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I–electrophoresis of deoxyhemoglobin S: Theoretical study of the deformation induced by the applied field and the mechanical stresses

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Abstract

A more efficient technique for the study of red cell is electrophoresis. More precisely, if a constant external field is applied to a protein, the later migrates to anode or cathode according to his net charge. Compared to Hemoglobin A (HbA) which is the normal constituent, it was observed that Hemoglobin S (HbS) have a charge difference which is due to a genetic mutation $\beta 6(A3)\, Glu \to Val$, ***and consequently, a less anodic migration [1]. For the patients with HbS disease, the red cell instead to stay binocave takes the sickle form when there are deoxygenated. This deformation leads to the vaso-occlusives crisis very painful which can provoke different lesions in organelles [2]. In this work, we paid our attention on a particular phenomenon, viz, the account of the deformation which result exclusively from the conjugated action of the applied field and the mechanical stress acting on the deoxy-HbS during the electrophoresis process. In these conditions, a molecule of deoxy-HbS which is not deformed is considered as a dieletric sphere with dielectric constant*** ε_o ***while a defined HbS molecule is considered as a dielectric ellipsoid of revolution with dielectric constant*** ε_i ***along the j axis where the field is applied. The results obtained clearly indicate that the deformation*** ς ***is an analytical function of the form:*** $\varsigma = f(E, \mu, \varepsilon_0, \varepsilon)$ ***where E is the electric field intensity,*** μ ***the slide modulus of the molecule, and*** ε

Keywords**:** Hemoglobine S, Electrophoresis, Deformation, Critical Value

1.0 The theoretical model

In this work, we paid our attention on a particular phenomenon, viz, the account of the deformation which result exclusively from the conjugated action of the applied field and the mechanical stress acting on the deoxy-HbS during the electrophoresis process.

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- We consider the medium used for the electrophoresis (acetate buffer pH 6,2) on agar, as a continuum medium with dielectric constant ε
- To simplify the mathematical difficulties related to the forms of sickle cell hemoglobin, we consider a normal deoxy-HbS as a aphere with dielectric constant ε_o, and a deformed deoxy-HbS as an ellipsoid of revolution elongated along j-axis with dielectric constant ε_j.

Hence, with this formulation, our biophysical problem reduces directly to an advanced problem of electrodynamics of continuum media [2]

In order to determined the eccentricity e of the ellipsoid, we suppose that the deformation is a uniform slide deformation along the volume of the body, more precisely, a deformation without change in volume.

If ς is the deformation, we get:

$$\varsigma = \frac{a_1 - a_2}{a};\ a_1 > a_2 \tag{1.1}$$

where a_1 and $a_2 = a_3$ are the lengths of the semiaxes of the deformed ellipsoid and a the radius of the sphere.

The equilibrium condition of the deformed body which also allows to express the deformation ς in terms of mechanical and electrical parameters, can be expressed as the condition for which the sum of the elastic energy and the electrostatic energy is minimum.

2.0 Derivation of the electrostatic energy ψ_{elast}

The electrostatic energy is obtained using the fundamental relationships of the theory of elasticity [3]. We have by definition:

$$\psi_{elast} = \frac{V\sigma_{ik}u_{ik}}{2} \tag{2.1}$$

where σ_{ik} is the elastic stress tensor, u_{ik} the strain tensor, and v the volume of the body. The conditions of the theoretical model read:

1˚) – No variation of the volume:

$$u_{ii} = u_{xx} + u_{yy} + u_{zz} = 0$$

2˚) – Because of the axial symmetry around the field direction, x-axis, only the components $u_{xx}, u_{yy} = u_{zz}$ are different from zero and we have:

$$\varsigma = \left(u_{xx} - u_{yy}\right) = \frac{a_1 - a_2}{a},\ u_{xx} = \frac{2}{3}\left(u_{xx} - u_{yy}\right) = \frac{2}{3}\varsigma$$

3˚) – Using the basic equations of the theory of elasticity [2], we get after some algebra the following relationships:

$$\sigma_{ii} = \sigma_{xx} + \sigma_{yy} + \sigma_{zz} = 0,\ \sigma_{xx} = \frac{2}{3}\left(\sigma_{xx} - \sigma_{yy}\right),\ \left(\sigma_{xx} - \sigma_{yy}\right) = 2u\left(u_{xx} - u_{yy}\right)$$

where μ is the slide modulus of the elastic molecule having a spherical form. Finally, using the relations obtained, the elastic energy (2.2) reads:

$$\psi_{elast} = \frac{v}{3}\left(\sigma_{xx} - \sigma_{yy}\right)\left(u_{xx} - u_{yy}\right) \tag{2.2}$$

which leads in term of the deformation ς

$$\psi_{elast} = \frac{2\mu V}{3}\left(u_{xx} - u_{yy}\right)^2 = \frac{2\mu V}{3}\varsigma^2 \tag{2.3}$$

3.0 Derivation of the electrostatic energy ψ_{elect}

Let P_x the component along x axis of the polarizaton vector $\vec{P}$, and ε_x the dielectric constant of the ellisoid along the same axis. In a medium of dielectric constant ε, the analytical exprssion of the electrostatic energy reads [4]:

$$\psi_{elect} = -\frac{P_x E}{2} = -\frac{VE^2}{8\pi}\frac{(\varepsilon_x - 1)\varepsilon}{[\varepsilon + (\varepsilon_x - \varepsilon)A_x]} \tag{3.1}$$

where E is the external field intensity, and A_x the coefficient of depolarization (or form factor). For an ellipsoid of revolution stretched along the x axis we get:

$$A_x = \frac{1-e^2}{2e^3}\left[\ln\left(\frac{1+e}{1-e}\right) - 2e\right] \tag{3.2}$$

If the ellipsoid is such that $(e << 1)$, we obtain with a good approximation:

$$A_x = \frac{1}{3} - \frac{2e^2}{15} \tag{3.3}$$

We have by definition: [2]

$$e^2 = 1 - \frac{a_2^{\,2}}{a_1^{\,2}} = \frac{(a_1 + a_2)(a_1 - a_2)}{a_1^{\,2}} \tag{3.4}$$

Hence, if we consider the following usual approximations:

$$a_1 + a_2 = 2a;\ \ a_1 = a$$

We obtained equation (3.3) the following relation for A_x, Viz:

$$A_x = \frac{1}{3} - \frac{4}{15}\left(\frac{a_1 - a_2}{a}\right) \tag{3.5}$$

It is worthy to note that equation (3.5) which useful for the derivation of the electrostatic energy; is also expressed in term of the deformation ς.

A dielectric tensor of a body slightly elongated, can be written in a series expansion limited to a second order terms, Viz :[2]

$$\varepsilon_{ik} = \varepsilon_0 + \gamma_1 u_{ik} + \gamma_2 u_{ll}\delta_{ik} \tag{3.6}$$

where ε_0 is the dielectric constant of the spherical body, γ_1 and γ_2 being to arbitrary constants

In particular, the dielectric constant ε_x reads according to equation (3.6):

$$\varepsilon_x = \varepsilon_{xx} = \varepsilon_0 + \gamma_1 u_{xx} = \varepsilon_0 + \frac{2\gamma_1}{3}\left(u_{xx} - u_{yy}\right) \tag{3.7}$$

Or in equivalent form:

$$\varepsilon_x = \varepsilon_0 + \frac{2\gamma_1}{3}\varsigma \tag{3.8}$$

Finally, using the relations (3.1) and (3.8) the expression of the electrostatic energy reads:

$$\psi_{elect} = -\frac{VE^2}{8\pi}\left[\frac{(\varepsilon_0 - 1)\varepsilon + 2\gamma_1\varepsilon\varsigma/3}{\varepsilon + (\varepsilon_0 - \varepsilon + 2\gamma_1\varsigma/3)A_x}\right] \tag{3.9}$$

Using equation (3.5) the electrostatic energy can be readily written in the following form:

$$\psi_{elect} = -\frac{VE^2}{8\pi}\frac{f(\varsigma)}{g(\varsigma)} \tag{3.10}$$

with

$$\begin{cases} f(\varsigma) = K_1 + K_2\varsigma \\ g(\varsigma) = K_3 + K_4\varsigma - K_5\varsigma^2 \end{cases} \tag{3.11}$$

with the following definitions for the coefficients K_i :

$$\begin{cases} K_1 = (\varepsilon_0 - 1)\varepsilon \\ K_2 = 2\gamma_1\varepsilon/3 \\ K_3 = (\varepsilon_0 + 2\varepsilon)/3 \\ K_4 = [2\gamma_1/9 - 4(\varepsilon_0 - \varepsilon)/15] \\ K_5 = 8\gamma_1/45 \end{cases} \tag{3.12}$$

The condition for the total energy $\psi_{tot} = \psi_{elast} + \psi_{elect}$ to be minimum reads:

$$\frac{4\mu\varsigma g^2}{3} = \frac{E^2}{8\pi}\left[g\frac{\partial f}{\partial\varsigma} - f\frac{\partial g}{\partial\varsigma}\right] \tag{3.13}$$

We observe as required that the condition (3.13) is independent of the volume V of the body. Hence after some algebra, and at first order term in ς we get:

$$\varsigma = \frac{k(K_2K_3 - K_1K_4)}{(K_3^2 - 2kK_1K_5)} \tag{3.14}$$

with:

$$k = \frac{(E^2/8\pi)}{(4\mu/3)} \tag{3.15}$$

In equation (3.14) the term $(2kK_1K_5)$ is very small compated to K_3^2, consequently, the expression for ς simply reads:

$$\varsigma = \frac{E^2(K_2K_3 - K_1K_4)}{8\pi(4\mu K_3^2/3)} \tag{3.16}$$

Substituting the coefficients K_i by their expreddions given equation (3.12) we get:

$$\varsigma = \frac{9E^2[(\varepsilon_0 - 1)(\varepsilon_0 - \varepsilon)\varepsilon + 5\gamma_1\varepsilon(1 + 2\varepsilon)/6]}{40\pi\mu(\varepsilon_0 + 2\varepsilon)^2} \tag{3.17}$$

which can be written in the following useful form:

$$\varsigma = \frac{9E^2}{40\pi\mu}\varphi(\varepsilon_0, \varepsilon) \tag{3.18}$$

where the factor $\varphi(\varepsilon_0, \varepsilon)$ depends only on the dielectric constants of unstretched body, and the dielectric constant of the medium

As indicated, we consider in this work the case of a deformation without change in volume, only the forms variation of the ellipsoid are taken into account by the variation of the parameter ς, namely are applied field strength E, and the dielectric constant of the medium. Indeed (ε_0, μ) are the characteristics of the deoxy-HbS.

4.0 Study of ellipsoid axes variation

We consider following the system of equations:

$$\begin{cases} a_1 - a_2 = a\varsigma \\ a_1 + a_2 = 2a \end{cases} \tag{4.1}$$

The first result from the definition (1.1) of the deformation, and the second from the approximation made to derive the form factor A_x. Solving the system (4.1 we get:

$$\begin{cases} a_1(\varsigma) = a(1+\varsigma/2) \\ a_2(\varsigma) = a(1-\varsigma/2) \\ a_3(\varsigma) = a_2(\varsigma) \end{cases} \tag{4.2}$$

The equation (4.2) indicates that the ellipsoid exes have a linear variations in terms of ς. However, for each increment $\Delta = a\varsigma/2$ of the axis $a_1(\varsigma)$ from the initial form (the sphere) correspond increment $-\Delta$ for axis $a_2(\varsigma)$. Hence the ellipsoid becomes more and more stretched.

It is interesting to observe that the variation of $a_2(\varsigma)$ indicates a limit for the deformation. More precisely, the following condition must be always satisfied.

$$a_2(\varsigma) \geq 0 \; so \; it \; \varsigma \leq 2 \tag{4.3}$$

The equation (4.3) clearly indicates that there exist a critical value E_c for the field intensity for which the HbS molecule is destroyed.

It is worthy to note this theoretical result is in excellent agreement with the experimental observations. Using equation (3.17) and the condition (4.3) we get for E_c:

$$E_c = \frac{4}{3}\sqrt{\frac{5\pi\mu}{\varphi(\varepsilon_0, \varepsilon)}} \tag{4.4}$$

For numerical calculation of the deformation ς, we suppose at first approximation that the dielectric mediums crossed by the light are not absorbing mediums. Consequently, the Maxwell equation can be readily used:

- $\varepsilon_0 = n_0^2$ for the dielectric constant of the sphere;
- $\varepsilon = n^2$ for the dielectric constant of the solution.

The intensity of the applied field is an experimental data, that is not the case of the slide modulus μ of the deoxy-HbS. However, the value of this parameter can be readily estimated through the theoretical results derived by Cerf [5] concerning the form fluctuations of a elastic spherical molecule

Hence, the formulae giving the mean square of the deformation coefficients reads:

$$< (u_{ii})^2 > = \frac{kT}{3V\mu} \tag{4.5}$$

where (u_{ii}) are the diagonal elements of the deformation tensor, k the Boltzmann constant, V the volume of the molecule, T the absolute temperature.

Moreover, in order to give insight to small fluctuations Cerf [5] used

$$< (u_{ii})^2 > \leq 0.01 \tag{4.6}$$

which leads to

$$\mu \leq \mu_0 = \frac{100kT}{3V} \tag{4.7}$$

Consequently, for a spherical molecule of radius *a* we get the desired result viz : :

$$\mu_c = \frac{100kT}{4\pi a^3} \tag{4.8}$$

5.0 Conclusion

The deformation of particles by homogenous electric fields is of importance in many physical problems, in particular in studies of disperse systems such as micelles, coaservates and emulsions.

When the particle is hemoglobin droplet, equilibrium deformation will be achieved when the electric and the elastic forces are balanced. And the free energy of the system is a minimum. It was assumed that the equilibrium shape of the deox-HbS would be an ellipsoid of revolution with symmetry axis parallel to applied field.

The sum of the electric and elastic free energies then was computed and minimized as a function of the deformation which depends of the eccentricity.

Finally, it worth noting that the theoretical treatment develop in this work will provides subsequent experimental measurement for a comprehensive studies of sickle cell disease.

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