perature. As pointed out in ref 4, the assumption that the dearth of configurational entropy in the region of the glass temperature is the essential basis of the glass transition accounts for the fact that the glass temperature is always found below the melting point in the case of crystallizable substances.
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(13) This expression for the entropy differs from that in ref 2 by a term $1/x \ln (Sx/2)$. This term was unintentionally omitted in ref 2.
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Fractionation of Electroneutral Polymer by Gel Electrophoresis in the Presence of Ionic Detergent

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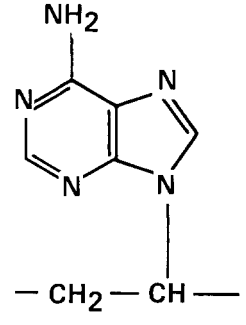
ABSTRACT: An electroneutral polymer, poly(9-vinyladenine), in aqueous solutions binds an ionic detergent and thus acquires electromobility; the polymer can then be fractionated by electrophoresis in polyacrylamide gels and size proportional fractionation is achieved through the sieving action of the gel. Binding of sodium dodecyl sulfate was measured by equilibrium dialysis, and values of 0.45 g of detergent/g of polymer were obtained at 3–4 mM detergent concentration and 0.05–0.1 ionic strength.

The estimation of molecular weight of macromolecules is of importance both in polymer chemistry and biochemistry. In recent years a method involving a modified system of gel electrophoresis[1,2] has gained wide acceptance in the field of protein research. In this method a protein is chemically reduced to a single polypeptide chain, or chains, and these are equilibrated with a solution of the anionic detergent, sodium dodecylsulfate (SDS). Polypeptides bind the detergent and this process is believed to confer on them a size proportional electric charge and also a more homogenous conformation.[3–5] Electrophoresis is then performed using gels of cross-linked polyacrylamide and polypeptides are separated according to their molecular weight through the sieving action of the gel.[1,2] In the field of synthetic polymers, electrophoresis has been used rarely (ref 6 lists 48 references). There is considerable evidence that, similarly to proteins, the synthetic polymers bind strongly the detergents in a similar manner to proteins. Anionic detergent was found to solubilize water insoluble polymers (e.g., poly(vinyl acetate)) and the solubilized polymers behave like polyanions with highly expanded chains.[7] SDS furthermore dissociates[8] some complexes formed by poly(9-vinyladenine) [poly(vA)]. This led us to study the applicability of the SDS gel electrophoresis method to a synthetic polymer. Using poly(vA) we found that the fractionation of the polymer is easily achieved.



poly (vA)

Results and Discussion

The anionic detergent, SDS, above a certain concentration exists in solution as an equilibrium mixture of monomeric and micellar forms.[3] The binding of SDS to poly(vA) was investigated by equilibrium dialysis and values around 0.45 g SDS/1.0 g poly(vA) were obtained (Table I). The conditions

Table I
Binding of SDS to Poly(vA)

Ionic conditions	SDS, total concn, mM	SDS monomer, mM	SDS in micellar form, mM	Binding obsd, g SDS/g poly(vA)	Av binding, g SDS/g poly(vA)
Sodium phosphate buffer, pH 7, ionic strength 0.05	3.37	2.30	1.07	0.45, 0.48	0.46
Sodium phosphate buffer, pH 7, ionic strength 0.05	4.41	2.30	2.11	0.46, 0.38 0.55, 0.43	0.45
Sodium chloride buffer, ionic strength 0.1	3.07	1.25	1.82	0.38, 0.44	0.41

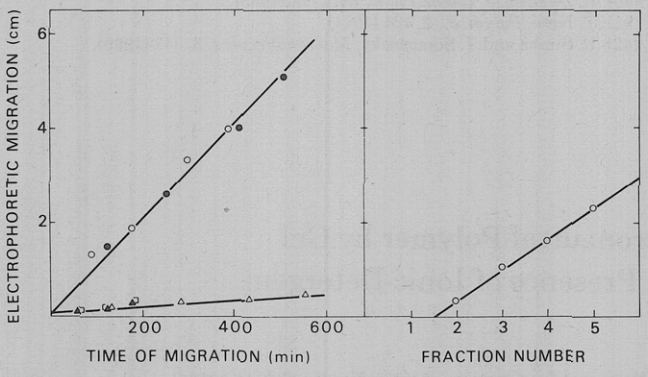


Figure 1. (Left) Effects of SDS concentration on the gel electrophoretic mobility of poly(vA): (△) 0.0% SDS; (▲) 0.001% SDS; (□) 0.01% SDS; (●) 0.1% SDS; (○) 0.2% SDS. Electrophoretic procedure and the detection of polymer are described in the Experimental Section. Unfractionated poly(vA) was used and the samples of polymer were, before electrophoresis, dialyzed against a large volume of solution containing SDS at the indicated concentrations. (Right) Electrophoretic migration of poly(vA) of different molecular weight, 0.1% SDS in buffer. Conditions are described in the Experimental Section; time of electrophoresis was 2 h.

used were similar to those used for gel electrophoresis and small variations in ionic conditions, which change the concentration of monomeric SDS, did not change the binding appreciably. An increase in the amount of micellar form of SDS did not effect binding (Table I); thus the polymer–micelle interaction is not important.

When poly(vA) was subjected to electrophoresis in polyacrylamide gels the mobility in the absence of SDS was negligible, but when SDS was included (0.1%) considerable mobility resulted; the distance travelled by the polymer increased linearly with the total amount of electricity passed through the electrolyte (Figure 1). To achieve any noticeable electrophoretic mobility of the polymer the SDS concentration must be above a certain threshold; the concentration of SDS which is routinely used for electrophoresis of proteins (0.1%) is saturating for poly(vA) as well, as a further increase does not change the mobility of polymer (Figure 1). The results show that any possible polymer–micelle interaction does not influence the electrophoretic separation. The electrophoretic mobility of the polymer through the gel is a function of the molecular size of the polymer. Poly(vA) was fractionated in the presence of SDS by gel filtration on a Biogel P300 column. When the fractions were tested for their electrophoretic mobility in gels a linear dependence between the column elution volume and the mobility was observed, lower molecular size fractions having higher mobility (Figure 1). In what follows, it is assumed molecular size is uniquely determined by molecular weight.

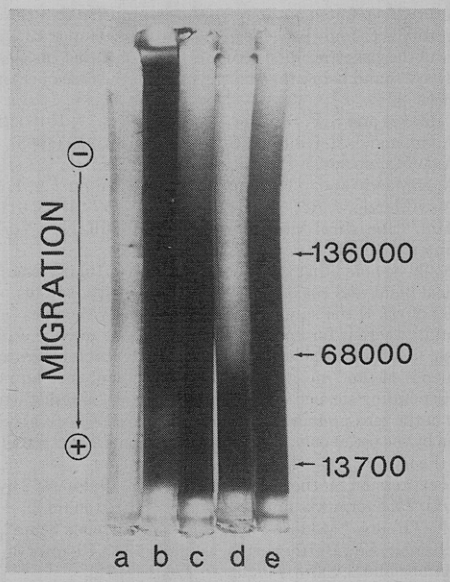


Figure 2. Electrophoresis of different preparations of poly(vA). Time of the electrophoresis was 4 h, 0.1% SDS in buffer, other conditions and detection were described in the Experimental Section. Gel a, no sample; gel b, product of polymerization of 9-vinyladenine in water prepared as described before.[9] In subsequent gels, products of polymerization of 9-vinyladenine (100 mg) in 10 ml of solvent (N_2 purged) by 8 mg of potassium persulfate for 10 min at 100 °C were analyzed. Gel c, solvent–water only; gel d, solvent 4:1 (v/v) water tetrahydrofuran; gel e, solvent 1:1 (v/v) water tetrahydrofuran. On the right-hand side are indicated positions of protein molecular weight standards, as measured in a separate electrophoresis run where the concentration of SDS was decreased to 0.009%.

Gel electrophoresis of electroneutral polymers in SDS solutions thus represents a suitable method for their fractionation. The method is extremely easy to apply as illustrated in the following example involving the low molecular weight preparation of poly(vA) which was required for biological testing. Various amounts of the chain transfer agent, tetrahydrofuran, were added to the aqueous polymerization mixture of 9-vinyladenine.[8,9] The polymeric fractions produced were isolated by dialysis and their molecular weights were compared by electrophoresis in gradient gels. In these gels the porosity is continuously increased and thus separation of compounds of very different molecular weights is possible. Results (Figure 2) obtained in the experiment which took half a day showed that a 4:1 mixture of water–tetrahydrofuran was the most suitable for the preparation of a polymer of uniformly small molecular weight.

The electrophoretic mobilities through the gels of polypeptides in SDS solutions are a function of their molecular weights and do not depend on their amino acid composition.[1–3,10] This very useful property is thought to be due to the uniform binding of SDS to protein.[3–5] If SDS concentration is over 0.02% around 1.4 g of SDS is bound/g of protein. At lower concentration of detergent (around 0.01%) only 0.4 g of SDS/g of protein is bound; this amount apparently coincides with the amount of SDS bound/g of poly(vA) at higher concentrations of detergent (Table I). If the arguments concerning the critical importance of the uniformity of SDS binding to proteins[3–5] are applicable to other macromolecules as well, then the molecular weight–electrophoretic mobility relation of poly(vA) could then be compared with molecular weight–electrophoretic mobility relation of proteins at low

concentrations of detergent; such a tentative comparison is made in Figure 2. Such a method which depends upon the comparison of polymers with proteins has the advantage that the molecular weights for proteins are well documented and hence the absolute molecular weight standards for synthetic polymers would readily become available. However, a definite evaluation of this comparison cannot yet be made.

The use of the electrophoretic method for the estimation of molecular weights of polymers presents the problem of detecting the polymer in the polyacrylamide gel. Three main detection methods are employed in biochemical applications and similar methods may be used for synthetic polymers. Direct detection, as illustrated in Figure 2, is possible when the polymer has a strong absorption band at 250 nm or at a longer wavelength. An alternative method involves the binding of an organic dye to protein[2] or to polymer;[7] some polymers can be made visible by iodine.[11] The last method involves a radioactive alkylation agent (Pitha, manuscript in preparation) or formaldehyde, which are available commercially. The distribution of radioactive label through the gel after electrophoresis can be measured easily.[2]

Experimental Section

Equilibrium Dialysis. Bags made of visking tubing containing 1 ml of solution of poly(vA) in SDS–buffer (Table I) were equilibrated at room temperature with 1 ml of SDS–buffer in stoppered tubes of about 3-ml volume. Slow rotation for 12 h was sufficient to attain equilibrium; the volume changes during dialysis were less than 10%. The concentration of SDS was assayed in the following way. A sample of 75 μl was added to the mixture of 15 ml of chloroform and 5 ml of methylene blue solution (400 mg in 1 l. of water) contained in a scintillation vial (20 ml size). The mixture was shaken for 1 h at room temperature then left to separate. The chloroform phase was cleared by centrifugation in a clinical centrifuge and the absorbance at 655 nm was recorded in a 1- or 0.5-cm cuvette. The concentration of SDS is a linear function of the recorded absorbance; the presence of the polymer does not influence the procedure. The amount of bound SDS was calculated from the difference of SDS concentrations inside and outside the bag.

Gel Electrophoresis. Gradient gels used in experiments in Figure 2 were purchased from Isolab Inc., Akron, Ohio. Standard gels (used in experiments in Figure 1) were prepared according to the Weber–Osborn[10] procedure. The gel buffer contained 7.8 g of $NaH_2PO_4 \cdot H_2O$, 38.6 g of $Na_2HPO_4 \cdot 7H_2O$, and 2 g of SDS in 1 l. of water. Acrylamide solution contained 22.2 g of acrylamide and 0.3 g of methylenebisacrylamide in 100 ml. To prepare the gel, 15 ml of gel buffer was mixed with 13.5 ml of acrylamide solution and 50 μl of *N,N,N′,N′*-tetramethylethylenediamine and the resulting solution was deareated by short evacuation. Then 1.5 ml of the solution of potassium persulfate (15 mg/ml) was added and the solution poured into glass tubes (100 mm long, i.d. 5 mm) and overlayered by a few drops of water; gelation occurred within 20 min and tubes were stoppered and stored.

The electrophoresis was performed in the gel buffer diluted 1:1 with water. Tubes were prerun at 60 V for 30 min, then the sample of the polymer was applied (about 100 μg of poly(vA) in 40 μl of solution of 1% SDS and 10% glycerol in water) and the separation was performed at 60 V for a specified period of time. The presence of poly(vA) could be visualized by uv light. A fluorescent TLC plate (e.g., PLC plate F-254, Merck, Darmstadt) was covered by a uv transparent plastic sheet (e.g., Handi-Wrap, Dow Chemical Co., Midland, Mich.) and the gels were layered on such a fluorescent screen. The presence of a uv abosrbing compound in the gels was detected by a mercury lamp equipped with a light filter (e.g. Mineralight UVS 12, Ultraviolet Products, Inc., San Gabriel, Calif.); see Figure 2 for results. Alternatively gels were scanned in a modified uv spectrophotometer at 265 nm and the position where absorbance was above 2 was taken as a front of the band; this procedure was used for the data in Figure 1.

Fractionation of Poly(vA). Polymer (180 mg) prepared according to the method of Kaye[9] was fractionated on Biogel P300 column (2.5 cm i.d., bed 23 cm long) using gel buffer diluted 1:1 with water.

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