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Computational Analysis of Thermal Denaturation of Human Hemoglobin by Non Linear Regression of Gushimana Yav Equation Using Origin Software Package

Koto-te-Nyiwa Ngbolua[1, 2*], Pius T. Mpiana[2], Gushimana Yav[2]
[1]University of Gbadolite, P.O. Box 111 Gbadolite, Nord-Ubangi Province, Democratic Republic of the Congo
[2]Faculty of Science, University of Kinshasa, P.O. Box 190 Kinshasa XI, Democratic Republic of the Congo
*Corresponding author: Koto-te-Nyiwa Ngbolua, Telephone: +243 81 68 79 527, Email: jpngbolua@unikin.ac.cd
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ABSTRACT:

Recent findings indicate that the number of newborns with Sickle cell anemia (SCA) will increase on the horizon 2050. Among the concerned countries, Democratic Republic of the Congo will probably still be the country most in need of policies for the prevention and management of SCA. The present fundamental research was carried out with the aim of providing data to help future design and the development of new generation of techniques for the low cost diagnosis of SCA patients from low income countries. The interest in the protein thermal denaturation is due to its potential to easily quantify any change in the hemoglobin stability.

The thermal denaturation of hemoglobin in human total blood samples was studied using molecular ultraviolet/visible absorption method. The result expressed as transition temperature (T_t) displayed the best discrimination between AA, AS and SS bloods at pH 7.40 and without ionic strength. Calculated values of T_t according to a non-linear regression analysis using the Gushimana Yav equation (a sigmoid plot) with the help of Microsoft Origin package are 65.2 ± 0.1 °C for AA blood, 61.0 ± 0.1 °C for AS blood and 58.0 ± 0.1 °C for SS blood. By considering T_t as a specific physical parameter, it is thus proposed to design and develop new generation of spectrophotometer for the biological diagnosis of SCA.

Keyword: *Sickle Cell Anemia, Hemoglobin S, Thermal Stability, Spectrophotometer, Diagnosis*

INTRODUCTION

Recent findings indicate that the number of newborns with Sickle cell anemia (SCA) will increase from about 305,800 in 2010 to about 404,200 in 2050. The Democratic Republic of the Congo (DRC) as well as Nigeria and India will probably still be the country most in need of policies for the prevention and management of SCA in 2050 [1].

The screening test of SCA patients usually uses electrophoresis and chromatographic techniques (HPLC). However, the continuous gels use makes these methods much cost, in particular, for the laboratories of the poor countries like DRC. It is therefore necessary to develop new techniques better adapted to these conditions by using knowledge deriving from biochemistry, physical chemistry and mathematical modeling [2]. The interest in the protein thermal denaturation in this case is due to its potential to easily quantify any change in the hemoglobin stability. The experiment is done by the unfolding of the protein determined from absorbance changes as a function of increasing temperature. Thermal denaturation assay in which the sample is slowly heated is more useful in providing quantitative comparisons [3].

The study of the transition between the native and denatured form of hemoglobin by thermal denaturation using spectrometry revealed that the sickle hemoglobin is thermally less steady than the hemoglobin A in aqueous solution. This thermal stability difference could be expressed by the difference of the transition temperature (Tt) for these two types of hemoglobin. This parameter (that represents the temperature to which 50% of the hemoglobin is denatured) is specific to each of these two proteins [2, 4, 5].

The necessity to develop new generation of spectrophotometer based on the transition temperature for the low cost screening of SCA patients by using total blood samples is its speed (not purification of hemoglobin is required) and its precision (the UV-Visible spectrophotometer being a physical method) thus justify the present research study.

THEORETICAL TREATMENT OF THE THERMAL DENATURATION

The hemoglobin stability data analysis can be done using a two-state model in which the hemoglobin is considered to be either in the native or the denatured state. It is thus assumed that the thermal denaturation reaction of hemoglobin is reversible and can be described according to the equilibrium shown below:

$N \overset{K}{\leftrightarrow} D$ (1). In diluted solution, $K = \frac{C_D}{C_N}$ (2); Where K, C_N and C_D are respectively the equilibrium constant, the concentrations of native and denatured hemoglobin. The absorbance of solution where the two forms (native and denatured) of hemoglobin coexist is given by the relation:

$E = d\,(\varepsilon_N\, C_N + \varepsilon_D\, C_D)$ (3)

Where ε_N and ε_D are the molar extinction coefficients of native and denatured forms of hemoglobin.

The value of the constant K can be formulated in terms of absorbance as follow:

$K = \frac{E - E_N^o}{E_D^o - E}$ (4)

Where E_N^o and E_D^o are the absorbencies of the native and denatured forms of hemoglobin. The equilibrium constant K depends on the intensive thermodynamic parameters like temperature, pressure and intensity of the electric field.

At both the constant pressure and the absence of electric field and according to the formalism of Gibbs, one has:

$d\ln K = (\frac{\Delta H^o}{RT2})\, dt$ (5)

Where ΔH^o is the standard molar enthalpy of denaturation, and T is the temperature. By integrating the equation (5) within the temperature domain in which the standard enthalpy of reaction remain constant and to the temperature T corresponding to transition temperature T_t (i.e temperature for which 50% of hemoglobin is denatured), the expression of K can write as:

$K = e^{\frac{\Delta H^o\,(\frac{1}{T_t} - \frac{1}{T})}{R}}$ (6). By combining the equations (4) and (6), the following equation is obtained:

$$E = E_D^0 - \frac{(E_D^0 - E_N^0)}{1+e^{\frac{\Delta H^0}{R}(\frac{1}{T_t}-\frac{1}{T})}} \quad (7)$$

Where R is the constant of perfect gases and the relation (7) is so called ***Gushimana Yav equation***.

EXPERIMENTAL PROCEDURE

❖ ***Preparation of the met-hemoglobin mother solution***

- To 20 mL of total blood previously diluted with a double volume of bi-distilled water;
- Add 1 mL of $K_3Fe(CN)_6$ solution at 20% ;
- Evaluate the concentration of mother solution at the wavelength of 500 nm ($\varepsilon_{500m} = 10.000M^{-1}.Cm^{-1}$) et à 630 nm ($\varepsilon_{630nm} = 4.400M^{-1}.Cm^{-1}$)

❖ ***Preparation of the working solution of met-hemoglobin***

The working solution of met-hemoglobin is freshly prepared by diluting the mother solution with citric buffer 0,01M. The pH is then adjusted at 7.40 with the help of concentrated solution of NaOH. The magnetic agitator IKAMAG REO is used for mixing the solution.

❖ ***Measurement of optical density of the working solution***

- Read the optical density/absorbance at 500, 575 and 630 nm in a Perkin Elmer Lambda 2 UV-visible spectrophotometer. The Lauda C_6 thermostat with digital reading of temperature is used to increase the temperature from 35 to 80 ^OC.

❖ ***Experimental data analysis***

The data analysis was carried out by non-linear regression of Gushimana Yav equation with the help of the Origin software package on a personal computer. In this analysis, the Gushimana Yav equation (GYE) is introduced as function of which the physical parameters like the transition temperature and the enthalpy are fitted according to GYE.

RESULTATS AND DISCUSSION

The figure 1 gives the absorption spectrum of met-hemoglobin formed in total blood sample.

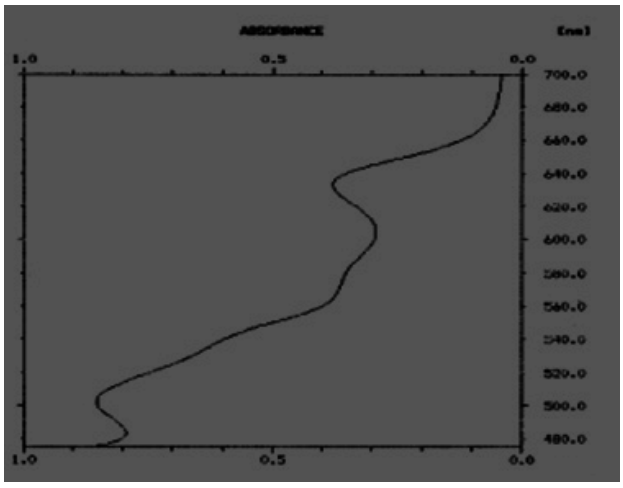

Figure 1: Absorption spectrum of met-hemoglobin in total blood at 26 ^OC and at pH 6 (Citric buffer 100 mM)

The met-hemoglobin aqueous solution presents characteristic absorption bands at three wavelengths: 500, 575 and 630 nm.

The figure 2 gives the curves of thermal denaturation of the met-hemoglobin A and S formed in total blood AA, AS and SS samples at pH 7.40 and the wavelength of 500 nm.

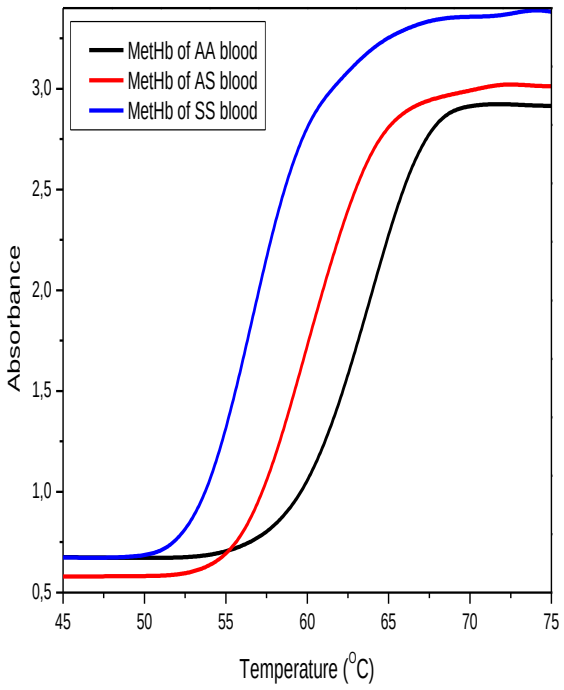

Figure 2 : Variation of absorbance of total blood MetHb according to temperature (Citric buffer 100 mM, pH 7.40, λ=500 nm)

The figure 2 shows the sigmoid curves characteristic of the thermal denaturation of aqueous solution of purified hemoglobin or the total blood sample. They are characterized by three zones: the first zone corresponds solely to domain in which hemoglobin exists in its native form in solution (low optical density). The second zone corresponds to the domain of transition between the native and denatured forms of hemoglobin. The optical density of the solution is in this case due to native and denatured forms of hemoglobin. It can notice that the narrowness of this zone as one can observe it on the figure 2 is an indication that steady intermediates does not exist between the native and denatured forms of hemoglobin in solution.

Finally, the third zone indicates that from a given temperature, the whole protein is entirely denatured (high optical density). Besides, one can also note that the hemoglobin S (SS blood) is thermally less steady than hemoglobin A (AA blood). Also, the mixture of hemoglobin A and S (AS blood) is thermally less steady than hemoglobin A (AA blood).

This observed difference between the two types of hemoglobin (normal and sickle Hb) can be exploited in order to develop a new technique for the diagnosis of SCA.

The calculated values of transition temperature and standard molar enthalpy of denaturation are given in table 1.

Table 1: Mean values of calculated parameters of thermal denaturation of methemoglobin (Citric buffer 100 mM, pH 7.40)

Methemoglobin	Transition temperature (T_t ^OC)	ΔH^O (kJ/mole)
Sang SS	58.0 ± 0.1	+455 ± 0.1
Sang AS	61.0 ± 0.1	+483 ± 0.1
Sang AA	65.2 ± 0.1	+502 ± 0.1

The table 1 revealed that the calculated values of T_t are 65.2 ± 0.1 °C for AA blood, 61.0 ± 0.1 °C for AS blood and 58.0 ± 0.1 °C for SS blood. These values displayed the best discrimination between AA, AS and SS bloods at pH 7.40 and without ionic

strength. Besides, the thermal denaturation is an endothermic process ($\Delta \mathbf{H^O > 0}$).

CONCLUSION AND FUTURE PROSPECTS

SCA is a tropical disease endemic to low income countries including Democratic Republic of the Congo. The use of existing techniques is much cost for the poor population. In the present study, we have demonstrated that molecular ultraviolet/visible absorption method constitutes an alternative for the characterization and screening of sickle bloods. Indeed, the thermal denaturation (a endothermic process) could permit to discriminate AA, AS and SS blood samples.

These results open thus the way for design, research and development of a new generation of spectrophotometers incorporating both a water programmable circulator (thermostat) and an automatic optical density tape recorder and possessing an artificial intelligence capable to solve in real time the Gushimana Yav equation in order to diagnose SCA.

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REFERENCES

1. Piel FB, Hay SI, Gupta S, Weatherall DJ, Williams TN. Global Burden of Sickle Cell Anaemia in Children under Five, 2010–2050: Modelling Based on Demographics, Excess Mortality, and Interventions. PLoS Med 10(7): e1001484, 2013. doi:10.1371/journal.pmed.1001484.
2. Ngbolua KN, Gushimana ZY, Mpiana PT, Bibelayi DD, Mangwala PK. Etude par spectroscopie UV-Visible de la dénaturation thermique de l'hémoglobine S : une nouvelle approche du dépistage rapide de la drépanocytose. Annales Africaines de Médecine 3 (4) :540-548, 2010.
3. Kenneth W. Olsen. Thermal denaturation procedures for hemoglobin Methods in Enzymology 231: 514-524, 1994.
4. Mpiana PT, Mavakala BK, Gushimana ZY. Etude de la dénaturation thermique de l'hémoglobine S. Annales de l'Institut de Recherche en Sciences de la Santé 1997 ; 1(1) : 38-42, 1997.
5. Ngbolua KN, Bibelayi DD, Mangwala PK, Kilunga PI, Mpiana PT, Gushimana ZY. Etude par spectrophotométrie UV-visible de la dénaturation thermique des hémoglobines A & S dans le sang total, Journal de l'Institut de Recherche en Sciences de la Santé 1(5) : 28-33, 2011.

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