J. Indian Chem. Soc.,
Vol. 94, September 2017, pp. 949-958

Studies on the interaction and complexation of pyrrole compound with hemoglobin

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Manuscript received 23 June 2017, accepted 15 July 2017

Abstract : Alkaloids such as pyrrole, tetrapyrrole etc. are gaining intensive research importance due to its structural and conformational similarities with porphyrin and heme proteins as well as possess a great biological significance to the living systems. Herein, we have reported a detail investigation into the nature of interaction and complexation of a pyrrole compound, methyl-4-(2-oxo-2-phenyl-ethyl)-5-phenyl-1*H*-pyrrole-3-carboxylic acid methyl ester (PyS) with an important blood protein Hemoglobin (Hb). The spectroscopic and computer generated docking calculation have been applied in this study to understand the molecular interaction and complexation. Systematic concentration dependent studies of the Hb-PyS complex in solution phase using absorption, steady state/time resolved emission have been reported in this paper. A number of residues have been identified as the interaction zone for the PyS compound within the Hb protein such as heme group, tryptophan (Trp), tyrosine (Tyr) and amide group through π-π interaction and hydrogen bonding as evident from simultaneous experimental and docking results. This study may contribute to the basic understanding of the biological and medicinal potential of the pyrrole based pure substituted alkaloid.

Keywords : Hemoglobin, pyrrole compound, complexation, interaction studies, spectroscopy, docking.

Introduction

Alkaloids such as pyrrole, tetrapyrrole and their derivatives are having structural similarities with porphyrin and heme proteins and due to its important biological significance, it is gaining intensive research thrust[1]. Especially after the discovery of the pyrrole based drug atorrastatin which are used for lowering blood cholesterol as well as prevention of events associated with heart diseases[1,2]. Tetrapyrrole derivatives occur abundantly in many biological systems such as in heme protein (iron porphyrin), in photosynthetic proteins as chlorophyll and porphyrin, and

in other proteins such as corrin (vitamin B_{12}). Their biological function ranges from O_2 transport (hemoglobin), conversion of solar energy to chemical energy (photosynthetic reaction centers), as well as other enzymatic reactions (peroxides, cytochromes P450 etc.)[3–5].

In this regard, the X-ray crystal structure study suggests that the non-planner geometry of the pyrrole ring within the heme group may be the reason for the biological activity or the conformational control of the heme protein. Non-planner geometry has been suggested to influence the redox properties of chlorophyll as well[3]. Thus the pyrrole compound with its various structurally manipulated forms may account significance importance for biological functions.

A group of small organic compounds related to pyrrole, such as tetrapyrrole and their derivatives have structural analogy to the group of porphyrin and heme proteins. These compounds may play a major role in controlling the biological activity of heme group, and its biological electron transfer processes[3]. In addition, the pyrrole compound has been proven as an electron transfer mediator compound for biosensor device fabrication[6]. These pyrrole related compound are now used as various kind of drug such as anticancer, anti-malaria, anti-inflammatory, and others anti-viral agent[1]. Still the basic understanding of how these compounds interact with blood protein such as Hb is lacking.

Herein, we present results from a preliminary investigation to gain insight into the nature of interaction and complexation of a substituted pyrrole compound namely methyl-4-(2-oxo-2-phenyl-ethyl)-5-phenyl-1*H*-pyrrole-3-carboxylic acid methyl ester (PyS). The spectroscopic and computer generated docking calculation have been applied to explore and understand the interaction and complexation between Hb and PyS. The details systematic concentration dependent spectroscopic studies of the Hb-PyS complex in solution phase using absorption, steady state/time resolved emission have been reported in this paper. The different experiments suggest a number of residues as the interaction zone for the PyS compound within the Hb protein such as heme group, Trp, Tyr and amide group through π-π interaction and hydrogen bonding. This study may contribute towards a better understanding of the potential of pyrrole compound for enhancing the biological activity of heme proteins.

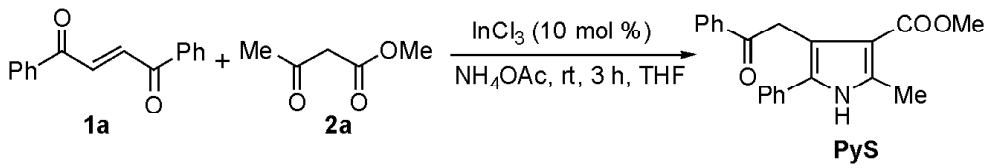
Experimental

Materials : The Human Hemoglobin, lyophilized and stored at 2–8 °C was purchased from Sigma Chemical Co. and used as received. The DMSO solvent was purchased from Merck, India.

Sample preparation and characterizations :

Synthesis of PyS : The PyS compound (Scheme 1) was synthesized following the protocol described by Mahato *et al*. with slight modification[7]. The compound was purified by crystallization rather than column chromatography. The isolated yield was also comparable with the reported method. Briefly, the reaction of *trans*-dibenzoylethylene **1a** (234 mg, 1 mmol) and methyl acetoacetate **2a** (1 mmol) in dry THF, anhydrous $InCl_3$ (10 mol%) and NH_4OAc (1.1 mmol) were added. The reaction mixture was then stirred at room temperature for 3 h. After completing the reaction, the PyS compound was purified by crystallization (Detailed in Supporting Information).

Preparation of Hb-PyS complex : The stock solutions of Hb and PyS compound were homogenously mixed in water-DMSO mixture (1 : 1). Triple distilled and deionized water purified using a Milli-Q Apparatus (Millipore, USA) was used to prepare the protein solution and protein-ligand solution. The pH and the resistivity of freshly prepared water were 6.8



Scheme 1. Synthesis of PyS from *trans*-dibenzoylethylene and methyl acetoacetate.

and 18.2 MW cm, respectively. The concentration of PyS in the Hb-PyS complex was varied from 1 to 1000 μM (1, 10, 100 and 1000). The mixture was maintained at 5 °C overnight and thereafter used for further measurements and characterizations.

Process of substrate cleaning : The silicon substrates used for FTIR measurements, were cleaned and made hydrophilic according to our earlier literature[8]. Briefly, all the substrates were cleaned in a liquid soap using an ultrasonic bath followed by repeated rinsing with Millipore water. They were then immersed in acetone and cleaned using Millipore water in an ultrasonic bath. A uniform layer of water onto the slide confirmed the hydrophilicity of the slide.

Spectroscopic characterizations : The steady state electronic spectroscopy such as UV-Vis absorption and fluorescence spectra of pure Hb and Hb-PyS mixtures were recorded using a cuvette having a path length of 1 cm by means of an absorption spectrophotometer (Shimadzu UV-Vis 2401PC) and fluorescence spectrophotometer (Hitachi F-4500) respectively.

The time resolved fluorescence lifetime measurements were done in time correlation single photon counting (TCSPC) system. The samples were excited at 280 nm using a picoseconds diode laser (IBH Nanoled-07) in an IBH Fluorocube apparatus. The repetition rate was 1 MHz. The fluorescence decays were collected on a Hamamatsu MCP photomultiplier (C487802) and were analyzed using IBH DAS6 software.

The FTIR spectra of film of pure Hb and Hb-PyS on silicon wafers were recorded by Magna-IR (Model No. 750 spectrometer, series II, Nicolet, USA). In all the cases, the data were averaged over 200 scans and the resolution of the instrument was selected as 4 cm^{-1}.

Molecular docking studies : To understand the molecular level interaction of ligand PyS and protein Hb, the molecular docking studies were carried out using AutoDock Tools 4.0[9]. Molecular docking studies were carried out on RHEL-5.0 Operating system installed on Dell Precision workstation with Intel core 2 quad processor and 8 GB RAM. Docking protocol was carried out as per the AutoDock manual. Ligand was prepared through PRODRG webserver[10]. 1.25 Å of oxy hemoglobin[11] with PDB code 2DN1 was used for docking interaction studies which was downloaded from Protein Data Bank namely RCSB webserver[12]. Water molecules were removed with subsequent addition of polar hydrogens. Gasteiger charges were applied as required for Lamarckian Genetic Algorithm (LGA) docking compilation. Based on the experimental results, docking studies were performed to know the interaction of ligand with heme moiety, Trp and Tyr residue. Three grids were prepared considering three binding sites. In the first grid, the grid center along x, y and z axes were set to 40.164 Å, 29.186 Å and 15.187 Å with a grid spacing of 0.375 Å. The grid dimensions were 40 Å along the x, y and z axes, which covered the heme moiety (site-1). In the second grid, the grid center along x, y and z axes were set to 32.823 Å, 46.901 Å and 22.097 Å with a grid spacing of 0.375 Å. The grid dimensions were 40 Å along the x, y and z axes which covered the Trp residue (site-2). In the third grid, the grid center along the x, y and z axes were set to 40.32 Å, 26.28 Å and 7.503 Å with a grid spacing of 0.375 Å, and grid dimensions as 40 Å along x and y axes and 54 Å z axis which covered the Tyr residues (site-3). Depending on the number in the cluster, the best position was considered for docking analysis. For docking, grid parameter file (.gpf) and docking parameter file (.dpf) were written using MGLTools-1.4.6[9]. Map types were generated using autogrid-4.0. Docking was carried out using Lamarkian Genetic Algorithm (LGA) along with following parameters : number of runs : 100, population size : 150, number of evaluations : 2,500,000 and number of generations : 27,000, using AutoDock 4.2. Analysis of docking results was done using MGL-Tools-1.4.6. The top scoring molecule in the largest cluster was analyzed for its interaction with the protein.

Results and discussion

UV-Visible absorption study : UV-Visible spectroscopy has been applied to understand the changes of the Hb in its conformation, structural, and residue specific in aqueous environment due to the presence of PyS and for its complexation with Hb by monitoring the characteristics spectral band of Hb protein[13,14]. Fig. 1 shows the UV-Vis spectra of pure Hb and Hb-

PyS complex with variation of concentration of PyS (1 μM to 1 mM). The concentration dependent (of PyS) UV-Vis absorption spectra of Hb-PyS complex shows that PyS has significant effect on the various absorption bands including Trp at 280 nm (arises due to the phenyl group of Trp and Tyr residues) and Soret band (406 nm) and oxy-deoxy band or Q-band (550–600 nm)[14,15]. The presence of the Soret band at 406 nm in pure Hb indicates the protein is in native form[16]. The Soret band appeared due to the π-π* transition within the heme group and its shape and position is dependent on the geometric position of the iron atom in the heme plane[15,17].

It is found from Fig. 1 that the Soret band intensity as well as position is changing and even fluctuating with the different concentration of PyS. This could be described as the PyS compound approaching the heme plane of the porphyrin residues in Hb. It is well documented in literature that if the heme plane changes its position from in-plane to out-of-plane then its signature is reflected in the Soret band by changing its shape (from Gaussian to non Gaussian) and position of Soret band spectrum[18]. Thus, the Soret absorbance band could provide important information about the possible unfolding and denaturation of protein during conjugation with other compounds[16,18]. At the concentration of PyS around 100 μM and above the intensity of Soret band has abrupt increment and position also blue shifted, indicating the distortion of heme plane due to the changes in chemical environment surrounding Hb in presence of PyS compound[15]. The heme group experiences a perturbation due to some kind of interaction of the heme group of the protein with the PyS compound. Overall UV-Vis absorption results indicate the formation of Hb-PyS complex[19]. This interaction has been further addressed in docking calculation.

Steady state emission spectroscopic study : Steady state fluorescence spectroscopy can visualize fluoropore residues and its surrounding information, which in case of protein can gives a picture about the conformational changes of the protein, degree of its exposure to a solvent or to an external compound[20]. Fig. 2 shows the emission spectra of Hb and its complex with PyS compound applying excitation at 280 nm. Generally, a protein has three fluoropore Trp, Tyr, and phenylalanine (Phe). But the fluorescence in most proteins is contributed mainly from Trp and Tyr alone, due to very low quantum yield of phenylalanine.

Hb has strong fluorescence and there are three Trp units (α214Trp, β215Trp, β217Trp) in each α and β chain[21,22]. Fig. 2 shows that when a fixed concentration of Hb (0.05 g/L) was titrated with varying concentrations of PyS compound, a change in fluorescence intensity of Trp was observed. Furthermore, there is a slight red shift of the maximum wavelength (λ_{max}) of Trp fluorescence, which reveals the presence of an interaction with the PyS compound[15,23–25]. This Trp emission is useful to understand the micro-environment around the Trp amino acid residues[26,27]. The present data indicates that Trp is one of the possible binding sites within Hb.

Interestingly, a broad peak ~420–480 nm is observed along with the Trp emission (Fig. 2 and its inset), which enhances in intensity along with the quenching of Trp in presence of higher concentration of PyS compound. This emission peak/peaks may be due to the inner filter effect or reabsortion effect[28] at 406 nm by the heme residue present in Hb.

We have plotted the ratio of the fluorescence intensity in absence (F_0) and presence (F) of a quencher i.e. F_0/F with the varying concentration of PyS compound (Fig. 3). The plot is called the Stern-Volmer (S-V) plot for the linear quenching of fluorescence intensity as represented by eq. (1)[29].

$$F_0/F = 1 + k_Q t_0[Q] \quad (1)$$

where, k_Q, t_0 are the fluorescence quenching rate constant and fluorescence lifetime in absence of the quencher.

Fig. 3 follows the linear type of S-V quenching plot with linear fitting having residual value = 0.999[30,31]. The value of $k_Q = 37.44 \times 10^{13}\ M^{-1}\ s^{-1}$. The linear type of quenching indicates the ground state complexation of the Hb protein with PyS compound.

Time resolved emission spectroscopic study :

To understand in more detail about the complexation mechanism surrounding Trp residue, we have

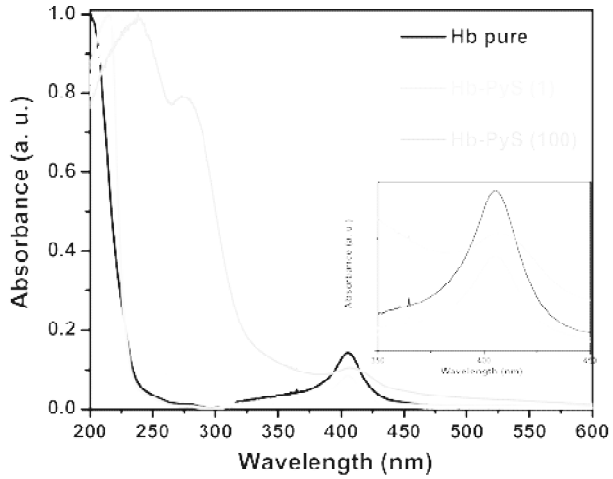


Fig. 1. UV-Visible spectra of Hb and Hb-PyS compound complex at different concentrations.

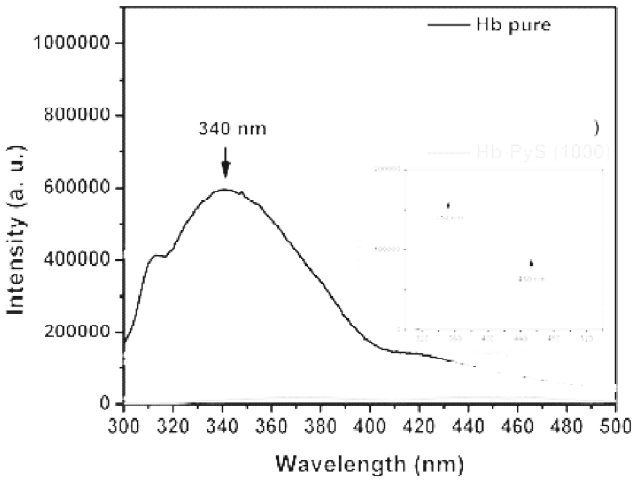


Fig. 2. Fluorescence spectra of Hb and Hb-PyS compound complex at different concentrations.

measured the Trp fluorescence decay average lifetime of the Hb with λ_{ex} = 280 nm for different samples.

The decay profiles were fitted using the following triple exponential function (eq. (2))[29].

$$f(t) = \sum_{i=1}^{3} B_i \exp(-t/\tau_i) \quad (2)$$

where, B_i's and τ_i's are the relative contributions and the lifetimes of the different components to the total decay. The residual plot as well as χ^2 values indicates that these Trp fluorescence decay profiles are triple-exponential in nature, which is also reported to be triple-exponential[32]. The relative contribution of each

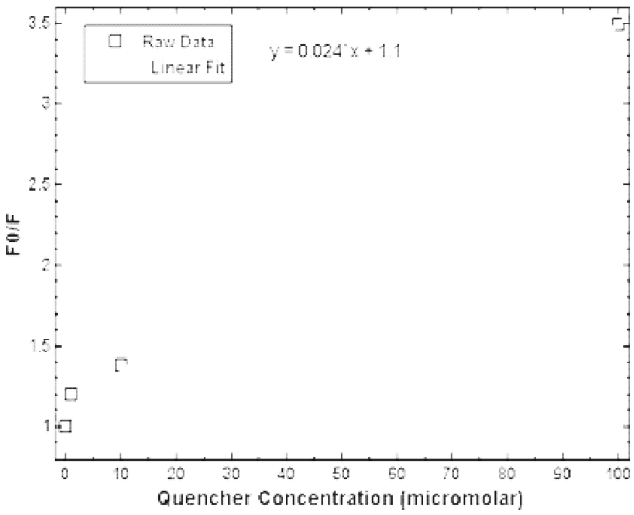


Fig. 3. Linear fitting of Stern-Volmer quenching of the fluorescence spectra of Hb Trp in presence of different concentration of pyrrole compound.

component to the total steady state fluorescence intensity has been defined as per (eq. (3))[30].

$$f(t) = B_i\tau_i / \sum_{j=1}^{3} B_j\tau_j \quad (3)$$

The values of f_1, f_2 and f_3 are shown in Table 1. From these values, we can infer that there is a sufficient contribution of each component to the total fluorescence intensity. The average lifetime of fluorescence decay was calculated using the following formula (eq. (4)). All the values are reported in the Table 1.

$$\langle\tau\rangle = \sum_{i=1}^{3} B_i\tau_i^2 / \sum_{i=1}^{3} B_i\tau_i \quad (4)$$

The average lifetime of Hb is reported in the range of 1.8 ns to 5.4 ns[32,33]. We found the decay lifetime of Hb pure (C_{Hb} = 0.04 g/L) ~6.41 ns which was closer with the earlier report[32,33]. Here the lifetime data show that the average lifetime of Trp increases suddenly in the presence of DMSO solvent, may be due to the confinement in the high viscous DMSO solvent[34]. With the presence of PyS compound the average lifetime of Trp decreases proportionately indicating the change of the micro-environment around Trp in the presence of PyS compound. It refers to the static type of quenching through forming ground state type of complex and the Trp as one of the binding residues to PyS compound[35].

Table 1. Fitting parameters of fluorescence lifetime measurements of pure Hb and Hb-PyS conjugates at different concentrations

Sample	B_1	B_2	B_3	τ_1 (ns)	τ_2 (ns)	τ_3 (ns)	f_1	f_2	f_3	$<\tau>$ (ns)
Pure Hb	0.45	0.39	0.16	2.47	0.83	10.21	0.362	0.105	0.532	6.41
Hb-DMSO	0.34	0.23	0.43	5.08	30.10	1.29	0.187	0.752	0.060	23.66
Hb-PyS (10)	0.40	0.12	0.48	3.18	13.50	0.98	0.377	0.482	0.139	7.86
Hb-PyS (100)	0.70	0.22	0.08	0.49	2.16	9.79	0.214	0.296	0.486	5.53
Hb-PyS (1000)	0.09	0.03	0.88	1.57	7.64	0.37	0.202	0.329	0.467	3.00

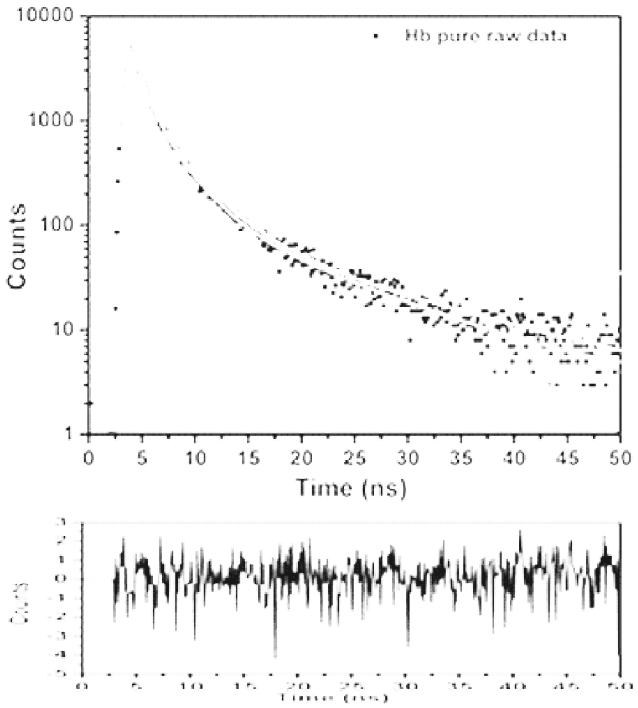


Fig. 4. Time resolved fluorescence decay spectra of Hb Trp in presence of different concentration of PyS.

It is important to note that the observed triple exponential decay reflects the presence of several tryptophans in the protein, with different decay times, resulting from different environments. The calculated value of F_0'/F than $\langle\gamma_0\rangle/\langle\gamma\rangle$ follows higher values in all the range of concentration of PyS compound, which again indicate static quenching in the presence of PyS compound[36]. This may be a possible quencher induced conformational reorientation of the protein Hb[37].

Here the Trp quenching to be close to diffusion control, the quencher should rapidly diffuse and interact with the Trp during the lifetime of the excited state. This can be easily diagnosed by the parallel drop of the fluorescence lifetime and the quantum yield of Trp with the quencher[38]. Our fluorescence lifetime and the quantum yield data showed the almost parallel drop indicating the Trp quenching to be diffusion limited.

Study in FTIR spectroscopy : Fig. 5 shows the FTIR spectra Hb, PyS and Hb-PyS compound in ATR mode on Silicon wafer. It indicates several vibrational frequencies (Table 2), among which 696 cm^{-1} (for C-N bond in pyrrole ring), 1678 cm^{-1} (C=O bond in carbonyl group), and 3258 cm^{-1} (for N-H bond in pyrrole ring), characterizes the pyrrole compound[4,5,39]. The amide-I (~1600 to 1700 cm^{-1}) and amide-II (~1500 to 1600 cm^{-1}) bands represent the Hb protein, which basically characterizes the protein content in Hb-PyS biocomposite[15,40]. The amide-I band resulted from carbonyl stretching vibrations of the peptide backbone and amide-II is due to the combination of N-H in-plane bending and C-N stretching vibrations of peptide groups[41]. Among them, the amide-I band represents the different components of secondary structure of the proteins[42,43]. We have applied Gaussian multipeak fitting tool to deconvolute

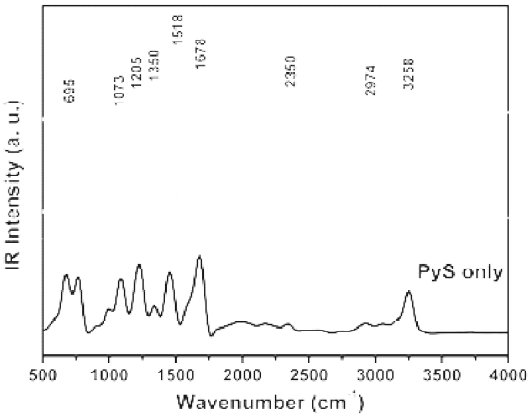


Fig. 5. Baseline corrected FTIR-ATR spectra of Hb, PyS and Hb-PyS complex film on Si wafer.

Table 2. Vibrational assignment of the ATR-FTIR spectra of pure Hb and Hb-PyS complex

Position in FTIR spectra (cm^{-1})	Assignment of bonds	Mode of vibrations
696	C-N bond	Out-of-plane deformation
1073	N-H bond	In-plane deformation
1205	C-O bond in ester	Stretching vibration
1350	Pyrrole ring	
1518	C-C bond in pyrrole ring	
1678	C=O bond in carbonyl	Stretching vibration
2974	C-H bond in methyl group	Stretching vibration (asymmetric)
3258	N-H bond in pyrrole ring	Stretching vibration

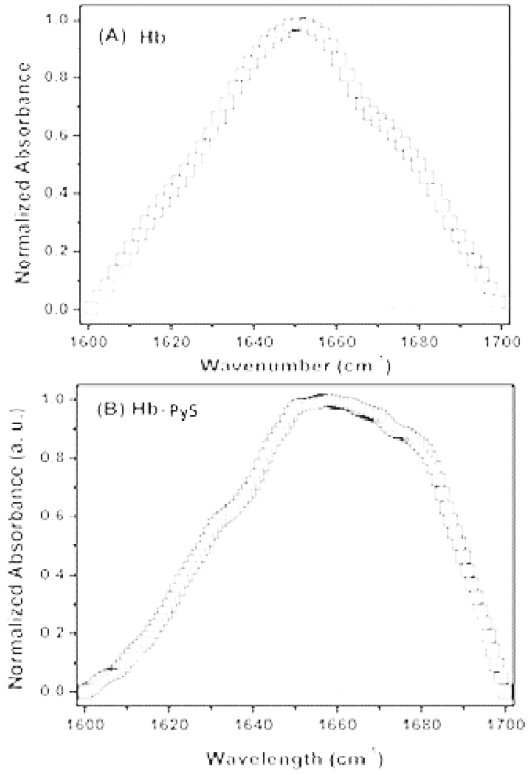


Fig. 6. The deconvoluted FTIR spectra of Hb and Hb-PyS in the amide-I region.

the amide-I band into its components of the secondary structural elements of protein. For multipeak fitting of the amide-I band, the Gaussian fitting is more appropriate than the Lorentzian one. Lorentzian fitting is more suitable at low temperature whereas at elevated or room temperature Gaussian fitting is more appropriate. At higher temperature different types of broadening come into the picture and perturb the line shape. Our experimental data is at room temperature and not at low temperature. Hence, it corroborate with the Gaussian fitting. Apart from this, the chi-square value also found to have best value in Gaussian fitting.

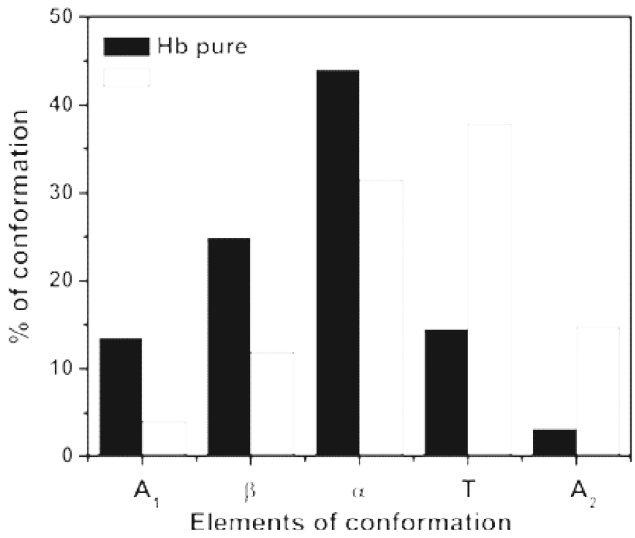


Fig. 7. Different conformation of Hb and Hb-PyS as obtained from deconvolution result.

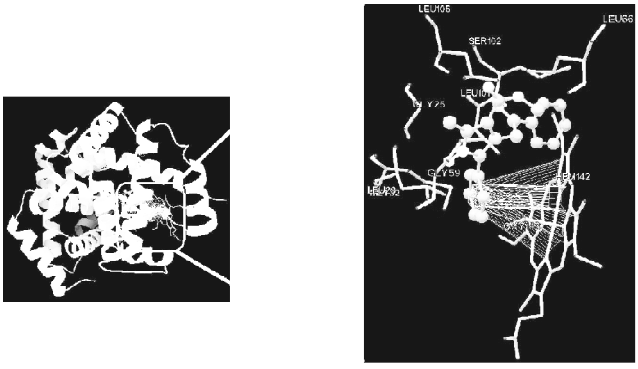


Fig. 8. Interaction of ligand PyS with heme portion of hemoglobin showing π-π stacking interaction (yellow lines).

Fig. 6 shows the deconvoluted FTIR spectra of Hb, and Hb-PyS compound in the amide-I band region. A rough assignment of the deconvoluted components of amide-I band suggested in most protein studies are as follows : 1651–1658 cm^{-1} (α-helix); 1618–1642 cm^{-1} (β-sheets); 1666–1688 cm^{-1} (turns); 1618–1623 cm^{-1} (inter-molecular aggregates); and 1683–1689 cm^{-1} band (intra-molecular aggregates) respectively[35,41].

Here, we have employed a multiple peak fitting technique with Gaussian profile to fit normalized amide-I band which allows one to identify different components and in particular to determine the corresponding peak frequencies[44,45]. The percentage area of the deconvoluted peaks gives the relative amount of the components. In the present work the maximum number of the components which can be identified in the deconvoluted amide-I band, was 5 for meaningful fitting. Unless use of lesser or more number of peaks, the fitting gives neither satisfactory result nor improves neither R^2 values nor do the deconvoluted peaks have definite positions.

Table 3 and Fig. 7, represent the summary of the deconvoluted results as obtained from FTIR spectra in the amide-I region. It indicates some structural and conformational perturbation occurred within secondary structure level of the proteins Hb due to PyS compound, which reflects by the decrement of intermolecular aggregate (A_1 = 13.50 to 4.00%), β-sheet (β = 24.87 to 11.88%), α-helix (α = 43.95 to 31.47%), and by the increment of random coil (T = 14.50 to 37.87%), intra-molecular aggregate (A_2 =

Table 3. Fitting parameters using multipeak fitting technique of amide-I band for Hb and Hb-PyS[a]

Conformers	Area (%)		Position (cm^{-1})		FWHM (cm^{-1})	
	A	B	A	B	A	B
A_1	13.50	4.00	1622.5	1615.8	22.26	14.54
β	24.87	11.88	1644.6	1629.4	22.79	14.92
α	43.95	31.47	1657.5	1647.9	33.93	19.78
T	14.50	37.87	1669.4	1667.8	19.59	22.09
A_2	3.18	14.75	1689.1	1684.3	14.52	15.38

[a]A and B represents Hb and Hb-PyS, respectively.

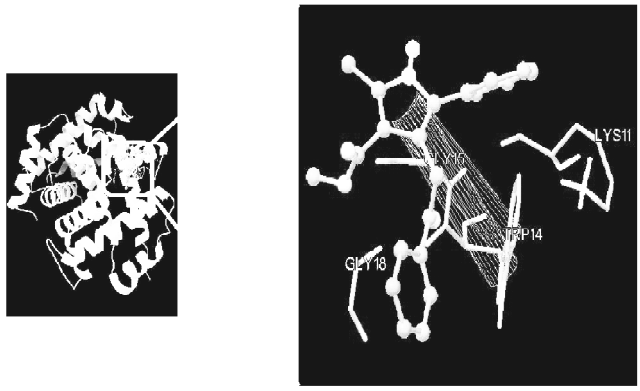


Fig. 9. Interaction of ligand PyS with Trp14 of the hemoglobin showing π-π stacking (yellow color lines).

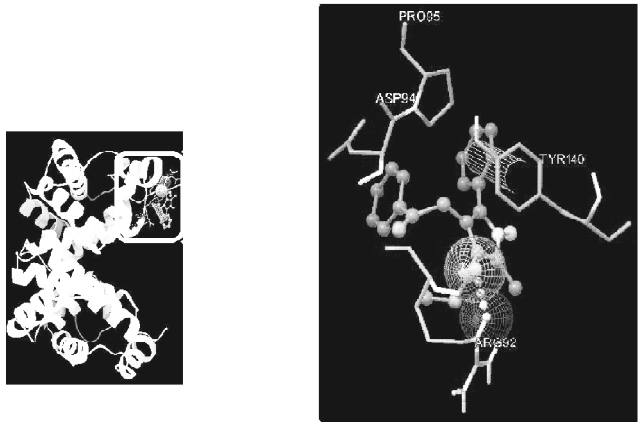


Fig. 10. Interaction of ligand PyS with Hb showing H-bond (green color balls) and π-π stacking interaction (yellow color lines).

Table 4. Tabular representation of docking result of Hb with PyS by AutoDock

Binding region	Binding energy (kcal mol^{-1})	Ligand efficiency	Cluster RMS	Component energy			
				ESTAT	HB	VDW	DSOLV
Site-1	–6.81	–0.27	0.0/44.79	–0.0750	0.3582	10.3296	2.6306
Site-2	–6.54	–0.26	0.0/33.13	–0.1309	0.4882	–7.1951	1.3811
Site-3	–6.13	–0.25	0.0/46.47	–0.0018	0.0113	–6.2510	1.0585

ESTAT – Electrostatic interaction energy; HB – Hydrogen bond interaction energy; VDW – Van Der Waals interaction energy; DSOLV – Desolvation energy. VDW includes π-π interaction energy.

3.28 to 14.75%) respectively.

Hb-PyS docking summary :

The top scoring clusters were analyzed for the interaction of ligand PyS with the hemoglobin protein (2DN1). Results of the interaction, in our docking studies of PyS with Hemoglobin containing binding energy, ligand efficiency, and respective component energies are summarized in Table 4.

From the docking studies, it is clear that, π-π stacking interaction is the major type of interaction of the ligand PyS with protein (Fig. 8).

Docking studies revealed that, the ligand may bind to protein at various sites. Majorly, the ligand binds to heme portion, tyrosine and tryptophan residues of the protein. The phenyl group adjacent to nitrogen of pyrrole ring of ligand PyS, has a strong π-π stacking interaction with porphyrin rings of heme moiety of the receptor 2DN1 (as shown in Fig. 8). Secondly, the pyrrole ring of ligand PyS has π-π stacking interaction with indole group of Trp14 of the receptor (Fig. 9).

Another major interaction is seen with Arg92 and Tyr140. Arg92 has a secondary amine acting as hydrogen bond acceptor which interacts with carbonyl oxygen in the ligand PyS acting as hydrogen bond acceptor, thereby making a hydrogen bond. This hydrogen bond makes the ligand to come closer to Tyr pocket making a π-π stacking interaction with the phenyl group of ligand PyS (Fig. 10) like earlier report[46,47].

The Auto Dock studies result summary indicate that the PyS compound has strong influence and interaction on some residues/groups such as heme-porphyrin group, Trp, Tyr, Arg etc. which are also evident from the spectroscopic experimental data itself. The information we got from the docking theoretical calculation is based on crystal structure which may has some difference with solution structure, from point of binding site view, it has good agreement with the spectroscopic experimental results.

Conclusion

Intensive research into basic interaction studies of biocomponent such as proteins, is important. Herein, we conducted our studies using fundamental techniques, such as spectroscopic and computer generated docking calculation to explore and understand the interaction and complexation between Hb and PyS. This study reveals the heme group, Trp residues and amide group as the interaction zone and complexation sites for the PyS compound within the big macromolecule named Hb protein.

This kind work may trigger the utilization of other experimental techniques in combination with theoretical supports to investigate and monitor other protein residues for interaction studies with potential organic compounds. In addition, this work may form the basis for future studies exploring the potential of the pyrrole compound for enhancing the biological activity of heme proteins like Hb.

Supporting information

Synthesis, characterization of PyS and the ^{1}H, ^{13}C NMR and EI-HRMS spectrum are available.

Acknowledgement

Authors acknowledge DST-SERB, Govt. of India, for financial support through two no. of SERB projects (No. EMR/2016/002634 and EMR/2016/004219) sanctioned to Dr. Mrityunjoy Mahato. MM also would like to thank Department of BSSS, NEHU, Shillong for providing laboratory working facility. The Career Point University Hamirpur is acknowledged for providing the facilities and author SKM wish to thanks University of South Africa (UNISA) for Post Doctoral Fellowship Award-2015.

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