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## Note

# Determination of the free electrophoretic mobility of proteins by polyacrylamide gradient gel electrophoresis: A new approach

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The calculation method that we developed earlier[1] provides the possibility of determining the free electrophoretic mobility ($m_o$) of proteins by polyacrylamide gradient gel electrophoresis from data obtained by the methods of Hedrick and Smith[2] and Manwell[3]. Knowing the acrylamide concentration *versus* distance relationship [$T = f(x)$], the protein retardation coefficient ($K_R$), the hypothetical limiting distance ($x_L$) and the time necessary to move the protein from the origin to $x_L/2$ ($t_{0.5}$), we derived the following equation:

$$m_o = \frac{1}{t_{0.5}} \int_0^{x_L/2} e^{2.3\, K_R f(x)} \cdot dx \tag{1}$$

However, it appears that for very large proteins (high $K_R$), having high mobilities, the value of the free electrophoretic mobility calculated by using the equation

$$m_o = \frac{1}{t_{x_L/n}} \int_0^{x_L/n} e^{2.3\, K_R f(x)} \cdot dx \tag{2}$$

is a decreasing function of $n$ [$T = f(x) > 0$] and always remains above its true value.

This experimental finding led us to re-examine the problem of the determination of $m_o$ and to develop a more accurate calculation method.

## THEORETICAL

According to the Ferguson relationship[4]

$$\log m = \log m_o - K_R T \tag{3}$$

The mobility, $m = dx/dt$, in a polyacrylamide gel in which the concentration is $T = f(x)$, can be written as

$$\frac{dx}{dt} = m_o e^{-2.3\, K_R f(x)} \tag{4}$$

(the electric field being arbitrarily chosen as 1 V/cm). Integration of eqn. 4 with respect to $x$ ($x < x_L$) gives

$$t_x = \frac{1}{m_o} \int_0^x e^{2.3\, K_R f(z)} \cdot dz \tag{5}$$

From eqn. 5, the apparent free electrophoretic mobility ($m_{o,a}$) can be calculated as

$$m_{o,a} = \frac{1}{t_x} \int_0^x e^{2.3\, K_R f(z)} \cdot dz \tag{6}$$

In fact, it has been proved that $\lim m_{o,a}$ when $x \to 0$ represents the true electrophoretic mobility ($m_o$).

As a matter of fact, the acrylamide concentration always presents a finite limit when $x \to 0$;

$$\lim_{x \to 0} f(x) = c \tag{7}$$

Let us set

$$g(x) = f(x) - c \tag{8}$$

Therefore,

$$\lim_{x \to 0} g(x) = 0 \tag{9}$$

This yields

$$\int_0^x e^{2.3\, K_R f(z)} \cdot dz = e^{2.3\, K_R c} \int_0^x e^{2.3\, K_R g(z)} \cdot dz \tag{10}$$

and so

$$\int_0^x e^{2.3\, K_R f(z)} \cdot dz \underset{x \to 0}{\approx} x \cdot e^{2.3\, K_R c} \tag{11}$$

Otherwise, the migration velocity of a protein in a concave polyacrylamide gradient gel is given by the Manwell relationship[3]:

$$\frac{dx}{dt} = k(x_L - x) \tag{12}$$

For a macromolecule moving in an increasingly dense medium, the mobility decreases and approaches zero when it reaches the hypothetical limiting distance $x_L$. With initial conditions $t = 0$ when $x = 0$, from eqn. 12 we obtain $t_x$:

$$t_x = -\frac{1}{k} \ln\left(1 - \frac{x}{x_L}\right) \tag{13}$$

Then,

$$t_x \underset{x \to 0}{\approx} \frac{x}{k x_L} \tag{14}$$

Thus, near zero, the free electrophoretic mobility will be

$$\lim_{x \to 0} m_{o,a} = m_o = k x_L \, e^{2.3 \, K_R c} \tag{15}$$

In the particular case of Gradipore (Uniscil) polyacrylamide gel slabs, in which $T = ax^2 + bx + c$, the free electrophoretic mobility is given by the general eqn. 15, and the initial tangent to the function 6 curve is obtained by a similar calculation using the limited development method:

$$\lim_{x \to 0} \frac{dm_{o,a}}{dx} = \frac{1}{2} k e^{2.3 \, K_R c} \, (2.3 \, K_R x_L b - 1) = \frac{m_o}{2} \left(2.3 \, K_R b - \frac{1}{x_L}\right) \tag{16}$$

## EXPERIMENTAL

For the free electrophoretic mobility determinations in polyacrylamide gradient gels we used the experimental conditions described previously[1] on the following set of proteins: ovalbumin (Serva, Heidelberg, G.F.R.) 5 mg/ml, bovine serum albumin (Sigma, St. Louis, Mo., U.S.A.) 20 mg/ml, horse spleen ferritin (Serva) 5 mg/ml and the multiple molecular forms ($C_1$, $C_2$, $C_3$, $C_4$) of human serum butyrylcholinesterase (sera were sampled by venepuncture on male volunteers: the mean activity was estimated at 4000 mU/ml by Ellman *et al.* method[5] using $10^{-3}$ *M* butyrylthiocholine iodide as substrate).

Numerical integration of eqn. 2 was solved by Simpson's method[15].

In order to confirm our results, and in addition to literature data, we also measured the free electrophoretic mobility of ferritin, ovalbumin and bovine serum albumin (monomer and oligomers) under the same set of experimental conditions [tris (0.041 *M*)–glycine buffer (pH 8.4), $\mu = 0.0225$, $\theta = +4°$, $\sigma_{(+4°)} = 0.604 \cdot 10^{-3}$ $\Omega^{-1} \cdot cm^{-1}$] by free zone electrophoresis according to Olivera *et al.*[6] at the Biophysical Laboratory of the Institut de Biologie Moléculaire et Cellulaire (Strasbourg, France) on the apparatus constructed by Ohlenbusch in this laboratory.

## RESULTS AND DISCUSSION

The study of the variation of the apparent free electrophoretic mobility ($m_{o,a}$) of the tested proteins worked out after integration of eqn. 2 for several pairs ($t_{x_L/n}$, $x_L/n$), and the calculation of the initial tangents to the curves [$m_{o,a} = f(1/n)$] by eqn. 16, show (Fig. 1) that the use of eqn. 2 for the calculation of the free electrophoretic mobility may give erroneous results. The errors become greater if the mobility and the size (large $K_R$) of the proteins are great. On the other hand, for middle- or small-sized proteins (small $K_R$) and/or those of lower mobility, the error is negligible. In Fig. 1, the extrapolation of curves for $x = 0$ ($n = \infty$) gave the same free electrophoretic mobility values as those calculated with eqn. 15 (Table I).

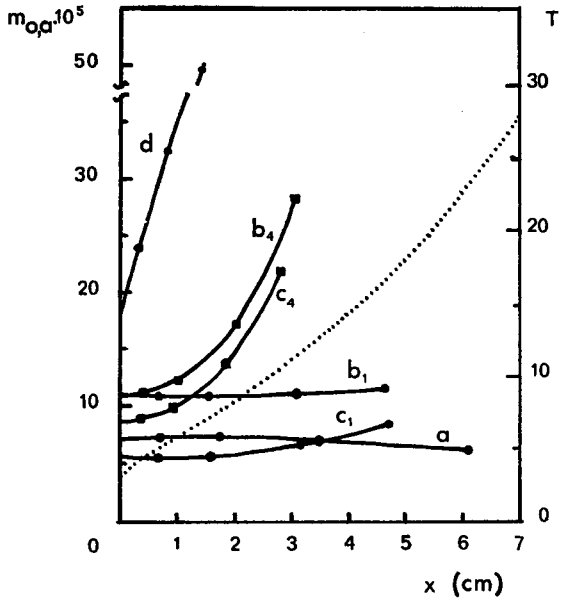


Fig. 1. Variation of $m_{0,a}$ for different proteins moving through a polyacrylamide gradient. a = Ovalbumin; $b_1$ and $b_4$ = bovine serum albumin monomer and tetramer; $C_1$ and $C_4$ = human serum butyrylcholinesterase monomer and tetramer; d = horse spleen ferritin. Experimental points correspond to mean values of $x_L/10$, $x_L/4$, $x_L/2$ and $3x_L/4$. The dotted line represents polyacrylamide concentration *versus* distance ($T = ax^2 + bx + c$).

TABLE I

FREE ELECTROPHORETIC MOBILITIES, $m_0 \cdot 10^5$ ($cm^2 \cdot V^{-1} \cdot sec^{-1}$), AT pH 8.4
$\mu = 0.0225$, $\theta = 4°$. Results are mean values ± standard errors.

| *Protein* | $K_R$ *(C = 3.5%)* | *Polyacrylamide gradient gel electrophoresis* | *Free zone electrophoresis* |
|---|---|---|---|
| Ovalbumin | 0.054 | 7.28 ± 1.40 | 9.24 ± 0.56 |
| Bovine serum albumin: | | | |
| Monomer | 0.081 | 11.35 ± 0.42 | 11.83 ± 0.59 |
| Dimer | 0.133 | 10.15 ± 0.69 | 11.50 ± 0.57 |
| Trimer | 0.185 | 10.16 ± 1.29 | 10.94 ± 0.55 |
| Tetramer | 0.232 | 10.83 ± 1.68 | 12.44 ± 0.87 |
| Human serum butyrylcholinesterase: | | | |
| $C_1$ | 0.097 | 5.74 ± 0.29 | Not tested* |
| $C_2$ | 0.138 | 7.66 ± 0.35 | |
| $C_3$ | 0.151 | 6.24 ± 0.39 | |
| $C_4$ | 0.257 | 8.54 ± 0.92 | |
| Horse spleen ferritin | 0.290 | 17.43 ± 2.07 | 13.19 ± 0.92 |

* $C_4$ component purified according to Lockridge and La Du[7] was not available in sufficient amounts for free zone electrophoretic measurements.

The results obtained for bovine serum albumin monomer ($M = 67{,}000$) are not significantly different from those published earlier[1], and still agree with literature data (ref. 1, Table III) and are fully correlated by free zone experiments (Table I). Otherwise, and in accordance with theory, the mobilities of bovine serum albumin and butyrylcholinesterase (monomer $C_1$, $M \approx 80{,}000$) $n$-mers ($n \geqslant 2$) are lower than those obtained in our previous work[1] and the results are less scattered around the mean values. The measurement of the free electrophoretic mobility of ovalbumin ($M = 45{,}000$) by polyacrylamide gradient electrophoresis gives lower values than those obtained by free zone electrophoresis but the two methods agree with literature data (Table II). On the other hand, for horse spleen ferritin ($M = 480{,}000$) polyacrylamide gradient electrophoresis gives higher results than free zone electrophoresis and literature values obtained under different experimental conditions (Table III). An explanation for this peculiarity is not easy as the concepts and the theoretical basis of polyacrylamide gradient gel electrophoresis have not yet been completely elucidated.

TABLE II

FREE ELECTROPHORETIC MOBILITY OF OVALBUMIN FROM LITERATURE DATA

| *Reference* | *Experimental conditions* | $m_0 \cdot 10^5$ $(cm^2 \cdot V^{-1} \cdot sec^{-1})$ |
|---|---|---|
| Longsworth[8] | Moving boundary method 0°, $\mu = 0.1$: | |
| | pH 7.83 | −5.92 |
| | pH 10.28 | −6.21 |
| Cannan *et al.*[9] | Titration, 0°, $\mu = 0.1$, pH 8.4 | ~ −5.80 |
| Morris and Morris[10] | Disc electrophoresis ($C = 3\%$), + 10°, $\mu = 0.05$, pH 8.76 | −9.00 |
| Rodbard and Chrambach[11] | Disc-electrophoresis ($C = 2.3\%$), 0°, $\mu = 0.1$: | |
| | pH 7.6 | ~ −5.00 |
| | pH 10.2 | ~ −7.50 |

TABLE III

FREE ELECTROPHORETIC MOBILITY OF HORSE SPLEEN FERRITIN FROM LITERATURE DATA

| *Reference* | *Experimental conditions* | $m_0 \cdot 10^5$ $(cm^5 \cdot V^{-1} \cdot sec^{-1})$ |
|---|---|---|
| Mazur and Shorr[12] | Moving boundary method, 0°, $\mu = 0.1$, pH 8.6 | − 6.10 |
| Ghosh and Moss[13] | Agar gel electrophoresis, 20°, $\mu = 0.05$, pH 6.8 | −10.5 |
| Rodbard and Chrambach[11] | Disc electrophoresis ($C = 2\%$), 0°, $\mu = 0.0034$, pH 8.88 | −10.97 |

In conclusion, the proposed calculation procedure, which is mathematically more accurate than our previous method[1], gives a satisfactory estimate of the free electrophoretic mobility of proteins in addition to size and geometric parameters (retardation coefficient, molecular weight, Stokes radius and quaternary structure) accessible by methods based on the Ferguson relationship[2,11,14] or on Manwell's method[3].

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