Effect of the Degree of Cross-Linkage of Polyacrylamide Gel on the Electrophoretic Mobility of Histones

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SUMMARY

The electrophoretic mobility in polyacrylamide gel of calf thymus histones was examined as a function of the degree of cross-linkage (C,%) of the gel in five different buffer systems. With increasing the degree of cross-linkage, the mobility once decreased and then increased, irrespective of total gel concentrations (T,%) used (5-15 %). The degree of cross-linkage of the gel where the mobilities of the histones became minimum (C_{min}), 7-15 %C, was larger than that where the mobilities of other high molecular weight proteins become minimum, 5-7 %C. The retardation coefficients (K_R) of the histones obtained from changes of the total gel concentration attained their maximum values in gels which have C_{min}. In gels of C_{min} an approximately linear relationship was obtained between the K_R and the molecular weight of the histones.

INTRODUCTION

Polyacrylamide gel electrophoresis has been widely used for estimation of molecular weight and separation of proteins (1-3). The effect of the degree of the cross-linkage (C,%) of gels has been investigated for proteins of high molecular weights, and it is known that the electrophoretic mobilities of proteins become minimum at the degree of cross-linkage of 5-7 % (4-7). However
in the case of histones, the low molecular weight proteins, only the gels of limited C values (0.66 and 2.6 %) have so far been used (8,9). In this paper I examined the relation between the electrophoretic mobility and the degree of cross-linkage in polyacrylamide gel of calf thymus histones in five different buffer systems. The relation between retardation coefficient \( K_R \) and the molecular weight of the histones was also investigated.

**EXPERIMENTAL PROCEDURE**

**Materials** — Acrylamide (S.P grade), N,N′-methylenebis-acrylamide (BIS) (S.P grade), urea (S.P grade), cetyltrimethylammonium bromide (CTAB) (G.R. grade) and sodium dodecyl sulfate (SDS) (S.P grade) were obtained from Nakarai Chemicals. Histones were prepared from calf thymus as described previously (8).

**Polyacrylamide Gel** — Five histones from calf thymus were electrophoresed in polyacrylamide gels containing five different buffers, i.e., 0.9 M acetic acid, 6.25 M urea-0.9 M acetic acid, 3 mM CTAB-0.1 M sodium phosphate, pH 7.0, 3 mM SDS-0.1 M sodium phosphate, pH 7.0, 3 mM SDS-0.1 M glycine-NaOH, pH 10.0 (8,9). Acrylamide-BIS stock solutions were prepared as 60 % (T%\(^1\), total percentage concentration of acrylamide monomers) solutions for 0.66, 1.3, 2.6, 3.9, 5.2, 7.8, 10.5, 15.8, or 21.1 % (C%\(^1\), percentage concentration of cross-linker) gels. N,N,N′,N′-tetramethyl-ethylenediamine (TEMED) and ammonium persulfate were used for the acetic acid, urea-acetic acid, and SDS gels. TEMED and riboflavin were used for the CTAB gel. Concentrations of these catalysts for polymerization were constant in the polymerization mixtures (8,9). Highly cross-linked gels became milky-opaque. Slightly cross-linked gels swelled during destaining.

\(^1\)The nomenclature of Hjertén (10) is used to describe gels.
Electrophoresis — Pre-electrophoresis was performed for all gels before the electrophoresis of histone samples. Electrophoresis using a single gel column (0.5 x 7.5 cm) was carried out at a constant current of: 2mA/tube (acetic-acid and urea-acetic acid gels), 5 mA/tube (SDS gels, pH 10.0) or 10 mA/tube (SDS gels, pH 7.0, and CTAB gels). The gel temperature was maintained at 20 °C using a Mitsumi electrophoresis chamber, model SJ-1060-DC. No marker dye was added to the sample solution, because it would run off the gel column before the end of electrophoresis under the conditions used. Staining and destaining of the gels after the electrophoresis were carried out according to our previous reports (8,9). The measured voltage across the gel was proportional to the supplied current per gel. Electrophoretic migration was linear with time and applied voltage. By assuming the apparent potential gradient in the gel, mobility (M) was calculated as M = migrated distance/ (apparent potential gradient x time) and was expressed in the unit of cm².volt⁻¹.s⁻¹ (2,3). The logarithm of mobility was plotted against T (%) according to the equation of Ferguson (1): \[ \log M = \log M_0 - K_R T, \] where \( M_0 \) is the free mobility and \( K_R \) is the retardation coefficient.

RESULTS AND DISCUSSION

Effect of the Degree of Cross-Linkage on Mobility — The gel electrophoresis of five molecular species of calf thymus histones was done in five different buffer systems. Both the cross-linking (C,%) and total concentration of acrylamide (T,%) were changed in each electrophoresis system. Typical gel electrophoretic patterns of the histones in urea-acetic acid gels are shown in Fig. 1. In Fig. 2A the mobility of H4 histone in urea-acetic acid gel is plotted as a function of C(%) for gels of three different T(%) values. Similar plots were obtained for all five histones electrophoresed in other gel systems. From
each plot the C(%) value where each histone exhibits a minimum electrophoretic mobility was obtained. Fig. 3 summarizes the results. It is obvious that the values of cross-linkage of gels at which histones exhibit minimum mobilities depend markedly on buffer systems. The mobilities of five histones appear to be minimum at C value of 15 % in acetic acid gels or C value of 14 % in urea-acetic acid gels. The mobilities of histones in CTAB or SDS gels become minimum at C=12-13 % or 7-8 %, respectively. In the previous work (8,9) we have observed that the $K_R$ value of histones increases in the order of, histones in acetic acid gel < histones in urea-acetic acid gel < histones in CTAB gel < histones in SDS gel. Therefore, the results in Fig. 3 indicate that the degree of cross-linkage which give minimum mobilities of histones in five different buffer systems inversely related with the magnitude of $K_R$ in five buffer systems.

These results suggest that the value of $C_{min}$ varies with the effective size of histones, that is, the effective molecular size of the histone-CTAB or -SDS complexes is larger than those of histone molecules in 0.9 M acetic acid or 6.25 M urea-0.9 M acetic acid (8,9). The minimum mobilities at C=5-7 % of high molecular weight globular proteins were explained in terms of the structural conversion of the gel meshwork around C=5 % (11, 12). In this respect, it should be noted that the mobilities of five histones, the low molecular weight proteins, are minimum at C=7-15 %.

$K_R$ in Various Cross-Linked Gels —— The plots of the logarithms of the mobilities of five histones versus T(%) (Ferguson plots) at various degrees of cross-linkage were obtained. All the plots were represented as straight lines, as are shown in Fig. 2B for H4 histone. Fig. 4 depicts the change of $K_R$ as a function of C(%) in urea-acetic acid or SDS gels.
The $K_R$ in urea-acetic acid or in SDS gel attains its maximum value at $\sim 15$ or $\sim 8\%$ cross-linkage, respectively. The $K_R$ is maximum in the cross-linked gel exhibiting the minimum mobility in each gel electrophoresis buffer system.

Fig. 5 shows the plots of $K_R$ versus molecular weight of five histones in urea-acetic acid and in SDS gels. In urea gels at 10.5 and 15.8 %C the plots are linear and the maximum molecular sieving effect is obtained. Although the linearity of the plot is obtained also at 0.66 %C as is shown in Fig. 5A, and has been reported previously (8), the resolution by molecular sieving is poor. In SDS gels, on the other hand, any linearity of the plot is not observed irrespective of the degree of cross-linkage (Fig. 5B). This would probably be due to some abnormality of SDS binding to histones or of conformation of SDS-histone complexes (8,9).

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REFERENCES

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Fig. 1 Polyacrylamide gel electrophoresis of calf thymus whole histones in different C(%) gels with a constant total gel concentration, T=15 % (A), and in different T(%) gels with a constant degree of cross-linking, C=0.66 % (B). Whole histones were electrophoresed in 6.25 M urea-0.9 M acetic acid at 2 mA/gel for 1 h (B) or 3 h (A). Gels were stained with Amido Black 10B. Migration was from the top (anode) to the bottom. Highly cross-linked gels became milky-opaque. Slightly cross-linked gels swelled during destaining.
Fig. 2  A, Relation between mobility and degree of cross-linkage of calf thymus H4 histone electrophoresed in polyacrylamide (T=5-15 %) gels containing 6.25 M urea-0.9 M acetic acid. B, Relation between mobility and total gel concentration of calf thymus H4 histone electrophoresed in polyacrylamide (C=0.66-21.1 %) gels containing 6.25 M urea-0.9 M acetic acid. Mobilities at C=0 % in (B) were obtained by extrapolating the plots in (A) to C=0 %.

Fig. 3  Relation between molecular weight (MW) and the degree of cross-linkage at the minimum mobility (C_{min}) of calf thymus histones in polyacrylamide gel electrophoresis containing 0.9 M acetic acid (A), 6.25 M urea-0.9 M acetic acid (B), 3 mM CTAB-0.1 M sodium phosphate buffer, pH 7.0 (C), 3 mM SDS-0.1 M sodium phosphate buffer, pH 7.0 (D), or 3 mM SDS-0.1 M glycine-NaOH buffer, pH 10.0 (E). C_{min} was estimated in T=15 % gels.
Fig. 4  Changes of retardation coefficient ($K_R$) as a function of cross-linkage (C, %) of calf thymus histones in polyacrylamide gels containing 6.25 M urea-0.9 M acetic acid (A) or 3 mM SDS-0.1 M glycine-NaOH buffer, pH 10.0 (B). $K_R$ was calculated from the slopes of Ferguson plots in Fig. 2B.
Fig. 5  Relation between retardation coefficient ($K_R$) and molecular weight (MW) of calf thymus histones in polyacrylamide gels containing 6.25 M urea-0.9 M acetic acid (A) or 0.3 mM SDS-0.1 M glycine-NaOH buffer, pH 10.0 (B). $K_R$ values in gels of various degrees of cross-linkages are shown. Molecular weights used are as follows: H1 (22,000–23,000) (13), H3 (15,324) (14), H2A (14,002) (15), H2B (13,775) (16), H4 (11,282) (17).