Use of polynucleotide/polyacrylamide-gel electrophoresis as a sensitive technique for the detection and comparison of ribonuclease activities

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A technique is described in which the incorporation of a polynucleotide substrate into the matrix of a polyacrylamide gel allows the use of electrophoresis for the detection of polycationic ribonuclease activity rather than simply the presence of protein. Because use is made of the catalytic properties of ribonucleases, polynucleotide/polyacrylamide-gel electrophoresis is approximately 10^5 times more sensitive for the detection of these enzymes than conventional gel electrophoresis with the use of protein-staining dyes. Initial studies showed that the poor migration, in the gels, of highly charged polycationic ribonucleases in the presence of negatively charged synthetic polynucleotides could be overcome by high concentrations of spermine. The positively charged polyamine, by neutralizing the polyanionic polynucleotide, enabled these basic enzymes to migrate considerable distances in the gel. Electrophoresis of the RNAases under conditions of low pH, and incubation of the gel at neutral pH followed by staining for polynucleotide, resulted in coloured gels containing clear bands that define regions of enzyme activity. Alterations in spermine concentration or substrate identity caused changes in the positions of these bands, suggesting a dynamic interaction among the enzyme, polyamine and polynucleotide. Because of the advantages, in terms of selectivity and sensitivity of polynucleotide/polyacrylamide-gel electrophoresis, this technique was used to demonstrate the nuclease homogeneity of three purified bovine muscle enzymes, and to compare these enzymes with each other, as well as with bovine pancreatic ribonuclease A.

In the preceding paper (Davies *et al.*, 1980) the properties of three polycationic RNAases from bovine muscle were described. In many respects these enzymes presented considerable difficulties in protein characterization because of their low tissue concentrations. The task, for example, of establishing the homogeneity of each RNAase becomes a formidable one. Similarly, the paucity of enzyme protein restricts any study of the interrelationships between the muscle enzymes and other bovine RNAases. These restrictions are a direct consequence of the limited sensitivity of the two stains (Coomassie Blue and Amido Black) most commonly used to locate proteins in polyacrylamide-gel electrophoresis. Further, although polyacrylamide-gel electrophoresis is a powerful tool for the separation and comparison of proteins, neither Coomassie Blue nor Amido Black is selective, so that such individual enzymes as the bovine muscle

RNAases are not distinguishable from other proteins.

In the present paper we describe a technique called polynucleotide/polyacrylamide-gel electrophoresis, in which the catalytic properties of the enzyme itself are used both to define its position within the gel and to give an indication of the nature of the catalytic activity present.

Although earlier attempts have been made to locate enzyme activities within gels (Wilson, 1969, 1971; van Loon, 1975), in general the techniques suffered from a lack of sensitivity, from denaturation of enzyme activity (Randles, 1968; Rabin & Weinberger, 1975; Rosenthal & Lacks, 1977) and from poor reproducibility.

The method described in the present paper obviates these difficulties by use of the following procedure. A synthetic polynucleotide is incorporated directly within the matrix of the gel. To permit the migration of a positively charged protein in the presence of a polyanionic polynucleotide,

Abbreviation used: RNAase, ribonuclease.

spermine was incorporated into the gel as well as the sample solutions and running buffer. After electrophoresis of the protein sample the gel is incubated to induce hydrolysis of the polynucleotide in the regions of the gel containing enzyme. It is then stained with a polynucleotide-specific dye; the clear regions in an otherwise coloured gel reveal the presence of RNAase activity. An important advantage of the method is that it increases sensitivity considerably, allowing detection, for example, of as little as 10pg of bovine pancreatic RNAase A. In addition, depending on the concentration of spermine, and the identity of the substrate, a single protein will have, not one, but a characteristic set of mobilities. These readily obtainable sets of mobilities for different enzymes can be used to facilitate comparisons among those proteins with a minimal expenditure of materials.

Experimental

Materials and general methods

Electrophoresis-purity reagents (acrylamide, *NN'*-methylenebisacrylamide, ammonium persulphate, *NNN'*-tetramethylethylenediamine and Pyronin Y), as well as select bore glass tubes (5.5 ± 0.1 mm inner diameter $\times$ 125 mm), were the products of Bio-Rad Laboratories, Richmond, CA, U.S.A. Methyl Green was the product of BDH Chemicals, Poole, Dorset, U.K. Coomassie Blue, polyamine hydrochlorides, highly polymerized yeast RNA (type XI) and β -alanine were obtained from the Sigma Chemical Co., St. Louis, MO, U.S.A. Synthetic polynucleotides were the products of Miles Laboratories, Elkhart, IN, U.S.A. RNAase A from bovine pancreas was supplied by Worthington Biochemical Corp., Freehold, NJ, U.S.A. Details of the purification of bovine muscle RNAases, as well as the procedure for measuring enzyme activity, are described in the preceding paper (Davies *et al.*, 1980).

Buffers and solutions for electrophoresis

Solution A was 0.48M-KOH containing 17.2% (v/v) acetic acid and 4.0% (v/v) *NNN'*-tetramethylethylenediamine (pH4.3). Solution B contained 30% (w/v) acrylamide and 0.8% (w/v) *NN'*-methylenebisacrylamide. Solution C was 0.48M-KOH containing 2.9% (v/v) acetic acid and 0.46% (v/v) *NNN'*-tetramethylethylenediamine (pH5.8). Solution D contained 12.5% (w/v) acrylamide and 2.0% (w/v) *NN'*-methylenebisacrylamide.

Small-pore gel solution. To 20 ml of either aq. 0.06% poly(U) or aq. 0.10% highly polymerized yeast RNA were added 5 ml of solution A and 10 ml

of solution B. Thorough degassing of the resultant mixture was accomplished at reduced pressure.

Large-pore gel solution. A 1 ml portion of solution C was added to 2 ml of solution D and 4 ml of water and the resultant mixture was degassed at reduced pressure.

Running buffer. This was 0.35M- β -alanine containing 0.8% (v/v) acetic acid (pH4.5).

Sample loading solution. This was 50% (v/v) glycerol containing 0.05% Methyl Green.

Staining solution A. This was 25% (v/v) methanol containing 7% (v/v) acetic acid and 0.25% Coomassie Blue.

Staining solution B. This was 7% (v/v) acetic acid containing 1.0% Pyronin Y.

Polynucleotide/polyacrylamide-gel electrophoresis

Preparation of gel tubes. All glass tubes were immersed in dichromate cleaning solution for a minimum of 4 h, then washed extensively with water. Drying was preceded by a final wash with 95% ethanol.

Preparation of polyacrylamide gels containing yeast RNA or poly(U). The following method was used for the preparation of 20 cylindrical gels having two sections: (a) a small-pore section, 7.5 cm in length, consisting of 7.5% acrylamide, 0.2% of *NN'*-methylenebisacrylamide and containing 0.03% poly(U) or 0.05% highly polymerized yeast RNA; (b) a short (0.8 cm) large-pore section consisting of 3.1% acrylamide and 0.5% *NN'*-methylenebisacrylamide. Spermine (25 mM) was present in both segments.

Freshly prepared ammonium persulphate solution (5 ml of 0.6%) was added to 35 ml of the small-pore gel solution containing 348 mg of spermine tetrahydrochloride. This mixture was dispensed into glass tubes to a height of 7.5 cm and overlaid with a small quantity of water. After 1 h, polymerization was complete and the water layer was removed. Ammonium persulphate solution (1.0 ml of 0.6%) was then added to 7 ml of large-pore gel solution containing 69.6 mg of spermine tetrahydrochloride. The tops of the small-pore gels were washed with this mixture, after which 0.25 ml of the mixture was added to each of the small-pore gels. Water was used to overlay the unpolymerized segment. Polymerization was completed in approx. 1 h.

Sample preparation and electrophoresis conditions. All operations were conducted at 4°C. Sample loading solution (50 μ l), containing 50 mM-spermine tetrahydrochloride, was added to 50 μ l of RNAase solution. This mixture was layered on the top of the gel and electrophoresis was accomplished at 8.0 mA/gel in running buffer containing 25 mM-spermine tetrahydrochloride. After 4 h, the very narrow (<1 mm) dye front traversed the length of the gel. A

disc-electrophoresis apparatus having a capacity of 18 sample tubes (model 3-175A; Buchler Instruments, Fort Lee, NJ, U.S.A.) was used.

Incubation, staining and destaining of gels. Gels were removed from the glass tubes with a gel tube eliminator (Bio-Rad Laboratories) and were incubated overnight (24°C) in 3.5 litres of 0.1M-Tris/HCl buffer, pH 7.6, containing 10mM-NaCl. A gel-electrophoresis diffusion destainer (Bio-Rad model 172A) without the cartridge of activated charcoal was used to ensure rapid flow of the buffer around the gels. The pH of the buffer changed by less than 0.1 pH unit as a result of gel incubation. Staining was accomplished with either staining solution A or B (Coomassie Blue or Pyronin Y respectively) for 1.5 h at 37°C. Gels were then destained overnight in a diffusion destainer with 7% acetic acid.

Spectrophotometric scanning of gels. Destained gels were scanned (5mm/min) at 546 nm with a dual-beam gel scanner (model 1310; Instrumentation Specialties, Lincoln, NE, U.S.A.) linked to an absorbance monitor (model UA-5, equipped with type 6 optical unit) and a multiplexer-expander (model 1133). Gels run without enzyme served as the reference. Reference gels could also be prepared after the electrophoresis of enzyme by incubating the gels for 15 min at 100°C before the staining. The reference gels obtained by either procedure were identical in all respects. Absorbances were automatically graphed by using a computer (model 3385A automation system; Hewlett-Packard, King of Prussia, PA, U.S.A.) that also listed the times elapsed from the beginning of the scan to each peak. These times provided the basis for the calculation of peak R_F values. All R_F values are relative to the Methyl Green dye marker run with each sample.

Results

In the absence of spermine, there was no migration of small quantities of bovine pancreatic RNAase A in gels containing 0.05% poly(U). As the amount of enzyme added to the gel was increased from 0.114 to 11.4 μ g, the clear band at the origin, representing enzyme activity, widened considerably, and resembled a streak ranging from the origin to R_F 0.63. The concentration-dependency of the width of this streak probably arises as a result of saturation of anionic substrate sites by the positively charged enzyme.

Effect of spermine on migration of RNAase A in polynucleotide/polyacrylamide gels

The binding of RNAase to polynucleotide can be decreased if the negatively charged sites on the polyanion are titrated before application of the enzyme to the gel. This may be accomplished by

using spermine in the preparation of polynucleotide/polyacrylamide gels. As shown in Fig. 1, raising the concentration of spermine within the gels, as well as in the running buffer, causes an increased mobility of RNAase A. The relationship between mobility and spermine concentration is fourth-order (see Fig. 1 and the Appendix), so that progressive increases in the concentration of the polyamine are accompanied by diminished increases in R_F . After some experimentation, satisfactory enzyme mobilities were obtained at a spermine concentration of 25 mM. At this polyamine concentration, a clear band of enzyme activity was seen at the same R_F over a wide range (0.1–114 ng) of RNAase A concentrations. This suggests that

Fig. 1. Mobility of bovine pancreatic RNAase A on polynucleotide/polyacrylamide gels as a function of spermine concentration

Polyacrylamide gels (7.5% as the monomer) containing 0.05% yeast RNA (○) or 0.03% poly(U) (●) were prepared as described in the Experimental section, except that the amount of spermine was varied as indicated (1.0, 10.0, 25.0 and 50.0 mM). The conditions for the preparation of sample, as well as those for electrophoresis, were as detailed in the Experimental section, except that the material applied to the gel consisted of 50 μ l of the loading solution, 50 μ l of bovine pancreatic RNAase A solution (2.28 μ g/ml) and a concentration of spermine identical with that in the gel. Also, at the highest concentration (50 mM) of spermine, 12.0 mA per gel instead of 8.0 was necessary to achieve satisfactory rates of migration. The running buffer contained the same concentration of spermine as that within the gel. After electrophoresis, gels were incubated for 2 h at 4°C in 3.5 litres of 0.1 M-Tris/HCl buffer, pH 7.6. They were then stained in Pyronin Y for 30 min at 37°C. Lines through the data points were fitted by the least-squares method and corresponded to the following equations: mobility (R_F) of RNAase A in gels containing yeast RNA = $2.35 [\text{spermine}]^4 + 0.80$ (----); mobility (R_F) of RNAase A in gels containing Poly(U) = $2.78 [\text{spermine}]^4 + 0.34$ (—). See the text and the Appendix for further details.

strong interactions between the enzyme and the polynucleotide within the gel did not take place during electrophoresis. The presence of polynucleotide, however, does influence the migration of enzyme to some extent. A decrease in the poly(U) concentration within the gel from 0.05 to 0.03%, for example, resulted in an increase of 30% in enzyme R_F . Decreasing the amount of polyacrylamide from 7.5 to 6.5% or 5.5%, however, had no effect upon RNAase A mobility.

Incubation conditions and the sensitivity of the staining reaction

After electrophoresis at pH 4.5, enzyme activity can be located by incubating the gels in Tris/HCl buffer, pH 7.6. This is conveniently and efficiently done by using a diffusion destainer wherein a high rate of flow of buffer past the gels is maintained. Use of a large volume of buffer ensures that no change in the pH of this buffer will occur as a consequence of gel incubation. As with any enzyme assay, the development of a band representing RNAase activity is responsive to changes in incubation conditions. After electrophoresis of RNAase A (specific activity 0.22 A_{260} unit/ng), and incubation of the gels at pH 7.6 for 1.5 h at 4°C, bands of enzyme activity could be detected only if at least 90 ng of enzyme protein were present. Changing the incubation conditions led to a substantial increase in sensitivity of the reaction. Thus, with the incubation period raised to 16 h and the temperature to 24°C, enzyme activity could be detected with as little as 11.4 pg of RNAase A per gel. Shorter incubation times (1–5 h) gave, at best, a very weak band. At temperatures higher than 24°C no increase in sensitivity was seen, regardless of incubation time. The small amount of enzyme (approx. 10 pg) necessary for detection of activity contrasts sharply with the minimum quantity (1.0 μ g) necessary for detection of protein by using Coomassie Blue protein staining techniques (Weber & Osborn, 1969).

It is noteworthy that the concentration of salt present in the sample applied to the gel before electrophoresis has little influence on the mobility of RNAases. Identical R_F values, for example, were obtained with samples of RNAase A containing 0–0.5 M-NaCl.

Effect of polynucleotide concentration change on migration of proteins

Once the procedure for the polynucleotide/polyacrylamide-gel electrophoresis of basic RNAases had been developed, the purities of bovine muscle RNAases I, II and III were examined and a comparison between these enzymes and bovine pancreatic RNAase A was made. On separate gels, RNAase I and RNAase A were found to have identical mobilities (Fig. 2). Furthermore, a sample

Fig. 2. Comparison of bovine pancreatic RNAase A and bovine muscle RNAase I by using polynucleotide/polyacrylamide-gel electrophoresis

Polynucleotide/polyacrylamide gels containing 0.03% poly(U) were prepared and subjected to electrophoresis as described in the Experimental section. Samples consisted of 50 μ l of loading solution and either 570 pg of bovine pancreatic RNAase A (a) or 1.5 units of bovine muscle RNAase I (b). After electrophoresis, the gels were incubated overnight at 4°C. These were then stained with Pyronin Y, destained, and the absorbance of the gels was monitored as described in the Experimental section. Gels electrophoresed without enzyme served as the reference. For each enzyme, the absorbance profile obtained in this manner is shown directly above a photograph of the corresponding gel. See the Experimental section for experimental details.

containing both RNAase I and RNAase A gave one band at the same R_F (0.71) as the individual enzymes. The fact that on electrophoresis of RNAase I only one band was visible indicates that the enzyme preparation is free of other ribonucleolytic activities. Under similar electrophoretic conditions, both RNAase II and RNAase III were

retained at the origin. No contamination of either enzyme with RNAase I was noted.

The low molecular weights of RNAases II and III (23 000 and 17 000 respectively) should not prohibit migration of these proteins in 7.5% polyacrylamide gels containing 0.03–0.05% polynucleotide. The finding that RNAases II and III do not move from the origin during gel electrophoresis suggests that these proteins are probably more basic than RNAase I or bovine pancreatic RNAase A, and in consequence are more tightly held by the polynucleotide than are the latter enzymes. Confirmation that polynucleotide binding was responsible for the lack of migration of RNAases II and III was obtained by simply lowering the concentration of poly(U) within the gels from 0.03 to 0.005%. At this low concentration, there is a definitive decrease in colour intensity of the gel after staining and, in consequence, a lessening of the contrast between regions of enzyme activity and unhydrolysed polynucleotide. Bands of RNAase activity are nonetheless readily apparent, and enzyme mobilities may be accurately measured. Under these conditions, R_F values of 0.21 and 0.17 were found for RNAases II and III respectively. For either enzyme, no contaminating RNAase activity could be detected either at the origin of the gels or at the same R_F as that measured for RNAase I or RNAase A (R_F 0.86).

Relationship between the polynucleotide substrate and protein migration

A second approach may be taken to compare one enzyme with another and to determine the homogeneity of individual enzyme preparations. As discussed above, the identity of the polynucleotide

substrate influences the mobility of RNAases in polynucleotide/polyacrylamide gels (Fig. 1). The use, then, of various substrates in the gels should facilitate comparisons of the purities of individual enzymes. This possibility arises because the mobility patterns obtained with different polynucleotides should vary with the identity of the RNAase examined. This was found to be the case for bovine muscle RNAases I, II and III, as shown in Table 1. With such substrates as poly(I,U), poly(A,U), poly(C,U) and poly(U) in polynucleotide/polyacrylamide gels, a different set of mobilities was obtained for each of the bovine muscle RNAases, indicating that the three enzymes are quite distinct, one from another. In addition, each enzyme gave only one activity band with each substrate, demonstrating the ribonuclease homogeneity of each enzyme.

Discussion

Polynucleotide/polyacrylamide-gel electrophoresis is a selective and sensitive technique for the assay of nucleolytic enzyme activity. This method differs from polyacrylamide-gel electrophoresis by the presence of a high-molecular-weight non-migrating polynucleotide substrate within the gel. The effect on enzyme migration of the inclusion of such polyanions as RNA or synthetic homo- or copolymers in the gel matrix may vary considerably, depending on the nature of the exposed residues and the net charge of the proteins under investigation. For example, the presence of a negatively charged polynucleotide may alter the rate of migration of a protein during electrophoresis by two different mechanisms. Hydrophobic interactions between the aromatic residues of the polynucleotide and those of the enzyme (Davidson & Fasman, 1969; Faiferman *et al.*, 1971; Faust *et al.*, 1973) would slow protein movement through the voltage gradient, particularly so at the high salt concentrations usually employed during electrophoresis. Interactions of a purely electrostatic nature may also be expected to occur (Guschlbauer, 1976; Montenay-Garestier *et al.*, 1976; Karpetsky *et al.*, 1979). These would be between the phosphate residues of the immobile polyanion and the positively charged groups on the surface of the migrating enzyme. These effects probably do not seriously deter the movement of most acidic, or negatively charged, proteins towards the anode in the presence of a polynucleotide at neutral or basic pH (van Loon, 1975).

If the enzyme proteins have a net positive charge, however, then the electrostatic interactions discussed above assume a primary importance during the electrophoretic migration towards the cathode, and may be of sufficient magnitude to prohibit migration entirely. To overcome these effects, the

Table 1. *Electrophoretic mobilities of bovine muscle RNAases on polynucleotide/polyacrylamide gels*
Conditions for the preparation and electrophoresis of polynucleotide/polyacrylamide gels were as described in the Experimental section, except that individual gels contained 0.005% of the polynucleotide substrates indicated below. Gels containing poly(U) were subjected to electrophoresis at an additional concentration of substrate (0.03%). RNAase solutions contained 0.15 unit of activity. Enzyme mobilities for RNAase I and bovine pancreatic RNAase A were identical.

Polynucleotide substrate	Mobility		
	RNAase I	RNAase II	RNAase III
Poly(I,U)	0.83	0.10	0.14
Poly(A,U)	0.74	0.24	0.60
Poly(C,U)	0.94	0.17	0.37
Poly(U)(0.03%)	0.71	0	0
Poly(U)(0.005%)	0.86	0.21	0.17

polyamines were chosen deliberately for inclusion in the gels. It was reasoned that the high positive charge density of these compounds and their well-characterized interaction with many types of RNA (Goldstein, 1966; Higuchi & Tsuboi, 1966; Matsuo & Tsuboi, 1966; Sprecher-Goldberger, 1967; Glasser & Gabbay, 1968; Szer, 1970; Janik *et al.*, 1972; Thierr *et al.*, 1972; Sakai *et al.*, 1975; Sakai & Cohen, 1976) would also ensure interaction with the polynucleotides during electrophoresis. Although the polyamines bind to a number of RNAases, this is probably beneficial, since these organic cations are known to enhance the activities of RNAase (Levy *et al.*, 1973; Frank *et al.*, 1975; Igarashi *et al.*, 1975; Schmukler *et al.*, 1975), and to protect the enzymes from inactivation (Frank *et al.*, 1975; Karpetsky & Levy, 1977). Preliminary studies with three polyamines, putrescine, spermidine and spermine, showed that putrescine was ineffective and that spermidine, although promising, did not support as high a protein migration as did spermine. Thus protein migration increased with the charge density of the polyamine used.

Because of the many interactions that may occur involving the enzyme, the polyamine and the polynucleotide substrate, the mobility of a particular enzyme during electrophoresis may be subject to considerable variation, and depend on both the concentration of polyamine and the identity of the substrate. On one hand, in the presence of a constant quantity of polynucleotide, the change in enzyme mobility as a function of spermine concentration may be explained on the basis of a titration by the positively charged polyamine of negatively charged sites on the polymer's phosphate backbone (Fig. 1 and Appendix). On the other hand, varying the identity of the polynucleotide in the presence of fixed amounts of spermine and enzyme may also cause a change in the rate of migration of the enzyme (Table 1). Although the reason for this is not clear, it should be noted that, if the magnitude of electrostatic and hydrophobic binding between protein and polynucleotide varied with the identity of the polynucleotide, another more specific interaction between these two polymers may be possible. An enzyme may have a greater affinity, in terms of weak binding under the non-catalytic conditions of electrophoresis, for types of linkages found in certain polynucleotides and not in others. These interactions would not lead to enzyme-catalysed degradation of substrate. In this connection, it is noteworthy that the rate of hydrolysis of the synthetic polymer by an enzyme does not serve as a guide to the migration of that enzyme in the presence of the polynucleotide (compare Table 1 with Table 3 of Davies *et al.*, 1980).

It is conceivable that the low concentrations of polynucleotides used cause changes in the average

pore size of the gel, and that this change is therefore related to individual polynucleotides. It is unlikely, however, that significant changes in enzyme mobility would result from this effect alone, since RNAase A mobility remained constant in the presence of yeast RNA or poly(U) as the concentration of polyacrylamide in these gels was varied from 5.5 to 7.5% (as acrylamide). Furthermore, if the change in polynucleotide identity were only related to a mechanical modification of the gel matrix and no interactions among enzymes and polynucleotides occurred, the consequences, in terms of enzyme mobilities, of alteration of one type of RNA for another should be the same for all enzymes investigated, i.e. the mobilities of all proteins should either increase together or decrease together on use of a different RNA within the gels. That this is clearly not the case is obvious from examination of Table 1.

One powerful consequence of the variation in enzyme mobility is that the relationships among several RNAases can be determined quickly with negligible quantities of enzyme. Thus it was possible, with four different substrates in 17 gels (Table 1 and Fig. 2), to establish simultaneously that muscle RNAases I, II and III were homogeneous, that each enzyme was distinct from the remaining two, and that RNAase I and bovine pancreatic RNAase A were similar, if not identical. The sensitivity that can be attained by using polynucleotide/polyacrylamide-gel electrophoresis is considerable, with the added advantage that resolution of different enzyme species may be readily achieved. The versatility of the technique coupled with its sensitivity should find further use in enzyme characterization, as well as in the examination of physiological fluids for RNAases present in low quantities.

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APPENDIX

The relationship between ribonuclease mobility and spermine concentration investigated by using polynucleotide/polyacrylamide-gel electrophoresis

The interaction of a positively charged enzyme with a polyanion contained within a polyacrylamide gel is assumed to prohibit migration of the protein during electrophoresis. The mobility of the enzyme can be increased, however, by titration of the polyanionic polynucleotide with a low-molecular-weight cation such as spermine (eqn. 1):

Therefore, in the presence of polyamine, enzyme mobility is assumed to depend directly on the amount of polynucleotide-spermine complex present in the gel. The higher the ratio of complexed anionic sites to free ionized phosphate moieties, the greater will be the mobility of the enzyme during electrophoresis as a consequence of the increased shielding of negative charge within the gel. The quantity of complex c (c) as a function of those of the reactants a and b (a and b) may be determined in the following manner (Moore, 1964). Assuming that the concentration of spermine is much greater than the amount of complex, the rate of complex formation may be expressed as eqn. (2):

$$\frac{dc}{dt} = k_{+1} \cdot (a - c) \cdot b^{\frac{1}{4}} - k_{-1} \cdot c \quad (2)$$

If m is defined as $k_{+1} \cdot a \cdot b^{\frac{1}{4}} / (k_{+1} \cdot b^{\frac{1}{4}} + k_{-1})$, then:

$$\frac{dc}{dt} = (k_{+1} \cdot b^{\frac{1}{4}} + k_{-1}) \cdot (m - c) \quad (3)$$

Solving eqn. (3) in the usual manner results in an expression for the concentration of complex (eqn. 4):

$$c = m \cdot \{1 - \exp[-(k_{+1} \cdot b^{\frac{1}{4}} + k_{-1}) \cdot t]\} \quad (4)$$

Expansion of this expression to obtain a first-order approximation (Hodgman, 1959) reveals a fourth-order relationship between the concentrations of complex and spermine (eqn. 5):

$$c \simeq m \cdot (k_{+1} \cdot b^{\frac{1}{4}} + k_{-1})t = k_{+1} \cdot a \cdot b^{\frac{1}{4}} \cdot t \quad (5)$$

Thus, if enzyme mobility is linearly related to the concentration of complex while the time of interaction (t) and the concentration of polynucleotide phosphate residues (a) are maintained constant, mobility (R_F) should be a linear function of $[\text{spermine}]^{\frac{1}{4}}$. This was found to be the case for gels containing either yeast RNA or poly(U) and concentrations of spermine in excess of those of polynucleotide phosphate (Fig. 1 of the main paper). Plots of R_F versus $[\text{spermine}]$, $[\text{spermine}]^{\frac{1}{4}}$ or $[\text{spermine}]^{\frac{1}{2}}$ were all curved.

It should be noted that the concentration of uncomplexed phosphate groups ($a - c$) is given in eqn. (6) or, to a first-order approximation, in eqn. (7):

$$a - c = a \cdot \left\{1 - \frac{m}{a} \cdot [1 - \exp-(k_{+1} \cdot b^{\frac{1}{4}} + k_{-1}) \cdot t]\right\} \quad (6)$$

$$a - c = a \cdot (1 - k_{+1} \cdot b^{\frac{1}{4}} \cdot t) \quad (7)$$

Therefore, if the total amount of phosphate moieties (a) is maintained constant, changes in

polyamine concentration (*b*) simply cause a shift to occur between the free and complexed forms of these residues. However, the situation is quite different if the polyamine concentration is invariant and the quantity of RNA is increased. In this case, although the absolute amount of complexed residues increases (eqn. 5), so does the concentration of free negatively charged groups (eqn. 7). For this reason, and in the absence of other effects influencing the R_F of enzymes, increasing the polynucleotide concentration will result in lower basic RNAase mobilities. In the limiting case, low-molecular-weight basic enzymes will remain at the

origin unless the concentration of polynucleotide is sufficiently low to permit migration [compare the mobilities, listed in Table 1 of the main paper, of RNAases II and III obtained with either 0.03 or 0.005% poly(U)].

References

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