacrylamide gel electrophoresis in the presence of urea or NaCl. In the presence of 6.25 M urea in the gel, five histones are resolved into four bands in the order of their molecular weights and exhibit essentially the same relative mobilities at either 20°C or 5°C (Fig.4A). When electrophoresis is carried out at the same temperature, the presence of 0.02 M NaCl selectively retards the relative mobility of calf thymus H2A histone, compared with that of the histone in the absence of NaCl (compare Fig.1-CT with Fig.4B). Histones migrate much slower at 5°C than at 30°C in gels containing 0.02 M NaCl, similar to the case when the electro-
phoresis was done in the absence of NaCl.

Fig.5 summarizes changes of relative mobilities of calf thymus histones with temperature in polyacrylamide gel (15%T-0.66%C) in 0.9 M acetic acid (Fig.5A) or in 0.9 M acetic acid in the presence of 6.25 M urea (Fig.5B) or 0.02 M NaCl (Fig.5C).

Gel chromatography

The elution patterns of polyacrylamide gel chromatography of calf thymus whole histone or a mixture of clupeine and calf thymus H1 histone in 0.01 M HCl at different temperatures are shown in Fig.5 or 7, respectively. The elution position of H1 histone is not changed over the temperature range of 2°C-30°C. The elutions of the other four histones and clupeine are retarded by cooling the column temperature and the elution volumes of these proteins, which are estimated approximately from the distribution in the effluents, increase at lower temperatures. It is also seen that, at lower temperatures, the elution volumes of H3 and H4 histones or clupeine increase considerably, while the elution volumes of H2A and H2B histones increase only slightly. Below 10°C H3 and H4 histones are not completely eluted even after the total column volume(Vt)-xerogel volume(Vg) has been passed. Furthermore, the retardation of the elution of the four histones and clupeine at lower temperatures, beyond the elution volume expected from a molecular sieving effect of the gel, is more prominent when chromatography is done in a highly cross-linked polyacrylamide gel such as P-4 or P-10 rather than P-60 or P-200.

Similar temperature dependency of the elution behavior of calf thymus whole histone is seen when the chromatography is done in 0.01 M HCl/0.05 M NaCl (Fig.8B). In the presence of 0.05 M NaCl the order of elution of the histones changes from that observed when the chromatography is done in the absence of NaCl (compare Fig.8B with Fig.6B).
In the presence of 5 M urea (Fig. 8A) the four calf thymus histones are eluted in the order of increasing molecular weight and their elution patterns are almost unchanged with temperature.

The effect of temperature on the fractionation of calf thymus H2A and H3 histones from mixtures of them was studied on a P-10 column in 0.01 M HCl, as shown in Fig. 9. The elution of H3, an arginine-rich type histone, is more markedly retarded with lowering temperature than the elution of H2A, a lysine-rich histone. A tailing of H3 fraction in the elution pattern occurs significantly below 10°C.

The elution behaviors of calf thymus histones in polyacrylamide gel chromatograph column in different eluents at different temperatures are summarized in Fig. 10. The distribution coefficient, K_d, was calculated from \( \frac{V_e - V_0}{V_t - V_g - V_0} \), where \( V_e \) and \( V_0 \) are the elution volume of histones and the void volume of column, respectively. It can be seen that the extent of retardation of chromatographic elution is a function of the operating gel temperature and increase in the order of H2A < H2B < H4 < H3 (Fig. 10A) or H2A < H2B < H4 < H3 (Fig. 10C). Thus there appears to be a general tendency that the higher the arginine content of histones the larger the retardation.

**DISCUSSION**

Histones and protamines are basic proteins rich in lysine and/or arginine residues. H1 and H5 are lysine-rich type histones. H2A and H2B are characterized as slightly lysine-rich histones and H3 and H4 are arginine-rich histones. Fish protamines such as salmine and clupeine contain only arginine as basic amino acid residues.

It has been shown in the present study that the migration or elution of histones H2A, H2B, H3 and H4 and protamines in the
polyacrylamide gel electrophoresis in 0.9 M acetic acid or the polyacrylamide gel chromatography in 0.01 M HCl, respectively, is markedly retarded at lower temperatures. The retardation was more pronounced in highly cross-linked polyacrylamide gels. Furthermore, the degree of retardation differed with individual protein species and H1 and H5 histones exhibited the least extent of retardation among the proteins examined. In the presence of urea no retardation of migration or elution of the basic proteins at lower temperatures was observed.

Polyacrylamide gel contains a little amount of carboxyl groups owing to slight hydrolysis of amide groups (17). The retardation of the elution of histones and protamines in polyacrylamide gel chromatography at lower temperatures, beyond the elution volume calculated from a molecular sieving effect of the gel, would occur mainly as a result of interactions between the carboxyl groups on the gels and positively charged groups of the proteins. The order of the chromatographic retardation with lowering temperature of calf thymus histones, H1 ≪ H2A ≪ H2B ≪ H4 ≈ H3 or H1 ≪ H2A ≪ H2B ≈ H4 ≈ H3, can be roughly correlated with their arginine contents but not basicity or helix forming capacity of them (18). The hydrophobic interactions between polyacrylamide and histones might not be important to strengthen the adsorption of the latter to the former, since the retardation of migration or elution of histones occurred at lower temperatures. Therefore the present results could be explained as that ionic interactions between guanido groups of arginine residues and carboxyl groups of the gel are strengthened at lower temperatures, leading to the increased retardation of the basic proteins of higher arginine contents. Recently, Kaye et al. (19) have also discussed about the retardation of elution of histones in polyacrylamide gels, although temperature effect is not given in their study.
Some of the present findings were successfully applied to get better separation of histones by polyacrylamide gel electrophoresis and chromatography. Calf thymus H2B and H3 histones have not been separated each other by the previous gel electrophoresis in 0.9 M acetic acid in the absence of urea operated at room temperature on 15%T-0.66%C polyacrylamide gels (1,2). In this study we could separate the five histones of calf thymus into five electrophoretic bands by performing the polyacrylamide gel electrophoresis on 15%T-5.28%C gel at 10°C (Fig.2). The fractionation of histones by polyacrylamide gel chromatography in 0.01 M HCl depends sensitively on gel temperature. Based on the fact we could get the best resolution between H2A and H3 histones of calf thymus on Bio-Gel P-10 at 15°C (Fig.9). For the fractionation of individual histones from a mixture of H2A, H3 and H4 histones, Ohe et al. controled the gel column of P-10 at 15°C (15,20). The adsorption mechanism with the highly cross-linked polyacrylamide gel (Bio-Gel P-2) has been used also for the separation of other basic proteins (21).

ACKNOWLEDGMENTS

Most of the experiments reported in this paper was done in the Institute of Endocrinology, Gunma University. The author thanks Drs. K. Iwai, N. Ui and Y. Ohe for their valuable suggestions. The author also thanks Dr. M. Zama of National Institute of Radiological Sciences for preparation of the manuscript. This study was supported in part by Scientific Research Grants from the Ministry of Education, Science and Culture of Japan.

Part of this work has already been presented in the form of published abstract(16).

REFERENCES


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Figure 1. Polyacrylamide gel electrophoresis of whole histones of calf thymus (CT), chicken erythrocyte (CE) and Tetrahymena (T) in 15%T-0.66%C polyacrylamide gel. Electrophoresis was carried out at 120 volts at 30°C (2.8 milliamperes/gel) for 190 min, 20°C (2.2) for 240 min, 10°C (1.6) for 330 min or at 5°C (1.4) for 370 min in 0.9 M acetic acid. Migration was from left (anode) to right.

Figure 2. Polyacrylamide gel electrophoresis of salmine and calf thymus H1 histone in 15%T-0.66%C polyacrylamide gel. Electrophoresis was carried out at 120 volts at 30°C (2.8 milliamperes/gel) for 60 min or at 5°C (1.4) for 60 min in 0.9 M acetic acid.
Figure 3. Polyacrylamide gel electrophoresis of calf thymus whole histone in 15%T-5.28%C polyacrylamide gel. Electrophoresis was carried out at 120 volts at 30°C (2.6 milliamperes/gel) for 240 min or at 10°C (1.7) for 430 min.

Figure 4. Polyacrylamide gel electrophoresis of calf thymus whole histone in 15%T-0.66%C polyacrylamide gel. Electrophoresis was carried out at 200 volts at 20°C (1.6 milliamperes/gel) for 300 min or at 5°C (1.0) for 470 min in 0.9 M acetic acid/6.25 M urea (A), or 120 volts at 30°C (9.0 milliamperes/gel) for 190 min or at 5°C (4.5) for 370 min in 0.9 M acetic acid/0.02 M NaCl (B).
Figure 5. Changes of relative mobility of calf thymus histones with temperature in polyacrylamide gel (15% T-0.66% C) in 0.9 M acetic acid (A), 0.9 M acetic acid/6.25 M urea (B) or in 0.9 M acetic acid/0.02 M NaCl (C).

Figure 6. Polyacrylamide gel chromatography of calf thymus whole histone in 0.01 M HCl on Bio-Gel P-10 (A), P-60 (B) or P-200 (C) at 2°C, 5°C, 10°C, 20°C or 30°C.
Figure 7. Polyacrylamide gel chromatography of clupeine and calf thymus H1 histone on Bio-Gel P-4 in 0.01 M HCl at 2°C or 20°C.

Figure 8. Polyacrylamide gel chromatography of calf thymus whole histone on Bio-Gel P-60 in 0.01 M HCl/5 M urea (A) or 0.01 M HCl/0.05 M NaCl (B) at 2°C or 20°C.
Figure 9. Polyacrylamide gel chromatography of calf thymus H2A and H3 histones on Bio-Gel P-10 in 0.01 M HCl at 2°C, 10°C, 15°C or 25°C.

Figure 10. Changes of distribution coefficient (Kd) of calf thymus histones with temperature on Bio-Gel P-10 in 0.01 M HCl (A), on P-60 in 0.01 M HCl/5 M urea (B) or on P-60 in 0.01 M HCl/0.05 M NaCl (C).