Electrophoretic Transport of Poly(ethylene glycol) Chains through Poly(acrylamide) Gel†

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Separation of a series of poly(ethylene glycols) (PEGs) by sodium dodecyl sulfate−poly(acrylamide) gel electrophoresis (SDS−PAGE) is investigated. Unlike for proteins, preincubation with SDS is not a prerequisite for the electrophoretic movement of PEGs; nevertheless, presence of SDS in the running buffer is essential. The bands are detected by staining with Dragendorff's reagent (iodobismuthate) which forms an insoluble complex with PEG. Exclusion of a stacking gel above the separatory gel better resolves the low molecular weight PEGs (<10000), but high molecular weight PEGs yield well separated bands by both methods. To elucidate the mode of transport, PEGs samples were electrophoresed alongside marker proteins of comparable molecular weight and detected by a two step staining process involving Dragendroff's reagent for the former and coomassie blue for the latter. PEGs moved along with proteins of twice the molecular weight. The possibility of PEG chains migrating with dodecyl sulfate ion (DS^-) cloud is discussed.

Introduction

Poly(acrylamide) gel electrophoresis (PAGE) is an indispensable tool for the analysis of proteins and nucleic acids.[1] While several factors contribute to the popularity of this technique, simplicity of the procedure for multiple samples and modest cost top the list. PAGE of proteins is routinely done in the presence of sodium dodecyl sulfate (SDS) to achieve uniform surface charge density.[2,3] All theoretical attempts to correlate the migration pattern and molecular weight of the charged species take into account the three-dimensional conformation of the macromolecule and the nature of space available to negotiate its path. It is well accepted that the mobility patterns of proteins and DNAs do not conform to a single model. The Ogston−Chrambach−Rodband sieving model[4] best explains the electrophoretic mobility of proteins and small DNA fragments (<1 kbp), while the mobility of large DNA chains (>20 kbp) are modeled on reptation, originally proposed by de Gennes for the movement of macromolecular chains in viscous solutions.[5−7] It is presumed that proteins and smaller DNA fragments assume roughly spherical form, while large DNA chains reptate through the gel matrix alternating between expanded and compact conformations during gel electrophoresis.[8] Synthetic macromolecules do not possess any preferential three-dimensional geometry; they are more flexible to assume any random conformation. Probing the electrophoretic mobility of poly(styrene sulfonates), Smisek et al.[9] and Arvanitidou et al.[10] pointed out the necessity to give more weightage to configurational effects which are not adequately represented either in the Ogston model or the reptation model. They proposed "entropic barriers" transport which takes into account the configurational and translational entropy of the migrating polymer chain.

Where does a macromolecule like poly(ethylene glycol) fit in? Detailed structural analysis has shown that poly(ethylene glycol) chains assume helical conformation in aqueous medium and random coil conformation in benzene.[11,12] A systematic investigation of this class of macromolecules seemed possible since they are easily available commercially in a series of molecular weight ranges and in relatively pure form. Moreover PEG is commonly employed as a biochemical reagent because of its ability to complex with proteins. Hence a comparative evaluation of the electrophoretic mobilities of these two classes of macromolecules seemed desirable. We had perfected the use of Dragendroff's reagent for detecting PEGs in TLC plate.[13] We felt this same technique could be extended to detect PEGs in gels.

Materials and Methods

Materials. PEGs were purchased from different sources as indicated in parentheses: PEG 35000 (Fluka); PEG 23200 (Poly Science, Inc.); PEG 20000 and PEG6000 (local market); and PEG 10000, PEG 8000, PEG 3400, and PEG 2000 (Aldrich). Protein Markers were from Sigma. Acrylamide, *N*,*N*′-methylene bis-acrylamide (BIS) teramethylethylenediammine (TEMED), ammonium persulfate (APS), and sodium dodecyl sulfate (SDS) were of electrophoresis grade from SRL, Bombay, India. Bismuth subnitrate, potassium iodide, tartaric acid, coomasie brilliant blue 250, and all other chemicals were from local market and of highest purity grade available. Doubly distilled water was used for all experiments.

Methods. SDS−PAGE was carried out on gels (1.0 mm thickness) using the gel apparatus of Bio-Tech, Madras, India. Electrophoresis buffer contained per liter3.03 g Tris, 14.4 g glycine, and 1 g SDS. For a typical experiment, after preelectrophoresis for 10 min at 50 V, 2 μL of a solution of PEG homologues, in 1% SDS, 0.1% bromophenol blue, and 10% glycerol, was injected into the wells, and the electrophoresis was carried out at constant voltage for the indicated times at room temperature without buffer recirculation. Experiments were repeated several times to ensure reproducibility and accuracy.

Gels with Separate Stacking Gel. Gels with discontinuous buffer system and a separate stacking gel were cast in two steps.

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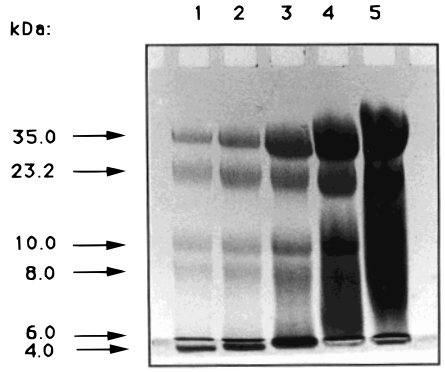

Figure 1. Electrophoresis of PEGs on 12% SDS−poly(acrylamide) gel without stacking gel. The gel was run at 10 V/cm for 100 min after preelectrophoresis for 10 min at 5 V/cm: Lane 1−5: 2 μg, 5 μg, 10 μg, 20 μg, and 40 μg, respectively, of each PEG homologue as indicated by arrow.

A pregel solution containing required amounts of acrylamide−BIS mixture (30.0:0.8), 0.1% SDS, 0.38 M Tris.HCl (pH 8.8), APS, and TEMED was poured into the glass mold and allowed to set. Stacking gel (4%) (1.3 cm) was cast on top of this separatory gel from a solution of required amount of acrylamide−BIS mixture (30.0:0.8), 0.1% SDS, and 0.125 M Tris.HCl (pH 6.8), with APS and TEMED as polymerization agents.

Gels without Separate Stacking Gel. The pregel solution poured as above to a level of 7.3 cm and without the stacking gel.

Staining of Gels. Dragendorff's reagent (iodobismuthate) was used to stain PEGs because it forms an insoluble orange stoichiometric complex with PEG.[14] The staining solution was prepared by mixing equal volumes of potassium iodide solution (40 g of potassium iodide was added in 100 mL ~10%(w/w) tartaric acid aqueous solution) and a saturated aqueous solution of bismuth subnitrate. For the gels containing both PEGs and proteins, the staining was done in two steps: first with Dragendorff's reagent for 10−15 min, second coomasie blue.

Gel Permeation Chromatography. GPC was run on a Shimadzu unit, fitted with a RI detector using Ashaipak amphiphilic column with water as an elutent at a flow rate of 1 mL/min. The calibration was done against PEG standards.

Results and Discussion

Staining Method. Kurfurst,[15] Zimmerman, and Murphy[16] report the use of barium iodide solution to stain the pegylated proteins and also PEGs after SDS−PAGE. In comparison, the present staining process is much faster; a few minutes compared to more than an hour as reported by them. The efficiency is up to 0.5 μg of PEG. Gels, when soaked in the Dragendorff's staining solution after electrophoresis, developed deep orange colored bands of PEG immediately. After 10−15 min, the staining solution was replaced and the gels were put into water for destaining. Without destaining, the band pattern can be stored for months. Destaining leads to fading of the bands but this could be regenerated by repeating the staining procedure. The staining solutions can be reused several times and can be stored for months.

Band Characteristics. We have observed that loading and molecular weight of the samples have bearing on the characteristics of the bands (Figure 1). Best results were obtained when PEG loading was low (~5 μg). Heavier loadings resulted in overlapping, broad bands. Zimmerman and Murphy[16] have discouraged the use of stacking gel to get better bands. However, we found this valid only for low MW PEGs (<10000); indeed,

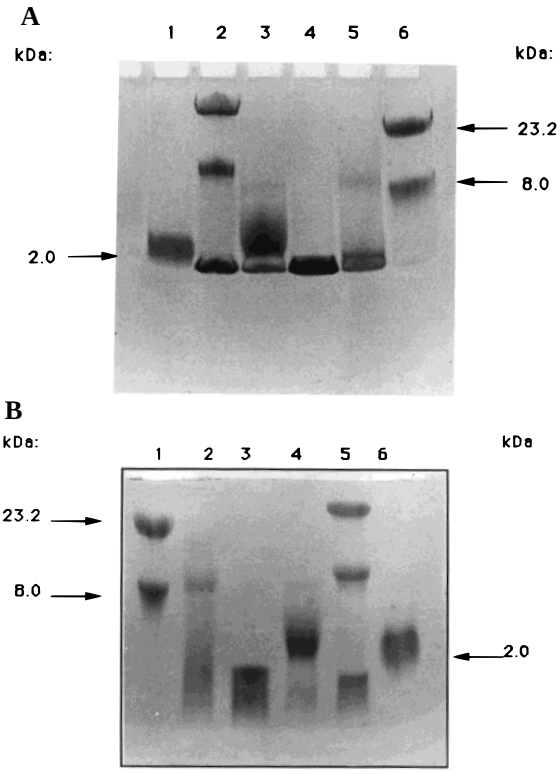

Figure 2. Electrophoresis of PEGs on 17% SDS−poly(acrylamide) gel with and without stacking gel. Both the gels were run at 10 V/cm for time as indicated after preelectrophoresis for 10 min at 5 V/cm: (A) without stacking gel, 100 min. The amount of each PEG sample loaded was 8 μg: lane 1, PEG 2000; lane 2, PEG 35000, 10000, and 4000; lane 3, PEG 6000; lane 4, PEG 3400; lane 5, PEG 20000; lane 6, PEG 23200 and 8000. (B) With stacking gel, 120 min. Same amount of samples were loaded in reverse order as lanes 1−5 in (A).

for higher PEGs the band quality slightly improved with stacking gel (Figure 2). We made a repeated observation that PEGs of MW less than 3400 did not move according to their sizes but migrated as broad zones (Figure 2). Zimmerman et al.[16] also report the difficulty in electrophoresing PEGs lower than 4000. It has been reported that the nature of the interaction of SDS with PEGs of molecular weights lower than 4000 is markedly different from that of SDS with high molecular weight PEGs.[17] This might be one of the possible reasons for the abnormal location of PEG 2000 in Figure 2.

We have used 10% − 20% gel both with and without stacking gel for separation of various PEGs. For calculating the relative mobility of the different PEGs, we have taken the midpoint of the band as the reference point. Figure 3 shows the plot of relative mobility against logarithm of the molecular weight of PEG for both with and without stacking gel. Visual comparison of the molecular weight distribution of PEGs by electrophoresis and GPC show good correspondence (Figure 4). We could carry out only visual matching. We did attempt a quantitative comparison by measuring the bandwidth in SDS−PAGE and calculating the mol.wt. spread. However, the error involved was too high because of the diffuse nature of the bands. A more accurate method would be to use the densitometer tracings of the PEG bands using a gel scanner. Unfortunately, this technique is not available to us. The polydispersed nature of the PEGs 20000 and 6000 is reflected as broad bands in SDS−PAGE compared to PEGs 35000, 10000, and 4000. We also tried separately other synthetic polymer like poly(vinyl pyrrolidone) (PVP) but the bands appeared as smears.

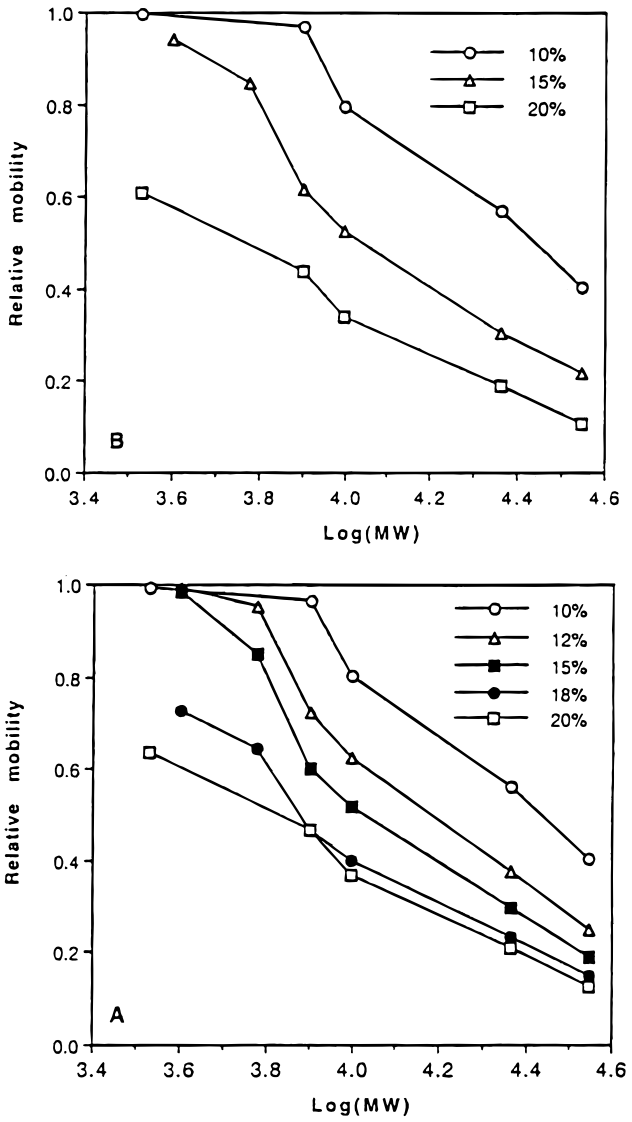

Figure 3. Relative mobilities of PEGs on 10%−20% SDS−poly(acrylamide) gels (A) with and (B) without stacking gel. The points are distance of migration of the bands expressed relative to that of bromophenol blue.

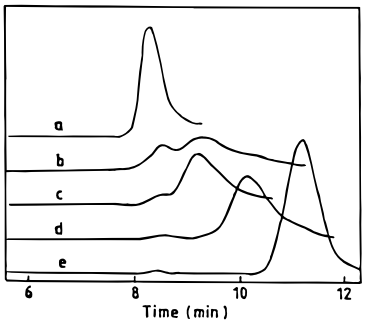

Figure 4. GPC profile of PEGs: a−e are PEGs 35000, 20000, 10000, 6000, and 4000, respectively.

Mobility of PEGs vs That of Proteins. We were successful in generating both proteins and PEG bands in tandem (Figure 5). This helped in the quantitative comparison of the mobility of the two classes of macromolecules. Proteins of comparable molecular weight moved faster than the corresponding PEG. PEG of molecular weight *M* moved alongside a protein of almost 2*M*. PEGs and proteins yielded nearly parallel straight lines when relative mobilities were plotted against logarithm of the

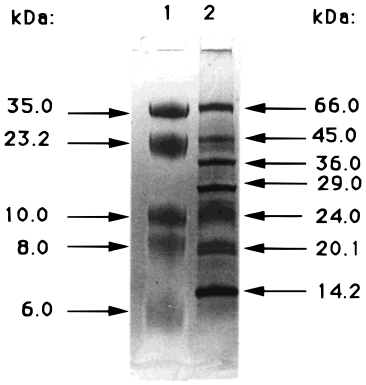

Figure 5. SDS−poly(acrylamide) gel showing the protein and PEG bands in tandem. The 15% gel without stacking gel was run at 12 V/cm for 90 min after preelectrophoresis for 10 min at 5 V/cm. Lane 1 and lane 2 represent PEG and protein bands, respectively. Two micrograms of each of the PEG and protein samples (as indicated by arrow) was loaded.

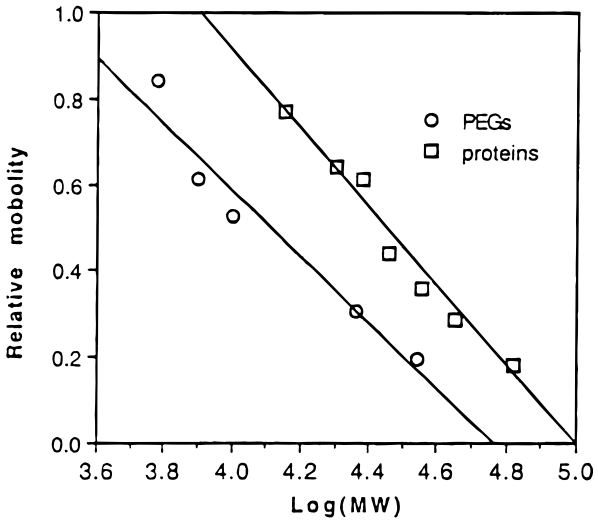

Figure 6. Relative mobilities of PEGs and proteins on 15% SDS−poly(acrylamide) gel without stacking gel. The points are distance of migration of the bands in lane 1 and 2 of Figure 5 expressed relative to that of bromophenol blue.

molecular weight (Figure 6). This is in agreement with the observations of Kurfurst.[15] Measurements of the intrinsic viscosities of [protein−SDS] complexes suggest a rigid prolate ellipsoid model for the complex.[18] In contrast, there are conflicting views on the structure of [PEG−SDS] aggregates and the location of PEG chain therein. A PEG chain in aqueous solution behaves as a highly mobile molecule with a large exclusion volume. Gel chromatography experiments also establish that PEGs are much larger in solution than many other molecules of comparable molecular weight especially proteins.[19,20] Thus the larger hydrodynamic size of PEG, together with the possibility that charge-to-mass ratio of the [PEG−SDS] complex could be lesser than that for proteins, satisfactorily accounts for the reduced mobility of [PEG−SDS] complex in comparison to that of [protein−SDS]. In fact, the sieving model implies that macromolecules of similar molecular weight but different mean molecular radii will have different mobilities.

On the Mechanism of Migration: The [PEG−SDS] Complex. To ascertain the electrophoretic mobility of the [PEG−SDS] complex, we carried out PAGE under different conditions: (i) preincubation of PEG with SDS and electrophoresis with 0.1% SDS in the running buffer, (ii) No preincubation with

SDS but with SDS in the running buffer, and (iii) preincubation with SDS with no SDS in running buffer.

We could observe electrophoretic mobility of PEG in the first two cases. This is in contrast with the behavior of proteins as reported by Stoklosa and Latz.[21] They observed that preincubation with SDS was enough for the electrophoretic mobility of proteins; the presence of SDS in the running buffer was not essential. It is indeed intriguing that just the reverse holds good for PEGs.

Several studies have been reported on the nature of interaction between SDS and PEGs.[17,22−27] While no consolidated, cohesive picture emerges from these studies, there is a general agreement that the interaction between PEG and SDS occurs at three distinct levels. At low SDS concentrations there is no interaction between the polymer and the surfactant. In a critical concentration range, the surfactant binds to the polymer chain in the form of aggregates; above this range these aggregates coexist with free SDS micelles. Just as for proteins, we used 1% (w/v) SDS for preincubation of PEGs. This corresponds to a situation where SDS micelles coexist with [PEG−SDS] complex and ensures saturation of PEG with SDS. The apparent distribution coefficient of PEG in SDS micellar system is reported to be dependent on the PEG−SDS concentration ratio, saturation by SDS occurring at a ratio of one SDS per one EO unit.[17]

It has been suggested that the PEG−SDS interaction is mostly electrostatic.[22−27] In comparison, the protein−SDS interaction is mostly hydrophobic in nature. According to Schwuger and Bartnik,[28] initially SDS molecules might preferentially interact with cationic amino acid residues such as lysyl, arginyl, histidyl, etc., at the protein surface, consequently unfolding the protein molecule. This would expose the hydrophobic domains of the protein for favorable interaction with the hydrophobic alkyl chains of the surfactants resulting in a stabler complex.

The electrophoretic mobility of [protein−SDS] complex in the absence of SDS in the running buffer implies that the complex is mostly intact under the applied dc field. The inability of the [PEG−SDS] complex to move under similar circumstances has been attributed to the weak binding of SDS to PEG by Zimmerman et al.[16] It could very well be that under the conditions of the experiment, the weak complex dissociates and that perhaps PEG is present as neutral species. PEG depleted of the SDS will not move since the primary driving force for the electrophoretic mobility of a species is the accelerating force exerted by the dc field on the electric charge of the species.[29] If SDS is present in the running buffer, then the continuous stream of DS^- ions might form a transient complex and drag the PEG chain forward. This drag will depend on the size of the PEG chain which conforms to the PEG chain migrating with a constantly renewing DS^- ion cloud.

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