A MODEL OF THE MOLECULAR AGGREGATE PROCESSES OF HEMOGLOBIN

S. ABSENCE OF CRYSTALLIZATION.

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ABSTRACT:
The molecular aggregate formation mechanisms play a major role in the interpretation of the
pathophysiology of Sickle Cell disease and in the selection of the therapeutic strategies to
follow. A mechanism and a mathematical model are proposed. The model postulates the
existence of defective microtubules formed by deoxy Hemoglobin S and oxy Hemoglobin S,
and explains the dependence of polymerization on Hemoglobin concentration, temperature,
and partial oxygen pressure. The analysis focuses on the Polymerization of Hemoglobin S in
the absence of crystallization. The action of other kinds of Hemoglobin in the molecular
aggregate formation process can be explained.
Keywords: Polymerization, Hemoglobin S, Sickle Cell, Modeling

I. INTRODUCTION:

Sickle-cell anemia remains a health problem worldwide [1]. This genetic disease is due to

an alteration in the hemoglobin amino-acids chain, which leads to the polymerization of Hemoglobin S (HbS) under low oxygenation conditions, the deformation of red blood cells, changes in membrane permeability and elasticity, modifications in blood rheology and several other physiological processes; thus producing the vase-occlusive crises that cause an intense, progressive deterioration of the patients, resulting in their death, generally at early ages [1].

Several factors determine the polymerization of deoxygenated HbS. Some of them are: the HbS oxygenation degree [2-4], HbS concentration *[*HbS*]* [2, 5, 6], temperature [7,8], the pH of the solution [2,6], and the presence of other Hemoglobins [5,9-12]. The main factor determining HbS polymerization is partial oxygen pressure p, because the oxygenated HbS - deoxygenated HbS ratio (oxyHbS/deoxyHbS) depends on it. Increasing temperature favors the formation of deoxyHbS aggregates; so does an increase in *[*HbS*]*. However, for this phenomenon to occur, a deoxyHbS concentration *[*deoxyHbS*]* greater than a minimum value ( ) is necessary [2, 3].

An interesting experimental fact is that, even at a low p, a fraction of deoxyHbS is not in molecular aggregate state and decreases with the increase of p [2, 3]. The molecular aggregates formation of HbS is a reversible phenomenon, since deoxyHbS aggregates dissolve under oxygenation or with a temperature decrease [1-3, 5].

The inhibiting effect of mixing HbS with other types of Hemoglobin (HbA, HbF, HbA$_2$) on polymerization is well known [2, 6, 9-12]. The increase of HbA and HbF in the total hemoglobin of sickle-cell anemia patients has also been associated with a decrease of clinical severity [1, 2, 12, 14].

HbS polymers have been studied using different techniques, such as X -ray diffraction [2, 15-17] and Electronic Microscopy (EM) [2, 18-22]. The structure of HbS molecular aggregates has been determined by EM [2, 20, 21], showing the structure of fibers (microtubules, μT) formed by 14-16 hemoglobin molecules. The dominions formation has also been observed inside erythrocytes in advanced gelation state. The kinetics of the microtubules and dominions formation has not been explained in detail [2, 19-24].
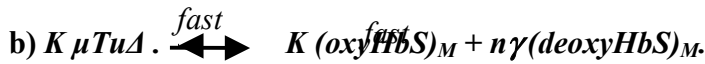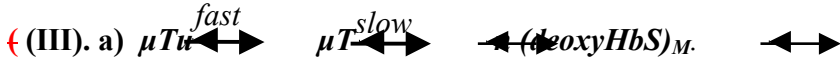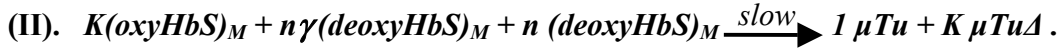
The molecular mechanisms of polymerization and depolymerization play a major role in the interpretation of the pathophysiology of the disease and in the selection of therapeutic strategies to follow [1-3, 23]. Different mechanisms and models have been proposed [2, 3, 22-30], which allow the interpretation of numerous phenomena.

In this paper, a polymerization mechanism and model are presented assuming that defective structural units are formed. The structural data related to the different number of strands forming the HbS fibers can be explained. The model also describes the influence of *[*HbS*]* and p on the polymerization process and the role of other Hemoglobins, including HbS itself, whenever its three-dimensional structure or contact points are modified.
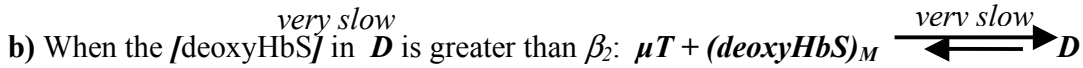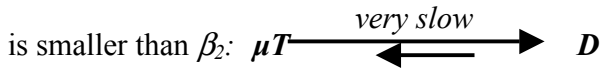
II. METHOD.
Let us assume that HbS can appear in red blood cells in the following states (Figure 1):
- **HbS Oxygenated** in a) Monomers *M*; b) Defective microtubule structural units *μTuΔ*
- **HbS Deoxygenated** in a) Monomers *M*, b) Microtubule structural units *μTu*, c) Microtubules *μT*, d) Domains *D*.
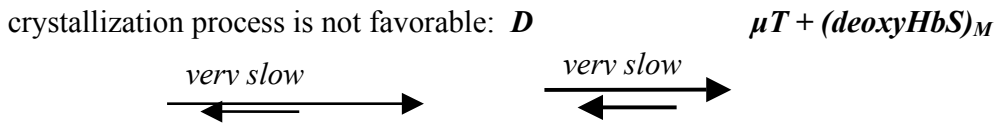
Considering the hypothesis that **µTu** (formed wholly by deoxyHbS molecules), coexist with **µTuΔ** (formed by both deoxy and oxyHbS) unable to form new tubules, we propose the following mechanism:

(I).   1(deoxyHbS)$_M$ + 4 O$_2$ $\xrightarrow{\text{\textit{fast}}}$ **1 (oxyHbS)$_M$ (in any state)**

(II).   K(oxyHbS)$_M$ + nγ(deoxyHbS)$_M$ + n (deoxyHbS)$_M$ $\xrightarrow{\text{\textit{slow}}}$ **1 µTu + K µTuΔ .**

(III). a) µTu $\xleftrightarrow{\text{\textit{fast}}}$ **µT** $\xleftrightarrow{\text{\textit{slow}}}$ **(deoxyHbS)$_M$.** $\longleftrightarrow$

   b) K µTuΔ . $\xleftrightarrow{\text{\textit{fast}}}$ **K (oxyHbS)$_M$ + nγ(deoxyHbS)$_M$.**

(IV). a) When the **[deoxyHbS]** in **µT** is greater than or equal to β_1 and the **[deoxyHbS]** in **D**

is smaller than β_2: **µT** $\xleftrightarrow{\text{\textit{very slow}}}$ **D**

b) When the **[deoxyHbS]** in **D** is greater than β_2: **µT + (deoxyHbS)$_M$** $\xleftrightarrow{\text{\textit{very slow}}}$ **D**

When the **[deoxyHbS]** in **µT** is smaller than β_1 and the **[deoxyHbS]** in **D** is smaller than β_2, the

crystallization process is not favorable: **D**   **µT + (deoxyHbS)$_M$**

   $\xleftrightarrow{\text{\textit{very slow}}}$ $\xleftrightarrow{\text{\textit{very slow}}}$

Processes **(II) - (IIIa)** constitute the polymerization mechanism, which includes the formation of **µTuΔ.** $n\alpha = n + n\gamma = n(1+\gamma)$ Where α: minimum **[deoxyHbS]** necessary for polymerization to occur; *n*: number of HbS molecules that form the **µTu**. As   can range from 1 to approximately 2, it is generally taken as such in the model, i.e. $\alpha=2+\delta$, where $/\delta/<1$. This allows us to explain the experimental data related to different numbers of molecules forming the **µT.**

The crystallization mechanism is described by **(IV)**. It has been supposed that the HbS change from **µT** state to **D**, only occurs when the **[deoxyHbS]** in **µT** surpasses a threshold β_1. Once small deoxyHbS dominions exist, they can grow either by addition of isolated deoxyHbS monomers or by addition of **µTu**. The **D** shall be disintegrated when the **[deoxyHbS]** in **µT** is smaller than β_1 and the **[deoxyHbS]** in dominions decreases below its threshold value β_2. Reactions **I - IV** are in agreement with the double nucleation model described (Homogeneous and Heterogeneous processes) [2].

III. RESULTS AND DISCUSSION.

The following mathematical model is proposed: Assuming the quick processes **(I)** and **(III)** as instantaneous compared to the rest, then the mass conservation law is stated by:

$$x(t) + n \cdot y(t) + n \cdot z(t) + w(p) = N \quad (1)$$

Where *x(t)*: **[deoxyHbS]** in monomer state or as part of **µTuΔ**; $n \cdot y(t)$: **[deoxyHbS]** in **µT** ; $n \cdot z(t)$: **[deoxyHbS]** in **D**; $w(p)$: **[oxyHbS]** depending on the *p* inside the erythrocytes; *N*: total **[HbS]**. In turn, the equation system describing the kinetic process is:

$$\frac{dx}{dt} = -n \cdot P(x) - (c - d)x + n \cdot b \cdot y \quad (2)$$

$$\frac{dy}{dt} = -(b + e - f) \cdot y + P(x) \quad (3)$$

$$\frac{dz}{dt} = \left(\frac{c - d}{n}\right) \cdot x + (e - f) \cdot y \quad (4)$$

Where: a_i *(i=1...2n)*, *b, c, d, e* and *f*, are the reaction coefficients. a_i: polymerization, *b:* depolymerization, *c*: crystallization by the addition of deoxyHbS monomers, *d:* decrystallization by separation of monomers, *e* crystallization by addition of *μT*; *f:* decrystallization by separation of *μT*. $P(x) = \sum_{i=1}^{2n} a_i \cdot x^i$ is a Polymerization function.

In this paper the fundamental properties of the solution of the (2) - (4) equation system are discussed and a phenomenological interpretation of these properties is given. The (1) - (4) non-lineal ordinary differential equation system has not been solved because of its complexity. However, several specific cases are interesting and simple enough to study them separately. The analysis focuses on the *Polymerization of HbS in the absence of crystallization*.
If at a given moment there are no dominions and at every subsequent moment *[HbS]* < β_l in *μT,* then crystallization will never occur and $z(t) = 0$. Thereby

$$x(t) + n \cdot y(t) + w(p) = N \quad (5)$$

$$\frac{dx}{dt} = -n \cdot P(x) + n \cdot b \cdot y \quad (6)$$

$$\frac{dy}{dt} = -b \cdot y + P(x) \quad (7)$$

The solution of the (6), (7) system is expressed as follows:

$$x(t) + n \cdot y(t) = Q = const. \quad (8)$$

i.e. a set of straight lines with *-1/n* slope. If we substitute (8) in (5), we get *w(p) = N – Q,* which indicates that *[oxyHbS]* is constant in this specific case. This system has an indefinite number of equilibrium positions, which can be seen in the following equation:

$$y = \frac{P[x(t)]}{b} \quad (9)$$

If the initial condition *x(t₀)=x₀*, *y(t₀)=y₀* is met, after a considerably long time, the solution of the (6) - (7) system approaches curve (9), moving across a set of straight lines with *-1/n* slope (See fig. 2A). This guarantees the stability of the equilibrium positions. In other words, if there is no crystallization, the *[deoxyHbS]* in monomer state forming part of *μT* tends to fixed values, whether these initial conditions are above or below the curve (9). A small increase in total *[HbS]* or a small decrease of *p* produces an increase of the *[deoxyHbS]* in *μT* [2]. If the temperature rises [2,3], the a_n/b ratio in (9) should grow, and so will *y(τ)*.

The stable solution of the (5)-(7) system can explain another experimental fact. The *y(τ) / x(τ)* ratio decreases with the increase of *p,* not only because *y(τ)* falls but also because *x(τ)* grows simultaneously [5]. This is illustrated in figure 2.

The parameter α was introduced in the model of the HbS molecular aggregate formation process. It should be interpreted as follows: When $p=0$ and all the HbS is deoxygenated, $\gamma=0$, then oxyHbS molecules able to participate in the formation of **μTuΔ** do not exist. Therefore, only n molecules of deoxyHbS are necessary to form correct structural units. On the other hand, when $p\neq0$, then $\gamma\neq0$, i.e., when a correct structural unit is formed (by n deoxyHbS molecules), K' defective units should be formed (by a total of $n\gamma$ deoxyHbS molecules). With the increase of p, the probability of interaction between oxy and deoxy molecules also increases. Then, at least one of the oxyHbS molecules will be part of a **μTuΔ**, instead of a correct one. In other words, $\gamma(p=0)=0$ and $\alpha(p=0)=1$ and both increase when p grows. From another viewpoint, when HbS is highly deoxygenated $(p=0)$, reaction **(IIIb)** is negligible and only correct structural units influence the polymerization - depolymerization equilibrium. On the other hand, when HbS is too oxygenated, reaction **(IIIa)** is negligible, and the defective structural units determine the polymerization - depolymerization equilibrium. The number of molecules $(n\alpha)$ forming the **μTu** varies according to the values of p at which these experiments are carried out. The dependence of α on p discussed above implies that the increase of *[*deoxyHbS*]* in **μT** produced by an increase in the total *[*HbS*]* is much more abrupt than that caused by a decrease of p.

Polymerization speed:

Supposing that under certain conditions the polymerization process is dominant and depolymerization is negligible, $a_{2n}>>b$, and considering that P(x) is determined by the last term a_{2n} then the (5), (6), (7) system approximately remains as follows:

$$\frac{dx}{dt} = -n \cdot a_{2n}x^{2n} \quad (10)$$

$$\frac{dy}{dt} = a_{2n}x^{2n} \quad (11)$$

For arbitrary initial conditions (x_0, y_0), the solution of (10) is:

$$x(t) = \left[\frac{1}{Bt + A}\right]^{\frac{1}{C}} \quad (12)$$

Where: fix A, B and C $A = x_0^C$; $B = nCa_{2n}$; $C = 2n - 1$

Then in this specific case after a considerably long time $(\tau \gg 1)$, the total polymerization of HbS will occur and the following expression can be obtained:

$$y(t) = \left[\frac{1}{Bt + A}\right]^{\frac{1}{C}} + \frac{x_0}{n} + y_0. \quad (13)$$

Expressions (12) and (13) can be used experimentally for the quantitative determination of a_{2n}

IV. CONCLUSIONS

The polymerization process can be described by a non-lineal ordinary differential equation system. The solution has an indefinite number of equilibrium positions. In other words, if there is no crystallization, the deoxyHbS concentration in monomer state forming part of microtubules **μT** tends to fixed values. A qualitative discussion properties of the solution to the mathematical model corresponding to the HbS molecular aggregate formation mechanisms is proposed.

The existence of defective microtubules formed by deoxyHbS and oxyHbS make possible explain the dependence of polymerization on HbS concentration, temperature and partial oxygen pressure. The number of molecules $(n\alpha)$ forming the microtubule units μTu varies according to the values of partial oxygen pressure p at which these experiments are carried out. The increase of deoxyHbS concentration in microtubules μT produced by an increase in the total HbS concentration is much more abrupt than that caused by a decrease of p, which is in agreement whit the literature.

The action of other types of Hemoglobin in the molecular aggregation process forms defective tubules with HbS, whose stability is considerably lower. It is possible to understand that the action of some chemical compounds (specifically the Vanillin) forming a relatively stable link to Hemoglobin in the polymerization process, increase the delay time [31]. This fact has important implications in the therapeutic strategies. The selection of the therapeutic strategy to follow must aim at reducing the a_i/b_i ratios, which would lead to the control of the parameters determining polymer formation: the molecular basis of the pathophysiology of the disease.

The conclusions derived from the discussion above are in accord with the experimental data known. Future papers will deal with models that supplement the one presented here and will include crystallization.

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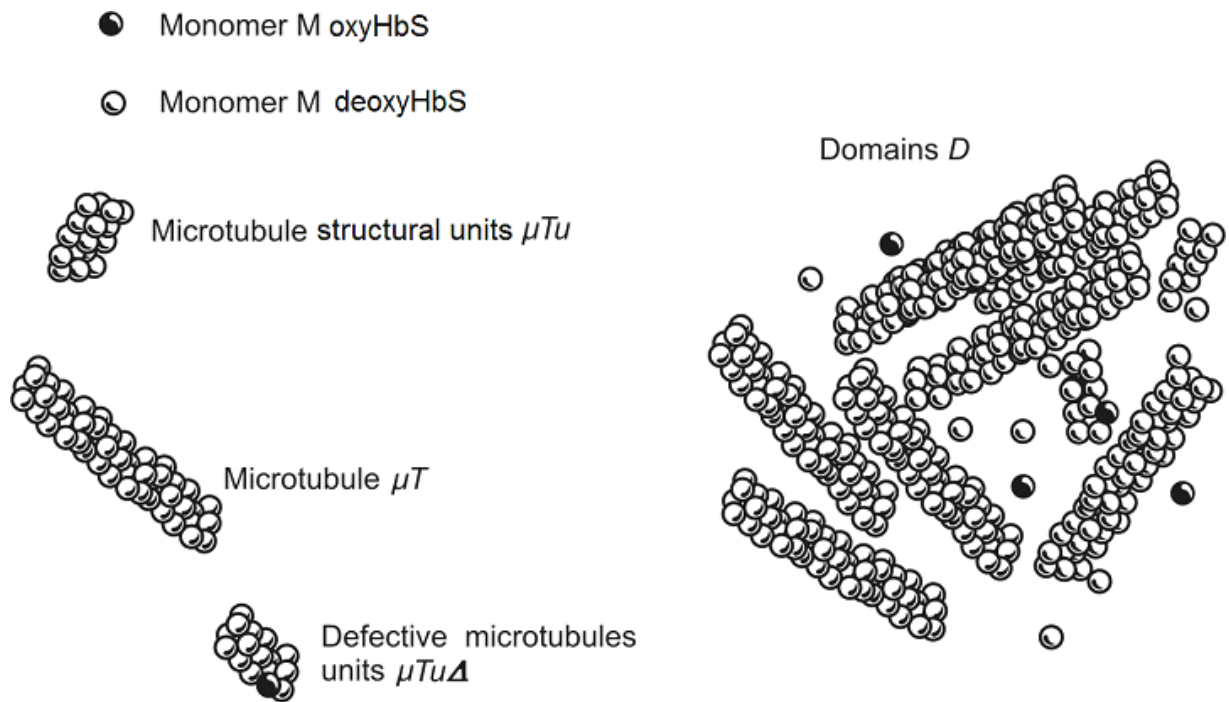
Figure 1. Different aggregation states of Hemoglobin S in the polymerization process.
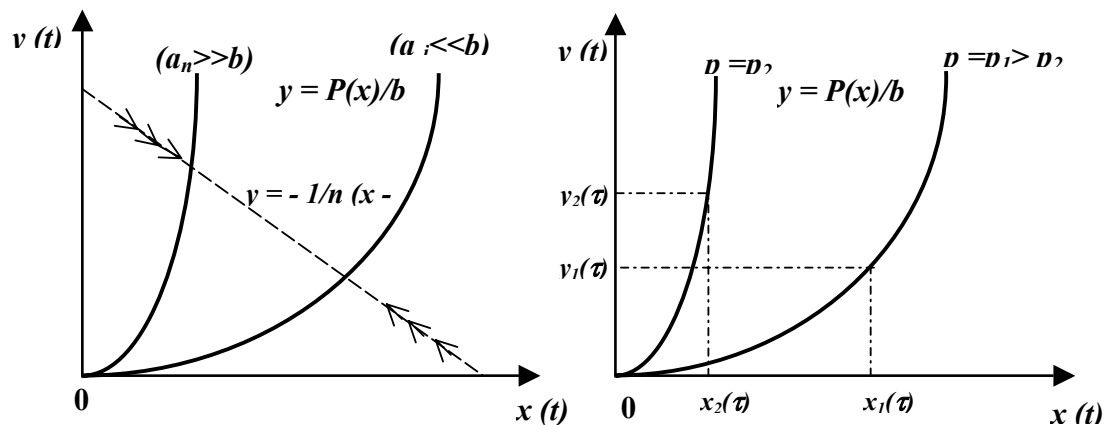
Figure 2 Dependence of the deoxyHbS concentration in microtubules *y(t)* at two extreme ratios of polymerization and depolymerization coefficients *a/b* (fig. 2A) and at two different partial oxygen pressures *p* (fig. 2B). This system has an indefinite number of equilibrium positions moving across a set of straight lines with -*1/n* slope (2A). If *p* rises, then x(τ) increases while y(τ) will decrease. Consequently, the ratio y(τ) / x(τ) will decrease very fast (2B).