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ON THE MECHANISM OF THE MOLECULAR-SIEVE EFFECT IN POLYACRYLAMIDE GEL ELECTROPHORESIS

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SUMMARY

A model for the mechanism of the molecular-sieve effect in polyacrylamide gel electrophoresis is presented for low gel concentrations. From mobility measurements at different gel concentrations, the model gives radii for protein molecules which are in good agreement with the values calculated from the known molecular weights.

INTRODUCTION

The linear relation between the logarithm of the mobility and the gel concentration in polyacrylamide gel electrophoresis has been established experimentally by several workers[1–3]. This relation

$$\log m(T) = \log m(0) - K_R T \tag{1}$$

where T is the gel concentration as defined by Hjertén[4], $m(T)$ and $m(0)$ are the mobilities at gel concentrations T and 0 (*i.e.*, only buffer) and K_R is a constant which is characteristic of the dimensions of the protein (retardation constant), has been connected by Morris[1] and Rodbard and Chrambach[5] with a theory of Ogston[6]. Ogston calculated the accessible volume fraction, f, for spherical particles of radius R in a uniform random suspension of fibres having radius r and a concentration of l cm/ml. He showed by statistical treatment that

$$f = \exp - \pi l(R + r)^2 \tag{2}$$

Morris[1] stated that $m(T)/m(0) = f$; Rodbard and Chrambach[5] arrived at the same conclusion, $m(T)$ proportional to f, by applying the principle of Delesse (See ref. 7) to the result of Ogston's theory.

These models provide a functional relation between experimental and molecular parameters. The molecular parameters of an unknown protein can only be obtained from this relation by the use of standard proteins. In the model which is

proposed in this report, a more detailed description of the behaviour of a spherical protein molecule in polyacrylamide gel electrophoresis will be given. From this model, values for molecular radii have been obtained without the use of standards. These values appear to be in good agreement with values calculated by Rodbard and Chrambach[8].

Description of the model

A particle moving under the influence of an electrical field through a polyacrylamide gel will either travel unhindered through the buffer solution or be in contact with a fibre. When travelling unhindered it will have a velocity V_0. In contact with a fibre it will have a variable velocity which is less than V_0. The particle can pass from one velocity state to another both by drift and by Brownian motion. The Brownian motion will interfere with the drift in the different velocity states. So it may be expected that the Brownian motion will have an effect on the mean velocity. The coupling of drift-velocity variations and Brownian motion is analogous to the problem of eddy diffusion, which has been studied among others by Giddings[9] and Roes[10]. Roes showed the existence of a dependence of the mean velocity on the rate of interruption by Brownian motion. This dependence appears to be of minor importance at high interruption rates. Therefore the Brownian motion will not be considered in the calculation of the mean velocity. Giddings[9] and Roes[10] showed additionally that the variance of the velocity at high interruption rates is determined completely by longitudinal diffusion.

Gel structure

As to the structure of the gel, we proceed on the assumptions made by Ogston[6]. Ogston considered the gel to be a random suspension of straight polymer fibres of radius r. All spatial orientations of a fibre have the same probability. From the work of Laurent and Öbrink[11] on fluorescence depolarization, one may conclude that the viscosity of the solution between the fibres is equal to the viscosity of a polymer-free solution. Komiyama and Fuoss[12] reached the same conclusion from conductance measurements in polymer solutions. Apart from the viscosity of the solution in the spaces between the fibres, the velocity of the particles will be determined by contacts with the fibres. In our model the concentration of polymer is assumed to be so low that a molecule does not make contact with more than one fibre simultaneously.

Behaviour of protein molecules in the gel

The protein molecules for which the model is constructed are considered to be spherical. Furthermore, it is assumed that the molecules slide along the fibres in such a manner that there is no energy dissipation due to rotation. This implies that there is no adsorption to the polymer fibres. As the diameter of the particle is great relative to the diameter of the fibre (in reality the ratio exceeds 10), the liquid stream around the particle will not be seriously disturbed. In addition, the relaxation time of the velocity of a molecule in water is small, so the velocity can be taken as proportional to the force in the direction of motion

$$V = \frac{K_m}{F} \tag{3}$$

where V is the velocity of the particle, K_m is the force acting on the particle in the direction of motion and F is the friction coefficient. If the particle makes contact with the fibre, the force in the direction of motion is the resultant of the field force and the reaction force exerted by the fibre on the particle. We will calculate the additional residence time of a molecule in a gel column with gel concentration T, in comparison with the residence time in a corresponding column with gel concentration 0.

In order to calculate the additional residence time of a particle at gel concentration T, the number of contacts with the polymer fibres and the additional time for a particle to pass along one fibre has to be accounted for. For the description of the movement along a fibre a Cartesian coordinate system is used. The z-axis of this system coincides with the axis of the fibre. The y-axis is taken in such a way that the yz-plane is parallel to the field direction (see Fig. 1). The direction of the field force acting on the particle makes an angle φ with the z-axis. The angle which the line, connecting the centre of the sphere with the tangent point of the sphere and cylinder, makes with the y-axis is θ. The centre of the sphere moves under the influence of the electrical field on the surface of the cylinder with radius $R + r$ from the z-axis. Contact with the fibre is lost when $\theta = \frac{1}{2}\pi$ rad. The resultant P of the field force K and the reaction force can be resolved into a component $P_2 = K \cos \varphi$ parallel to the z-axis and a component $P_1 = K \sin \varphi \sin \theta$ parallel to the xy-plane. The corresponding velocity components, with the assumption that the friction factor F remains constant, are

$$V_1 = \frac{K \cos \varphi}{F} = V_0 \cos \varphi \tag{4}$$

$$V_2 = \frac{K \sin \varphi \sin \theta}{F} = V_0 \sin \varphi \sin \theta \tag{5}$$

At the same time the relation

$$V_2 = (R + r) \cdot \frac{\mathrm{d}\theta}{\mathrm{d}t} \tag{6}$$

has to be met.

By combining eqns. 5 and 6 the time interval can be calculated from the first contact with $\theta = \theta$ rad until $\theta = \frac{1}{2}\pi$ rad. This time interval is

$$\Delta t(\theta,\varphi) = \int_{\theta=0}^{\pi/2} \frac{(R + r)\,\mathrm{d}\theta}{V_0 \sin \varphi \sin \theta} = -\frac{(R + r) \ln \tan (\theta/2)}{V_0 \sin \varphi} \tag{7}$$

As a result of this movement a change, ΔS, in the position of the centre of the sphere occurs, with the components

$$\begin{aligned} \Delta S_x &= (R + r)(1 - \sin \theta) \\ \Delta S_y &= (R + r) \cos \theta \\ \Delta S_z &= V_1 \Delta t(\theta,\varphi) = -\frac{(R + r) \ln \tan (\theta/2)}{\tan \varphi} \end{aligned} \tag{8}$$

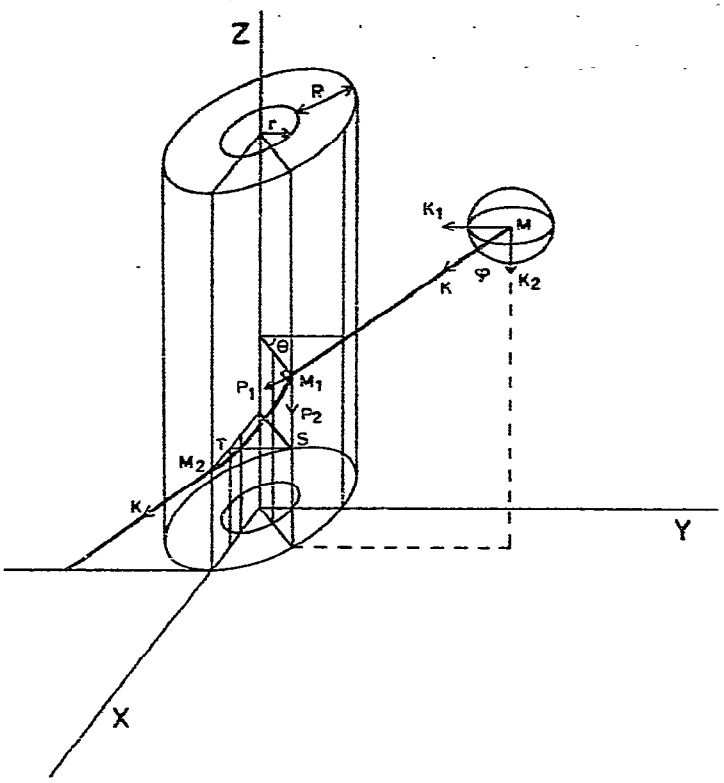

Fig. 1. Cartesian coordinate system used: r = radius of the fibre; R = radius of the sphere; M = centre of the sphere; M_1 = position of the centre of the sphere at the moment of first contact ($\theta = \theta$); M_2 = position of the centre of the sphere at the moment of last contact ($\theta = \pi/2$); P_1 and P_2 = components of P as defined in the text; $TM_2 = \Delta S_x$, $TS = \Delta S_y$ and $M_1S = \Delta S_z$ = components of the displacement vector ΔS; K = field force, with components K_1 and K_2 in y and z direction, respectively. The curve M_1M_2 is the track of M during contact of the sphere with the fibre.

Only the displacement of the particle in the direction of the field is relevant to the problem, so the magnitude of the projection of ΔS on K has to be calculated. The magnitude of this projection is

$$S(\theta,\varphi) = \frac{K_x\Delta S_x + K_y\Delta S_y + K_z\Delta S_z}{|K|} \tag{9}$$

The components of K are

$$\begin{aligned} K_x &= 0 \\ K_y &= K \sin\varphi \\ K_z &= K \cos\varphi \end{aligned} \tag{10}$$

By substitution of eqns. 8 and 10 in eqn. 9

$$S(\theta,\varphi) = \frac{(R + r)\,[\sin^2\varphi \cos\theta - \cos^2\varphi \cdot \ln \tan(\theta/2)]}{\sin\varphi} \tag{11}$$

is obtained.

If the particle moves a distance S in the direction of the field while suffering n collisions of the type (θ, φ), the time needed will be

$$t(n,\theta,\varphi) = n\Delta t(\theta,\varphi) + \frac{S - nS(\theta,\varphi)}{V_0} \tag{12}$$

This residence time for a particle in a column of length S is a function of the three random variables n, θ and φ. In order to be able to calculate the mean residence time of a particle, the probability-density functions of n, θ and φ have to be known. The angle φ between the axis of the fibre and the field direction has a probability-density function[6] given by

$$p_1(\varphi) = \sin\varphi,\ 0 < \varphi < \pi/2 \tag{13}$$

In order to calculate the probability-density function of θ, we assume that the projections of the first contact points on the x-axis are distributed homogeneously between the points $x = 0$ and $x = R + r$ on the x-axis. The probability of the angle of first contact being less than θ equals the probability of the projection of the first contact point on the x-axis being less than $(R + r)\sin\theta$. The probability-density function for θ is found to be

$$p_2(\theta) = \cos\theta,\ 0 < \theta < \pi/2 \tag{14}$$

It is apparent that, from the probability function of n, only the mean is needed for the calculation of the mean residence time $\bar{t}$.

The mean residence time of a particle is obtained from

$$\bar{t} = \sum_{n=0}^{\infty} \int_{\theta=0}^{\pi/2} \int_{\varphi=0}^{\pi/2} t(n,\theta,\varphi)\, p_3(n)\, p_2(\theta)\, p_1(\varphi)\, \mathrm{d}\varphi\, \mathrm{d}\theta \tag{15}$$

With eqns. 12–14 this expression reduces to

$$\bar{t} = \frac{S}{V_0} + \frac{\pi^2}{16} \cdot \frac{N(R + r)}{V_0} \tag{16}$$

Here $N = \sum_{n=0}^{\infty} n \cdot p_3(n)$ is the mean number of contacts of a particle with the fibres, while covering a distance S. Ogston[13] derived an expression for N

$$N = \frac{1}{2}\pi\, l\, (R + r)\, S \tag{17}$$

l is the concentration of the fibres expressed in cm of fibre per ml of gel. The same result is obtained by the method of Cornfield and Chalkley[14]. Substitution of eqn. 17 in 16 yields

$$\bar{t} = \frac{S}{V_0} \cdot \left\{1 + \frac{\pi^3}{32} \cdot (R + r)^2 \cdot l\right\} \tag{18}$$

The fibre concentration, l, expressed in terms of the gel concentration, T, is

$$l = \frac{N_A}{M} \cdot a \cdot 10^{-2} \cdot T \tag{19}$$

where N_A is Avogadro's number ($6.03 \cdot 10^{23}$), M is the molecular weight of the acrylamide monomer (71) and a is the length of acrylamide monomer[15] ($2.5 \cdot 10^{-8}$ cm).

Finally, the mean residence time of a particle in a column of length S is

$$\bar{t} = \frac{S}{V_0} \cdot \left\{1 + 2.06 \cdot 10^{12} \cdot T (R + r)^2\right\} \tag{20}$$

If the second and higher centred moments of the distribution of the residence time are small compared with the mean

$$V = V_0 / \{(1 + 2.06 \cdot 10^{12} \cdot T(R + r)^2\} \tag{21}$$

is a good approximation of the mean velocity, $\bar{V}$. This approximation is justified experimentally[16] because the mean distance $\bar{S}$ travelled by the molecules is found to be proportional to the time t. The straightforward calculation of $\bar{V}$ results in the calculation of the mean of $1/t$, which is mathematically more complex.

EXPERIMENTAL

The proteins used in this study were: myoglobin (horse) and ovalbumin from Serva, Heidelberg, G.F.R.; bovine serum albumin, apoferritin (horse) and γ-globulin (human) from Schwarz-Mann, Orangeburg, N.Y., U.S.A. Horse haemoglobin was isolated in our laboratory according to Drabkin[17]. Transferrin (human) was a kind gift from the Central Laboratory, The Netherlands Red Cross Blood Transfusion Service, Amsterdam.

Gels were prepared from ingredients supplied by Bio-Rad Labs., Richmond, Calif., U.S.A. For the measurements of the velocities at various gel concentrations, eight gels were prepared in quartz tubes (length, 16 cm; diameter, 0.7 cm). The cross-linking was chosen as $C = 5\%$ at all gel concentrations, because at this concentration the retardation is optimal[18] and hence the variation in the velocity caused by variations in C will be minimal. For the preparation of a gel two solutions were used: monomer solution (A) and ammonium persulphate solution (B). Solution A contained acrylamide, N,N'-methylenebisacrylamide (Bis) and tris(hydroxymethyl)aminomethane (Tris)–hydrochloric acid buffer in such amounts that the following concentrations resulted in the gel: $T = 2.5$, 3, 4, 5, 6, 7, 8 or 9%; $C = 5\%$; HCl = 6 mequiv. per 100 ml of gel; Tris = 4.57 g per 100 ml of gel. Solution B contained 560 mg of ammonium persulphate per 100 ml of water (final concentration, 140 mg of persulphate per 100 ml of gel).

Solutions A and B were first deaerated *in vacuo*. To solution A, N,N,N',N'-tetramethylethylenediamine (TEMED) was added (29 μg per 100 ml of gel). Subsequently, three parts of solution A were thoroughly mixed with one part of solution B. The quartz tubes were filled with this monomer solution to a height of 6.5 cm. After

polymerization, the gels were subjected to pre-electrophoresis at 2.5 mA per tube with Tris–glycine buffer (43.8 g of Tris + 3.52 g of glycine per litre) as electrode buffer. In this way, gels were obtained which had a sufficiently low extinction at 280 nm to be able to detect the protein bands with an UV monitor. The compositions of the Tris–HCl and the Tris–glycine buffers were chosen in such a way that they were comparable with the functions described by Ornstein[19] for a moving boundary (eqns. 9 and 18 of appendices D and E). In order to check whether the electrode buffer met the moving boundary conditions, we compared the pH of this buffer with the pH of the gel. The pH values were found to be 9.43 and 9.39 respectively at 21°, which is a satisfactory agreement.

The pH of the gel after pre-electrophoresis was measured by extracting the gel with an equal volume of carbon dioxide-free distilled water. The electrophoresis of the proteins was performed in the resulting continuous buffer system at 2.5 mA per tube. The protein samples, dissolved in water or diluted buffer with 5% sucrose, were layered on top of the gels. Nitrophenol was added to the samples in order to have a control for irregular behaviour of a gel column. Each run consisted of eight tubes with eight different gel concentrations. The apparatus used was essentially the apparatus described by Davis[20].

A glass cooling coil was placed in the lower buffer compartment in order to control the temperature. The gels were completely immersed in the lower buffer. In order to avoid deformation of the gels by a staining procedure, the gels were scanned in the quartz tubes at 280 nm in an ISCO gel scanner, combined with an ISCO UA-4 UV monitor. The displacements of the protein bands after 60 min, representing the velocities in cm/h, are given in Table I. All of the values given in Table I are the means of duplicate measurements.

The velocity of a protein band in buffer ($T = 0$) was estimated by electrophoresis in a sucrose density gradient. The velocities in the density gradient were corrected for the viscosity ratio of the buffer and sucrose solution as described below. The measurements were performed in the following way. A polyacrylamide gel ($T = 5\%$) plug was polymerized at the lower end of a quartz electrophoresis tube in the way described for gel electrophoresis. On top of the plug was layered a linear sucrose gradient[21] (3.6–10% sucrose in Tris–glycine buffer). The protein samples were applied to the top of the gradient in Tris–glycine buffer with 2% sucrose. After

TABLE I

VELOCITIES (cm/h) OF THE PROTEIN BANDS AT 2.5 mA

T (%)	*Myoglobin*	*Ovalbumin*	*Horse haemoglobin*	*Bovine serum albumin*	*Transferrin*	*γ-Globulin*	*Apoferritin*
2.5	1.48	2.99	2.49	3.08	3.57	0.43	2.09
3	1.40	2.91	2.34	2.93	3.41	0.34	1.76
4	1.36	2.56	2.05	2.53	2.91	0.26	1.20
5	1.17	2.28	1.75	2.15	2.44	0.19	0.74
6	1.10	1.85	1.45	1.80	2.02	0.14	0.44
7	1.00	1.77	1.27	1.52	1.71	0.12	0.26
8	0.91	1.54	1.10	1.28	1.43	0.08	0.12
9	0.85	1.32	0.91	1.05	1.14	0.04	0.07

TABLE II

COMPARISON OF OBSERVED AND MEASURED VALUES OF ln V_0

	Myoglobin	*Ovalbumin*	*Horse haemoglobin*	*Bovine serum albumin*	*Transferrin*	*γ-Globulin*	*Apoferritin*
ln V_0 (sucrose)	0.74	1.63	1.36	1.74	1.73	−0.36	1.64
ln V_0 (ext)	0.61	1.44	1.32	1.58	1.75	−0.01	2.21

electrophoresis at 0.5 mA per tube for 5 h, the positions of the bands were detected with the ISCO gel scanner. This low current strength was necessary to avoid heat convection in the gradient. Temperature control by cooling at higher current strengths led to destruction of the density gradient by thermal convection. For this reason, the temperatures of the gel-electrophoresis experiments were adapted to the temperatures of the corresponding gradient-electrophoresis experiments. The dependence of the viscosity of the buffer on the temperature and the sucrose concentration was measured in the range wherein the electrophoresis conditions lay. As the temperature change during the electrophoresis is small (less than 0.5°) and the viscosity is a linear function of the displacement of the zone in the column, we may take for the viscosity in the gradient the mean ($\bar{\eta}$) of the viscosities at the start and at the end of the zone. The corrected velocity, V_0 (sucrose), was found from the measured velocity (V_m) by

$$V_0 \text{ (sucrose)} = \frac{\bar{\eta}}{\eta(0)} \cdot V_m$$

In Table II, ln V_0 (sucrose) is compared with the ln V_0 (ext) values obtained from the gel-electrophoresis experiments.

RESULTS AND DISCUSSION

From the above it will be clear that formula 21 may only be used at low gel concentrations. This means that expression 21 represents the equation of the tangent at $T = 0$ to the real curve of $1/\bar{V}$ against T. Empirically, it has been established that the actual curve is represented by the exponential relation[1-3]

$$\frac{1}{\bar{V}} = \frac{1}{V_0} \cdot \exp K_R T \tag{22}$$

The reason for the deviation from linearity of the function $1/\bar{V}$ against T has to be sought in the fact that at higher gel concentrations, apart from single contacts, other effects have to be considered. A "basket" effect, as mentioned by Ogston *et al.*[22], is probably the most important. This will lead to an additional retardation because the molecules can only escape from the baskets by diffusion. If formula 21 is the equation of the tangent at $T = 0$ to the empirical function 22 then

$$K_R = 2.06 \cdot 10^{12} \cdot (R + r)^2 \tag{23}$$

TABLE III

COMPARISON OF THE OBSERVED VALUES OF THE MOLECULAR RADII (IN Å) WITH VALUES FROM THE LITERATURE

R = the radius obtained from the values in Table I with eqn. 23; R_{lit} = the radius calculated by Rodbard and Chrambach[8].

	Myoglobin	*Ovalbumin*	*Horse haemoglobin*	*Bovine serum albumin*	*Transferrin*	*γ-Globulin*	*Apoferritin*
R	18.8 ± 0.9	23.6 ± 1.5	25.1 ± 0.5	26.8 ± 1.1	26.4 ± 0.7	35.7 ± 3.1	45.8 ± 3.1
R_{lit}	17.3	23.3	26.6	26.9	29.7	36.0	50.9

From the velocities at the various gel concentrations, K_R was obtained by the least-squares method as the slope of the best fitting line $\ln \bar{V} = \ln V_0 - K_R T$. The intercepts $\ln V_0$ (ext) of the regression line with the $\ln V$-axis compared with the values $\ln V_0$ (sucrose) from the sucrose-gradient experiments are given in Table II. The agreement between the measured and calculated values at $T = 0$ shows that formula 22 also holds at $T = 0$ (compare Morris[1]).

With $R = (K_R/2.06 \cdot 10^{12})^{\frac{1}{2}} - r$, we calculated the radii of the protein molecules from the corresponding K_R values. The radius r of the polyacrylamide chain is estimated[15] to be 2.5 Å. In Table III are given the values of the radii calculated from the data in Table I, together with the 95% confidence limits. These values are compared with values calculated by Rodbard and Chrambach[8] (R_{lit}) from the molecular weights. They assumed that the protein molecules were spherical and unhydrated, and estimated the partial specific volume to be 0.74. Considering the assumptions involved, the model presented here yields values for the molecular radii which are in good agreement with the values calculated by Rodbard and Chrambach. The radius obtained in this way cannot be interpreted as the Stoke's radius of a molecule. It is an open question which relation exists between the two radii.

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