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Comparison between two different hemichromes of hemoglobins (HbA and HbS) induced by *n*-dodecyl trimethylammonium bromide: Chemometric study

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Abstract

The interaction of *n*-dodecyl trimethylammonium bromide (DTAB) with oxyhemoglobin A and oxyhemoglobin S is investigated using UV–visible absorption spectra and chemometric resolution techniques. Oxyhemoglobins (A and S) induced to partial oxidized form (ferrihemoglobin) by DTAB and finally transform to fully oxidized hemichrome. Hemichrome mole fractions of HbS are more than HbA because of more hydrophobic interaction of DTAB–HbS in second set of binding site relative to DTAB–HbA. The visible spectra between 500 and 650 nm are used for identifying the present components in solution because each species of hemoglobin has a specific spectrum in this region. The number of components and mole fraction of mentioned species were determined by employing chemometric resolution techniques. Subspace comparison was used for determination of the number of components in each concentration of hemoglobin and DTAB. After the determination of components, multivariate curve resolution-alternating least square (MCR-ALS) by initial estimates of spectral profiles and proper constraints, was used to resolve the data matrix into pure concentration and spectral profiles. The results show that both number and mole fraction of components which were formed during hemoglobin (HbA and HbS) oxidation by DTAB were initial hemoglobin concentrations independent. Furthermore, in average the mole fraction of hemichrome of HbS is 14.4% more than HbA. On the other hand, the mole fraction of HbA ferrihemoglobin is 15.6% higher than HbS averagely.

Keywords: Ferrihemoglobin; Hemichrome; Chemometric resolution techniques; Dodecyl trimethylammonium bromide; Normal hemoglobin and Sickle hemoglobin; Multivariate curve resolution-alternating least square

1. Introduction

Hemoglobin (Hb), a kind of respiratory protein of vertebrate erythrocytes, is important in oxygen transport, H_2O_2 dispersion and electron transfer to all organs and parts of the body [1]. Normal adult hemoglobin (hemoglobin A) is a symmetrical tetramer consisting of two unlike subunits or chains, designated α and β [2]. Many changes in the structure of hemoglobin have arisen by mutations in the human population that they often involve substitution of one amino acid for another. One of the most serious unusual hemoglobins is hemoglobin S (HbS), which is present in individuals suffering from sickle cell anemia [3]. Hemoglobin S is caused by an A-to-T mutation within the sixth codon of the β-globin coding region [4]. As a result, the charged glutamic acid residues at the sixth position (A3) of the two β-subchains in normal adult hemoglobin, hemoglobin A (HbA), are replaced by non-polar valine [5,6]. This single change in the primary sequence causes a marked change in the surface charge distribution and so net charge of protein, conformation of the protein and the nature of the protein-solvent interactions in the immediate vicinity of the substitution. This probably explains in large measure the differences in the surface activities of Hb S and Hb A as reported by Elbaum et al. [7]. This surface activity difference is almost certainly the origin of the greater susceptibility of HbS

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to precipitation, presumably as a result of surface denaturation [8–10].

Now, we have understood hemoglobin structure, the mechanisms of its oxygen transport function and its electron transfer on electrode surface [11–15], but the research on its interaction with other molecules are almost limited to a few biological molecules, such as bacterial endotoxin [16,17], hydroxyurea [18] and trehalose [19], with the conclusion that some biological molecules can convert oxyhemoglobin (oxyHb) to methemoglobin (metHb) and hemichrome, while some others may slow or reverse the auto-oxidation process of Hb [16,19]. Since hemichrome accumulation in red cells is typical of some blood diseases [20,21] and aging of the erythrocyte [22], the interrelated study of the conversion between metHb and hemichrome will be worthwhile not only in theory but also in practice.

Surfactant–protein interactions are very common in the fields of medicine, chemistry, biology, and so on [23–33]. The presence of DTAB causes the destabilization of protein and results in a decrease in the temperature of unfolding with an increase in the DTAB concentration [34,35]. Several pyridinium compounds which contain a hydrocarbon chain longer than 12 carbon atoms were examined in order to find a reagent which causes a drastic conformational change in proteins by interacting with the hydrophobic amino acid residues. It is reported that these cationic surfactants cause reduction in the α-helix content, decreasing the Soret band and oxidiation of oxy- to metmyoglobin [36]. The effect of cationic surfactant, dodecyl trimethylammonium bromide (DTAB) on human and greylag goose (Anser anser) hemoglobins was studied and it was reported that methemoglobin and hemichrome are the products of hemoglobin oxidation by DTAB [36,37]. Isothermal titration microcalorimetry and binding study of DTAB with wigeon hemoglobin were investigated by Bordbar et al. [38]. The influence of anionic surfactant, sodium *n*-dodecyl sulfate (SDS) on hemoglobin was studied and it was resulted that the reaction products are aquamethemoglobin and hemichrome [39].

Multivariate resolution methods make up a set of mathematical tools that may be applied to the analysis and interpretation of spectroscopic data recorded when monitoring a physical or chemical process with multichannel detectors. The aim of resolution methods is the recovery of chemical and/or physical information from the experimental data. Such data include, for example, the number of intermediates present in a reaction, the rate or equilibrium constants, and the spectra for each one of those intermediates [40–42]. For example, singular value decomposition (SVD) and global analysis were used to determine the kinetics of the binding of the CO to the HbS polymers that was recorded by time resolved optical spectroscopy, then results were compared with CO-deoxy HbA complex [43], or to determine the re-equilibration rate of carbon monoxide binding to HbS polymers is determined by time-resolved measurements of linear dichroism spectra [44]. Multivariate curve resolution-alternating least squares (MCR-ALS) [45] is a well-known resolution methodology that has been used to model processes [46–48]. Being a soft modeling methodology, MCR-ALS is able to extract the information of the evolving systems without using the underlying kinetic/enzymatic model. MCR-ALS is applied to the series of spectroscopic measurements recorded during unfolding to recover the concentration profiles and the spectra of the protein conformations involved in the process [46,47,49]. This method has already allowed for the successful detection and modeling of intermediate conformations in similar processes, induced by other agents and monitored with different techniques [50,51]. The resolution results provide an interpretation of the mechanism of the unfolding process and of the thermal evolution of the species involved. The resolved spectra tell about the nature of the conformations. In the present work, we recorded the interaction between DTAB and two different kinds of hemoglobin (HbS and HbA) and then the number of components present in the reaction mixture has been determined by the use of subspace comparison [52]. Also the concentration and spectral profiles of intermediates and products were resolved via MCR-ALS as a chemometric resolution technique.

2. Theory

2.1. Subspace comparison

Subspace comparison compares two subspaces, each of which described by a set of orthonormal vectors selected by a method suitable for variable selection such as orthogonal projection approach (OPA) [53], simple-to-use interactive self-modeling mixture analysis (SIMPLISMA) [54,55] and principal component analysis (PCA) [56]. Although the different methods select different key variables, if correct number of variables has been selected, the vectors selected by different methods span the same subspace of the full row or column space of the matrix. Suppose two subspaces are defined as $\mathbf{A}=\{a_1, a_2, \ldots, a_k\}$ and $\mathbf{B}=\{b_1, b_2, \ldots, b_k\}$, where K is the number of key variables or factors selected by different variables selection methods and is the same for both matrices. The vectors in **A** and **B** are orthogonalized by the Schmidt procedure [57]. The next step is to calculate tr(K) as

$$\mathrm{tr}(K) = \mathrm{Trace}(\mathbf{A^T B B^T A}) \quad (1)$$

where tr(K) varies between 0 and K. Subspace discrepancy function, $D(K)$, is calculated as follows:

$$D(K) = K - \mathrm{tr}(K) \quad (2)$$

$D(K)$ is the measure of that part of the subspaces which is in orthogonal complement of the other. This becomes zero when two subspaces are identical. Eigenvalues are calculated on ($\mathbf{A^T B}$) matrix and are utilized as:

$$\sin^2(\nu_k) = 1 - g_k \quad (3)$$

where $\sin^2(\nu_k)$ is the largest principal angle between subspaces that are selected by different variable selection methods and represent the degree of agreement between variables for determination of the number of chemical species and g_k is the eigenvalue. Shen et al. [52] Compared $D(K)$ and $\sin^2(\nu_k)$ as a measure of disagreement between the subspaces by plotting both values for K components. The number of factors becomes