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Effect of glycerol on the separation of nucleosomes and bent DNA in low ionic strength polyacrylamide gel electrophoresis

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ABSTRACT

We report that glycerol changes the separation characteristics of polyacrylamide nucleoprotein gels in which it is included as a stabilizing agent. Polyacrylamide gel electrophoresis fractionates DNA and nucleosomes according to net negative charge, mass and conformation. With glycerol included, fractionation seems to be largely based on particle mass and charge. The conformation factor in separation is progressively lost with increasing glycerol concentrations. Nucleosome positions on the same DNA fragment are no longer resolved, while the difference in electrophoretic mobility between core particles and nucleosomes carrying longer DNA becomes smaller and is eventually lost. The retardation of bent DNA is also much reduced. Using the differences in separation characteristics between glycerol-containing and regular nucleoprotein gels could be a new means to obtain information on macromolecules in solution.

INTRODUCTION

Polyacrylamide gel electrophoresis is a widely used powerful technique for separating nucleic acids as well as proteins. It is also used to identify bent DNA and DNA binding proteins. Early on, the technique has been applied to studies of nucleosome particles (1, 2). Nucleosomes containing linker histones or HMG proteins for instance can be distinguished by their electrophoretic mobility (3, 4, 5).

We have recently described an additional resolving power of the nucleoprotein gel electrophoresis technique (6, 7). We have shown that different nucleosome positions migrate as separate bands. The electrophoretic mobility of positioned nucleosomes is a function of their proximity to the center of the DNA fragment (7). Maximal retardation was found for nucleosomes located centrally on the DNA, similar to the behavior found for bent DNA (8). Recent observations using a different system have led to similar conclusions (9). By exploiting this feature, nucleoprotein gel electrophoresis can serve as a sensitive tool to study nucleosome positioning and its dynamics (7).

Surprisingly, only one previous report is known to us that describes a differential electrophoretic mobility attributed to nucleosome positioning (10). In less homogeneous samples, the band heterogeneity caused by differences in DNA length or histone content can mask this additional parameter in determining electrophoretic mobility. Also, nucleosome positions on shorter DNA fragments are less well resolved (7).

This paper gives another explanation as to why this resolving ability of gel electrophoresis has not received more attention. A comparison of gel conditions has indicated that glycerol is more than an innocuous ingredient of polyacrylamide gels. Glycerol is often included in polyacrylamide gels to prevent dissociation of nucleosomes and other protein-DNA complexes during electrophoresis (5). Because it is added solely as a stabilizing agent, the separation is assumed to be indifferent to the presence (up to 30%), or absence of glycerol. We show that the concentration of glycerol during electrophoresis strongly affects the separation characteristics of polyacrylamide gels. The resolution of different nucleosome conformations and positions and the retardation of bent DNA were compared on polyacrylamide gels as a function of glycerol content. Sucrose, which shares some properties of glycerol, was used for comparison.

Our observations offer some new insights into the migration of bent DNA and nucleosomes (and possibly other nucleoprotein complexes) through polyacrylamide gels. The different characteristics of gel electrophoretic separations depending on the presence of glycerol as identified here, could be used to obtain additional information on nucleoprotein particles.

MATERIALS AND METHODS

Preparation of DNA substrates

Monomer 207 bp sea urchin 5S rDNA fragments were generated from the tandemly repeated insert of plasmid p5S207-18 (gift from Dr. R.Simpson (11)) by *Ava*I restriction digestion. A 186

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bp bent DNA insert of nucleosomal origin was excised from M13223 rf with *Eco*RI and *Bam*HI (12). DNA fragments of interest were purified on a Mono Q column (Pharmacia), eluting with a 0.7M−0.8M NaCl gradient in 0.2 mM EDTA, 20mM Tris-HCl (pH 8.0).

Preparation of histone octamers

$HeLaS_3$ cells were grown as described in (13), with optional butyrate treatment to obtain hyperacetylated histones. Long chromatin was prepared from HeLa and chicken erythrocyte nuclei as previously described (7). Histone octamers were purified as in (14). Briefly, long chromatin was depleted of linker histones by sucrose gradient centrifugation at 600 mM NaCl. The depleted chromatin was redigested with micrococcal nuclease and histone octamers were separated from the DNA by hydroxyapatite FPLC, eluting with 2 M NaCl, 10 mM sodium phosphate (pH 6.8). Specific procedures for hyperacetylated octamers are described in (15).

Nucleosome reconstitution procedure

Reconstitutions were carried out at 7 to 10°C. Histone octamers and 207 bp DNA substrate were mixed in a 0.9:1 (w/w) ratio to a final A_{260} between 2 and 5, in 2 M NaCl, 0.2 mM EDTA, 10 mM Tris-HCl (pH 7.4). Small volumes were dialyzed to decreasing NaCl concentrations in 1 hr steps (2 M−1.5 M−1 M−0.75 M−0.5 M NaCl, each including 0.2 mM EDTA, 10 mM Tris-HCl (pH 7.4)), and a final overnight step to 10 mM NaCl, 0.2 mM EDTA, 10 mM Tris-HCl (pH 7.4). 0.5mM sodium butyrate was included in all buffers whenever hyperacetylated HeLa histone octamers were used in the reconstitutions.

Preparation of chicken erythrocyte core particles

Long chromatin was prepared from chicken erythrocyte nuclei as in (7). The long chromatin was depleted of histones H1/H5 by centrifugation through a sucrose gradient containing 600 mM NaCl, 0.2 mM EDTA, 10 mM Tris-HCl (pH 7.4) at 4°C. The depleted chromatin was redigested with micrococcal nuclease (Cooper Biomedical) at 37°C in 10 mM NaCl, 0.2 mM EDTA, 10 mM Tris (pH 7.4), adding 0.5 mM $CaCl_2$. Core particles were isolated by sucrose gradient centrifugation at 4°C in the same buffer.

Gel electrophoresis

5% polyacrylamide nucleoprotein gels (29:1 acrylamide to bisacrylamide ratio) contained 30 vol% glycerol or 30% (w/v) sucrose where indicated. The viscosities of 30 vol% glycerol (= 35 wt%, 6.5 ± 0.2 cp at 0°C or 4.4 ± 0.2 cp at 10°C (16)) and 30% (w/v) sucrose (6.7 cp at 0°C or 4.5 cp at 10°C (17)) are identical within the error of dilution. Polymerizations were carried out with the solutions at room temperature. All gels were run at 4°C in 45 mM Tris, 45 mM boric acid, 1.25 mM EDTA (pH 8.3) (0.5 TBE) at 10 V/cm for 1.5 mm thickness. The gels were pre-run for at least 1 hr at 4°C. Samples were loaded in 3.5% ficoll, 0.5 TBE and tracking dye.

Glycerol (or sucrose) gradient gels were cast to form a 0%−30% glycerol (sucrose) gradient over the width of the slots. The glass plates were first mounted with the direction of electrophoresis parallel to the bench. A narrow tube was inserted straight down the middle. The tube was run through a pump and connected to a linear gradient former. A volume of 22+3 ml of 5% acrylamide/bisacrylamide (29:1), 0.03% ammonium persulfate in 0.5 TBE was prepared, and 22 ml of the same solution containing 35% glycerol (or sucrose). 10 μl TEMED was added to each solution. First, 3 ml of the light solution was pumped in (for the control lane, which was not supposed to contain any glycerol or sucrose). Then 38 ml of 0%−30% linear gradient was slowly pumped in until the solution reached the top minus 5 mm (the width of a gel spacer). A total of 6 ml was left in the gradient former and the tubing. The tube was retracted and the gel was allowed to polymerize. The gel spacer that occupied the area where the slot-forming comb was to be inserted was removed. On the side left open at the top of the gradient, a gel spacer was inserted. The gel was mounted vertically, this time with the direction of electrophoresis perpendicular to the bench. The top was filled with a 5% acrylamide solution and the comb inserted.

For the pore size control experiment, three identical polyacrylamide gels were cast, the glass plates were removed and the gels were soaked in 0.5 TBE, in 0.5 TBE plus 30% glycerol, or in 0.5 TBE plus 30% sucrose for at least 24 hrs with several buffer changes. All gels swelled equally and were then cut to a size that fitted the glass plates of the electrophoresis apparatus. Because the 45 ml gels swelled to a volume of about 55 ml, a starting acrylamide percentage of 6.1% was taken. The samples were thus electrophoresed through identical pore sizes that approximated the pore size of a 5% polyacrylamide gel, some mechanical deformation notwithstanding. All gels were stained with ethidium bromide. Apparent lengths for bent DNA were determined using pBR322 *Msp*I standard curves.

RESULTS

Glycerol affects the relative migration of different nucleosomes

We have determined the effect of glycerol on the separation of core particles and different 207 bp 5S rDNA nucleosomes (207 bp nucleosomes) on nucleoprotein gels. A core particle consists of a histone octamer and one and three quarter turns of DNA (146 bp) and is the result of a pause in a micrococcal nuclease digestion of chromatin. 207 bp nucleosomes contain six additional helical turns of DNA that protrude from the particle. Histone octamers are assembled onto 207 bp of 5S rDNA in a limited set of positions (14, 18) that separate into three bands in low ionic strength gel electrophoresis. The electrophoretic migrations of these nucleosomes are a function of the position of the histone octamer relative to the middle of the DNA fragment (6, 7), in analogy with the retardation of bent DNA (8). We show that glycerol, which is often included in gel recipes as a stabilizing agent for macromolecular complexes (5), is not without consequences for the separation characteristics of polyacrylamide gels.

Fig. 1 shows that core particles migrate considerably faster than 207 bp nucleosomes in regular 5% polyacrylamide gels. However, they move slower than these nucleosomes when the polyacrylamide gel contains 30% glycerol. The same observation is made when core particles are compared to H1 depleted chromatosomes (S. Muyldermans, personal communication). Fig. 1 also shows that 207 bp nucleosomes migrate as three bands in the regular gel, which reflects the positions of the histone octamer on the 207 bp 5S rDNA fragment (6). In the presence of 30% glycerol, however, these three bands condense into one band that migrates about the same distance relative to the DNA marker.

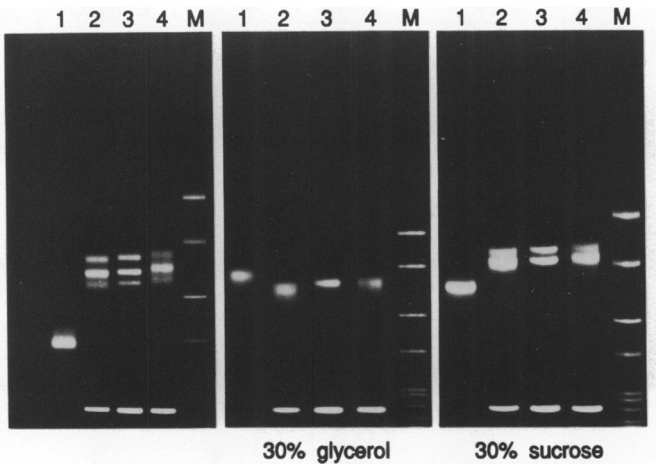

Figure 1. Identical samples loaded on 5% polyacrylamide gels containing respectively: no glycerol, 30% glycerol and 30% sucrose. All gels were run in 0.5 TBE at 4 °C. Lanes 1: nucleosome core particles from chicken erythrocytes; lanes 2 to 4: nucleosomes reconstituted on a 207 bp 5S rDNA fragment, using chicken erythrocyte histone octamers (lanes 2), non-acetylated Hela octamers (lanes 3), acetylated Hela octamers (lane 4). Lane M: pBR-MspI marker.

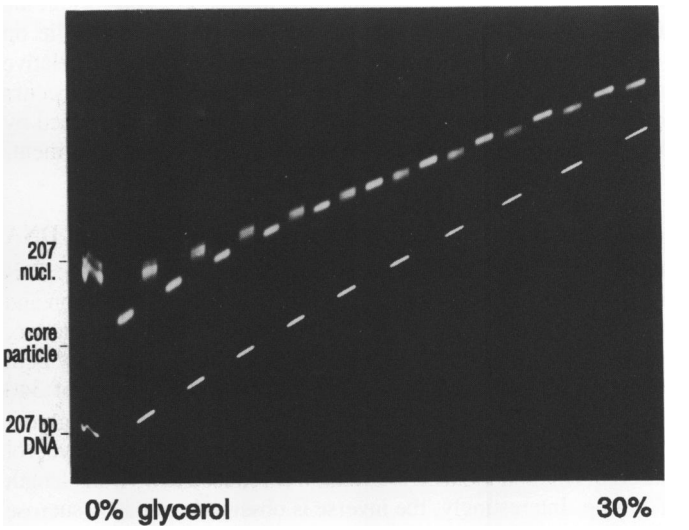

Figure 2. 5% polyacrylamide gel containing a (0 to 30%) linear glycerol gradient. Even lanes: nucleosome core particles from chicken erythrocytes; odd lanes: reconstituted nucleosomes containing 207 bp of 5S rDNA.

Because of the important contribution of viscosity to friction in the gel, as well as the lower dielectric constant of the electrophoretic medium, macromolecules have a reduced electrophoretic mobility in glycerol gels. This is evidenced by the approximately 4 times longer running times of the gels. When sucrose, another stabilizing agent, is included in the gel at the same viscosity as glycerol, very different effects are observed, however. As shown in Fig. 1, the three 207 bp nucleosome bands are now reduced to two bands. There is also a significant retardation of core particles relative to 207 bp nucleosomes in sucrose but no inversion of the bands. A two dimensional nucleoprotein/nucleoprotein gel, in which a strip containing the three bands cut from a regular gel was run in 30% glycerol in the second dimension, revealed that the coinciding three bands still have minor differences in migration. This makes it unlikely that it is the positioning of these nucleosomes that is altered instead of their mobility. This analysis also showed that the lower and middle band comigrate in 30% sucrose (not shown).

207 bp nucleosomes reconstituted from different sources of histone octamers display the same electrophoretic behavior (Fig. 1). Without glycerol, the three bands indicative of the positioning of the nucleosome are resolved to the same extent with acetylated as with non-acetylated histones. If the gel senses the position of the histone octamer the same way it senses the position of a bend in a DNA fragment, then at least by this criterion, the path of DNA extending from the core particle is not changed by the acetylations in the histone terminal domains. Note that the three bands from hyperacetylated nucleosomes are not as sharp as the ones from the non-acetylated nucleosomes. Because acetylation is the only difference between these histone octamers, this band width must be due to the heterogeneity in the number of acetylated lysine residues per octamer. Mixtures of histone subtypes could also produce fuzzier bands.

Nucleoprotein gel electrophoresis separates particles according to their mass, net negative charge and conformation. The fact that the positions are no longer resolved in the presence of 30% glycerol could indicate that glycerol negates this conformation

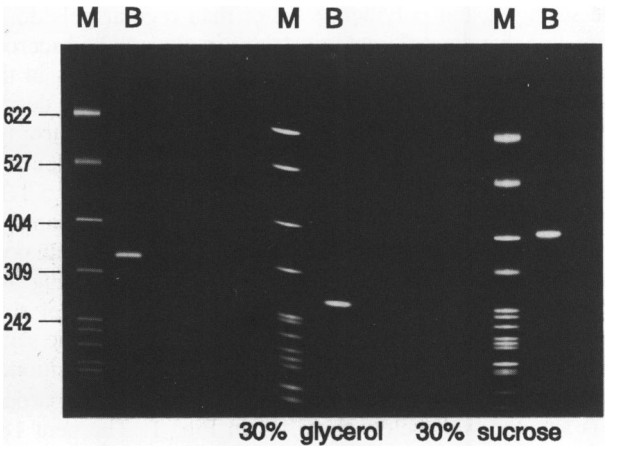

Figure 3. Migration of a 186 bp bent DNA fragment (B) on 5% polyacrylamide gels containing respectively: no glycerol, 30% glycerol, and 30% sucrose. Marker (M) is pBR-MspI.

factor. A slower migration of core particles relative to 207 bp nucleosomes supports this. Core particles are not expected to migrate much faster than 207 bp nucleosomes unless a relevant conformation factor is valid, because the lack of 60 bp of linker DNA in core particles has a far greater impact on net negative charge than it does on mass of the particle.

There clearly is a difference between the effects of sucrose and glycerol on the separation of the nucleoproteins. Comparison of separation characteristics of gels with a gradient of glycerol or sucrose across them shows that the difference is not merely quantitative. A 207 bp nucleosome reconstitution and isolated core particles were applied alternatively on these gradient gels as in Fig. 2. One can track 207 bp free DNA, core particles and 207 bp nucleosomes through the linear gradient. The transition from 0 to 30% sucrose is completely linear for all three macromolecules and the comigration of the lower and middle

band happens gradually (not shown). In glycerol (Fig. 2) on the other hand, 207 bp nucleosomes and 207 bp free DNA lie on a straight line but core particles follow a curve. The main relative decrease in electrophoretic velocity of core particles occurs between 0 and about 10% glycerol. The three bands, caused by the positions of the nucleosome on the 207 bp fragment, comigrate before 10% glycerol is reached.

Glycerol reduces the electrophoretic retardation of bent DNA

Bent DNA migrates slower through gels than projected (8). This is an obvious example of a conformation induced retardation and presents an attractive test of our findings. Three gels (native, glycerol, sucrose) were run in parallel (Fig. 3). A strongly bent DNA fragment of 186 bp is slowed to a virtual length of 340 bp in the native gel (at 4°C; at room temperature, this fragment migrates as 250 bp of DNA (12)). However, in 30% glycerol this conformation induced retardation is reduced to a virtual length of 270 bp. Interestingly, the inverse is observed with 30% sucrose where the DNA migrates as a 420 bp fragment.

Glycerol and sucrose affect acrylamide polymerization

We noticed that glycerol containing gels are mechanically weaker and swell more in staining baths than gels without glycerol but containing the same percentage of acrylamide and bisacrylamide. Sucrose containing gels, on the other hand, appear to have better tensile strength, and polymerize faster than regular gels do. It was possible that the different migrations in glycerol and sucrose containing gels were merely a consequence of changes in the polyacrylamide gel matrix, caused by glycerol or sucrose during polymerization. We therefore performed a pore size control for glycerol and sucrose containing gels. Three identical polyacrylamide gels were equilibrated against 0.5 TBE, 0.5 TBE and 30% glycerol, or 0.5 TBE and 30% sucrose. The samples were thus electrophoresed through identical gel matrices with pore sizes approximating those of a 5% polyacrylamide gel (Fig. 4). The native gel shows that the electrophoretic behavior of core particles, 207 bp nucleosomes and bent DNA is about the same as for an unsoaked 5% gel. The three bands of the positioned 207 bp nucleosomes are again well resolved and the core particle band is situated well below them, as in Fig. 1. The bent 186 bp DNA fragment is again slowed to a virtual length of 330 bp (within experimental error of the measurement of 340 bp for the native gel in Fig. 3).

The 30% glycerol-soaked gel of Fig. 4 shows the comigration of the three bands of 207 bp nucleosomes, just like in the glycerol containing gels of Figs. 1 and 2. Core particles migrate significantly slower and closer to 207 bp nucleosomes, although they are not 'overtaken' by them as in Fig. 1 and 2. The bent DNA retardation is reduced as in Fig. 3, but to a lesser extent (the virtual DNA length here is still 300 bp as opposed to 270 bp for the glycerol containing gel of Fig. 3). We conclude that the effect of glycerol in polyacrylamide electrophoresis is not caused by a reduction in matrix density. Moreover, comparison with lower percentage regular acrylamide gels (e.g. 3.5%) shows that neither the comigration of the three 207 nucleosome bands nor the slowing down of core particles is typical for an increased pore size. Instead all these bands migrate faster relative to the DNA marker at a reduced resolution (not shown). However, glycerol does affect acrylamide polymerization as illustrated by two observations. First, the exaggerated reduction in retardation of the bent DNA in the glycerol containing gel in Fig. 3 as

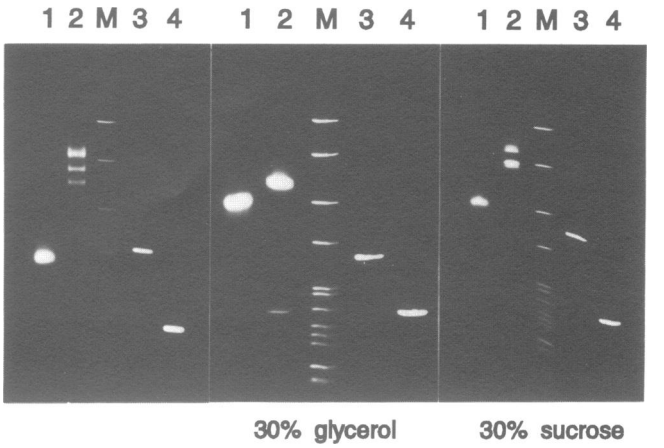

Figure 4. 5% polyacrylamide gels presoaked in respectively: 0.5 TBE, 0.5 TBE-30% glycerol, 0.5 TBE-30% sucrose. Lanes 1: chicken erythrocyte core particles; lanes 2: reconstituted 5S rDNA nucleosomes; lanes M: pBR-MspI marker; lanes 3: 186 bp bent DNA fragment; lanes 4: 207 bp 5S rDNA fragment.

opposed to the soaked gel in Fig. 4 could be due to loosening of the gel matrix when glycerol is present during the polymerization. Secondly, core particles are overtaken by 207 bp nucleosomes when glycerol is added prior to gel polymerization (Fig. 1), whereas core particle is still the faster particle of the two for the soaked gel (Fig. 4). The observation of Fig. 1 seems a combination of the fact that core particles migrate closer to 207 bp nucleosomes in looser polyacrylamide gels (e.g. 3.5%) and the fact that glycerol reduces the conformation factor in electrophoretic separation as in the glycerol-soaked gel of Fig. 4.

For the 30% sucrose-soaked gel, we again see two bands for the 207 bp nucleosomes (Fig. 4). The core particles are again slowed relative to 207 bp nucleosomes, in comparison to the native gel, but to a lesser extent than in Fig. 1. Again, comparison with higher percentage regular acrylamide gels (e.g. 7%) also shows that the migration behavior in sucrose containing gels cannot be explained by a smaller pore size alone (not shown). The bent DNA fragment is more retarded in the sucrose-soaked gel than in the native gel but not as much as shown in Fig. 3 (the virtual DNA length of the 186 bp fragment was 420 bp for the sucrose containing gel of Fig. 3 but 360 bp when the pore size of the gel is controlled as in Fig. 4). It therefore seems clear that the observed effects of Figs. 1 and 3 are due to the presence of sucrose, as demonstrated in Fig. 4, with the contribution of a denser matrix, reflected in the 'extra' retardation of the bent DNA fragment. It must be noted that although the bent DNA fragment displays the general shifts in migration relative to the DNA marker as expected for lower or higher percentage polyacrylamide gels, this effect is not observed at the nucleosome level. This indicates that the effects of glycerol and sucrose on polymerization of the gel matrix may be more than a mere increase or reduction of the mean pore size.

Glycerol inhibits acrylamide polymerization and sucrose enhances it, which renders the interpretation of experiments more complex. However, the gel pore size controls show that the basic conclusion holds up: glycerol drastically reduces the conformation factor in electrophoretic separation of certain macromolecules in polyacrylamide gels.

DISCUSSION

Polyacrylamide gel electrophoresis fractionates macromolecules according to net negative charge, mass, and conformation. It is the method of choice for analyzing the composition (5) and the positioning of nucleosomes particles (10, 6, 7). The molecular mechanisms at the basis for the resolving power in polyacrylamide gels are still not completely understood, however.

Nucleosomes have a basic disc shape of about 110 Å wide and 60 Å high. These dimensions do not account for the flexible histone C- and N-terminal domains, the location of which is not established (reviewed in (19)). They also do not include any additional lengths of DNA entering or exiting the core particle. The nucleosomal dimensions are therefore significantly larger than the mean pore size in 5% polyacrylamide gels, which was estimated to be around 50 Å (20).

Neither reptation models for migration of long DNA molecules, nor molecular sieving models for separation of globular particles explain the electrophoretic mobility of nucleosome particles satisfactorily. The separation of nucleosomes may follow similar mechanisms as for curved or bent DNA. These models take the elasticity of the gel fibers into account. In one recent model, the total volume of the molecule as sensed by the gel was proposed as the parameter on which separation acts (21).

In the case of nucleosomes varying only in the position of the histone octamer relative to the DNA fragment it seems clear that the lengths of DNA extending from the core particle provide sufficient conformational distinction for separation, presumably by their contribution to frictional drag. The volumes of these differently positioned nucleosomes would hardly differ, but they would sweep out different volumes of gel fibers during electrophoresis. That is, if the pieces of extra DNA would leave the core particle at a certain angle, as with protein-induced DNA bending (8). Centrally located nucleosomes would go slower than end-positioned nucleosomes, and this is what is observed (7, 9). This behavior is not dependent on the source of histones nor their state of acetylation.

Glycerol is often included in polyacrylamide gels because it preserves and even promotes macromolecular assemblies. It also stabilizes protein conformation and enzyme activity (22). Glycerol is expected to lower electrophoretic mobility because it both increases the viscosity and reduces the dielectric constant of the electrolytic medium. The effects of glycerol are often pleiotropic, however, and our study shows that more than a mere reduction of mobility is involved. A clear indication of the more specific action of glycerol is provided by the different effects observed when sucrose, another stabilizing viscosogen, is used instead of glycerol at the same viscosity.

The stabilizing effect of glycerol and sucrose is due to the preferential hydration of macromolecules in glycerol and sucrose solutions (22, 23). These solvent components are excluded from the macromolecular domain, leaving an effective layer of water around the macromolecules. This thermodynamicly unfavorable situation drives protein stabilization and protein assembly by minimizing the surface of contact between macromolecules and solvent (22, 23). Although the effect of glycerol and sucrose is similar, their modes of action differ. In the case of sucrose, which increases the surface tension of water, the free energy required to form a cavity in a solvent with a higher cohesive force is the major factor in stabilization (23). With glycerol, the main reason for preferential hydration and stabilization is the unfavorable interaction of glycerol with nonpolar groups, amplified by enhanced solvent ordering. The effect of glycerol is dependent on protein polarity (22). We propose that the different electrophoretic effects of sucrose and glycerol are due to their different modes of action, and may be explained by the high concentration of polar and charged groups on the macromolecular surfaces.

The distinction between differently positioned nucleosomes may be lost if the stabilizing action of glycerol brings the extending DNA into a different conformation, either by its tendency to reduce the surface of macromolecular complexes, or because the lower dielectric constant favors ionic interactions in the particles. The angle between the pieces of DNA that leave the particle could be reduced, or this extending DNA could be forced closer to the core. Another possibility would be that the layer of preferential hydration around the complex would shield these conformational differences upon interaction with the gel matrix. Conversely, an association of glycerol with the gel fibers could alter their interaction with particles.

The reduction of the retardation of bent DNA also suggests the loss of the conformation factor in gel electrophoretic separations when glycerol is present. Although glycerol has been reported to cause DNA helix unwinding as measured by DNA supercoiling in closed circular DNA (24), CD spectra for linear DNA in up to 60% glycerol did not evidence changes in DNA structure (25). It is not clear how the reduction in retardation of bent DNA in glycerol gels and its enhancement in sucrose gels should be interpreted. These clearly opposite effects of glycerol and sucrose again point to the differences in their modes of action, and not merely to the contribution of viscosity to friction and rotational diffusion during electrophoresis.

At higher percentages of glycerol, core particles are no longer separated from nucleosomes carrying longer DNA. Inspection of the glycerol gradient gel shows that this effect is not a linear function of the glycerol concentration. An insensitivity to the DNA length associated with nucleosomes in 30% glycerol containing nucleoprotein gels has been reported (4). The same study has shown that bound H1 or HMG molecules cause distinct band shifts of nucleosomes. This indicates that glycerol gels do remain very sensitive to mass or charge changes of nucleosomes. It is significant that in 30% glycerol gels, 207 bp nucleosomes migrate faster than core particles. Because the higher net negative charge is the only parameter that could be responsible for this result, it must mean that this component has more influence on electrophoretic mobility at higher glycerol concentrations. With the concomitant loss of the conformation factor, the negative charge may cancel out the contribution of the extra DNA to particle mass. Consequently, glycerol gels show an insensitivity to the DNA length contained in nucleosomes in a wide range of glycerol concentrations.

In addition, glycerol seems to affect the polymerization of polyacrylamide, resulting in a looser gel matrix for the same percentage of gel and acrylamide:bisacrylamide ratio. Just the opposite effect is observed with sucrose. It is conceivable however that not the mean pore size itself is affected, but other properties of the gel matrix, like the size distribution or shape and structure of the pores. Differences in gel pore characteristics affect the electrophoretic mobility of particles by altering the molecular sieving in the gel, and this seems to contribute to some of the glycerol-linked observations.

In conclusion, this study identifies the stabilizing agent glycerol as a factor affecting the sensitivity of polyacrylamide gel

electrophoresis to DNA conformation and possibly macromolecular conformation in general. This insight may lead to a more effective use of regular low ionic strength nucleoprotein gel analyses in the study of nucleosome positioning. On the other hand, inclusion of glycerol in the gel remains clearly indicated if it is the aim to analyze for nucleosome binding proteins or to analyze the composition of macromolecular complexes in general. In these cases, the analysis will also benefit from the stabilizing effect of glycerol.

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