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Micro-Raman spectroscopy study on the allosteric regulation of inositol hexakisphosphate on hemoglobin

Xing Gao a, Chang-chun Zeng * b, Han-ping Liu a, Yao-yan Lu b

Abstract

Inositol hexakisphosphate (IHP) is an important allosteric factor, which could affect the quaternary conformational equilibrium of hemoglobin (Hb) toward the T state. In this study, micro-Raman spectroscopy was used to analyze the Hb allosteric effect of IHP. Raman spectra of Hb by IHP of different molar ratios under different oxygen partial pressure (PO2) levels were obtained, linear fits and peak values for Raman intensity of Hb / IHP sodium salt of different ratios at 1357 cm⁻¹, 1377 cm⁻¹, 1545 cm⁻¹, 1585 cm⁻¹, 1606 cm⁻¹, and 1640 cm⁻¹ were analyzed. With the increase of molality of IHP sodium salt, the intensities and peak ratio values of 1357 cm⁻¹, 1545 cm⁻¹, and 1606 cm⁻¹ decreased while the intensities and peak ratio values of 1377 cm⁻¹, 1587 cm⁻¹, and 1640 cm⁻¹ obviously increased. There is a good linear relationship between the proportion of IHP sodium salt and its function of promoting the release of oxygen in oxygenated hemoglobin under 10, 20, 30 mmHg, with the R² all more than 0.910. The results confirmed that the interactions of Hb with IHP could promote the release of oxygen in the oxygenated Hb, especially in lower PO2 levels. It is promising that the results could provide a reference for allosteric regulation of IHP and other allosteric factors on hemoglobin oxygen affinity by Raman spectroscopy.
1. Introduction
Hemoglobin (Hb) is an important protein, which transports O\textsubscript{2} for the tissue in the vertebrates\textsuperscript{1,2}. In adult humans, the most common hemoglobin type is a tetramer called hemoglobin A, consisting of two α subunits and two β subunits. Every subunit is composed of a peptide chain tightly associated with a non-protein heme group and has an oxygen-binding site\textsuperscript{3-4}. In physiological condition, the peptide chain folds like a ball, which holds the heme group inside\textsuperscript{5}. The heme group consists of an iron (Fe) ion held in a heterocyclic ring, known as a porphyrin. X-ray crystallographic studies have demonstrated the presence of two distinct quaternary structures, called T (tense) and R (relaxed) states, which correspond to the low- and high-affinity states, respectively\textsuperscript{6-7}. Heterotropic effectors, such as 2, 3-diphosphoglycerate (DPG), inositol hexakisphosphate (IHP) and bezafibrate (BZF), are known to affect the quaternary conformational equilibrium toward the T state\textsuperscript{8-10}.

Inositol hexakisphosphate (IHP), which is also known as phytic acid, is the storage form of phosphorus in seeds, and it is especially rich in cereals grains, oilseeds and legumes\textsuperscript{11}. There are six phosphate ester bonds in IHP which are not on the same plane and have a good chelation ability to metal ions, such as Na\textsuperscript{+}, K\textsuperscript{+}, Fe\textsuperscript{3+} (see Fig. 1). This character gives IHP unique chemical properties, physiological and pharmacological properties, which have a wide range of applications in chemical engineering\textsuperscript{12}, medicine\textsuperscript{13-20}, food\textsuperscript{21-24}, etc. IHP can be found not only in the seeds of plant, but also in the red cells of animals in the form of IHP natrium, IHP calcium, IHP magnesium and IHP potassium.

![Structure of dodecasodium salt of IHP.](image)

Raman spectrum is a non-elastic light scattering spectroscopy with the merits of high sensitivity, non-destructive, rapid detection and without complicated sample pre-treatment\textsuperscript{25}. Through the assignment of band position, analysis of the symmetry terms and local coordinates, we can obtain the information of a molecular and its variation, like the functional groups, chemical bonds, electron density and so on. In 1972, Strekas and Spiro found that the Raman spectrum could be used in the study of hemoglobin\textsuperscript{26}. Recently, Raman spectroscopy has been shown to be an attractive optical technique to provide direct access to the state of hemoglobin. Based on the shifts to higher frequency upon oxidation of ferrous derivatives to ferric derivatives, Raman scattering provides information about the oxygenation state, as well as the spin state of the heme iron\textsuperscript{27-29}. In addition, oxygen saturation (SO\textsubscript{2}) can reflect oxygen supply and demand balance, and the tissue metabolism of the whole body. The SO\textsubscript{2} could be measured \emph{in vitro} by analyzing the maker bands of oxygenated and deoxygenated Hb in Raman spectrum, like \nu\textsubscript{4}, \nu\textsubscript{10}, \nu\textsubscript{19}\textsuperscript{30-31}. Combined with Principal Component Analysis (PCA) or other analysis methods, the Raman spectrum could identify the abnormal hemoglobin, like thalassanemia\textsuperscript{32}.

There is a long history to study the effect of IHP on the \textsubscript{O_2} dissociation of oxygenated Hb. Benesch \emph{et al} studied the effect of IHP on the \textsubscript{O_2} binding efficiency for the first time\textsuperscript{33}. Arnone \emph{et al} revealed that the DPG...
and IHP bind HbA at the same site, in the central cavity between the $\beta_1$- and $\beta_2$-subunits, at the molar ratio of 1:1 through X-ray crystallographic method. Perutz et al. and Lalezari et al. found the HbA bind at least two molecules of BZF through X-ray crystallographic method. Perutz et al. and Lalezari et al. found the HbA bind at least two molecules of BZF through X-ray crystallographic method. Various spectroscopic methods have been used to study the allosteric regulation of IHP and other heterotropic effectors on Hb. Marden et al. measured the flash photolysis kinetics for ligand recombination to Hb in the presence of BZF and IHP. Kanaori et al. clarified the various structural interpretations about the binding properties of IHP and L35 to Hb by NMR spectroscopy. Ascenzi et al. investigated the cooperative effect of IHP and BZF on the nitric oxide derivative of ferrous human hemoglobin (HbNO) by EPR spectra. A lot of Raman studies were also committed on the synergic effect of the BZF and IHP. In this study, we analyzed the influence of IHP on Hb allosteric effect at different molar ratios and different oxygen partial pressures ($P_{O_2}$) by using Raman spectrum.

2. Experimental

2.1 Separation and purification of Hb

Informed consent of the volunteers was obtained. All experiments in this work were carried out in compliance with the relevant laws and institutional guidelines in South China Normal University and Guilin Medical University of China. Blood was extracted from healthy volunteers with HbA and placed in anticoagulant tube with liquaemin sodium as an anticoagulant. The blood was centrifuged in centrifuge tube at 1,500 rpm, 4°C for 10 min to remove upper white blood cells and soterocytes, and the underneath of the tube was the erythrocytes. Then the erythrocytes were washed with isotonic phosphate buffered saline (PBS) at the rate of 1:1 three times. Hemoglobin was obtained by mixing with tetrachloride and double distilled water at the proportion of 1:0.4:1 and centrifuged at 3,000 rpm, 4°C for 20 min after 30 min of shaking. The mixture formed three layers. The upper liquid was the Hb solution. The hemoglobin was extracted to reduce the interference from the scattering effects of the red cell suspension during the Raman experiment. The concentration of the Hb solution was measured by using the HiCN method. The Hb solution was diluted 10 times with PBS (pH 7.4) to reach the final concentration, which is 0.2 mmol/L and stored at 4°C for further use.

2.2 Materials and reagents

Inositol hexakisphosphate sodium salt was obtained from Sigma-Aldrich. Purified water was prepared by an Elga water purification system (ELGA, London, UK). A stock solution of 2 mmol/L IHP sodium salt was prepared. 1 ml Hb solution was mixed with 2 mmol/L IHP sodium salt according to the molar ratios of 1:1, 1:0.8, 1:0.6, 1:0.4, 1:0.2, and 1:0. Every mixture solution was stored at 4°C before used.

2.3 Control of $P_{O_2}$ levels and sample preparation

![Fig. 2 Device used to collect of Hb and IHP sodium solution under different $P_{O_2}$ levels.](image)

$P_{O_2}$ was controlled by stabilizing the fluid flow of $N_2$ at a rate of 500 ml/min in the equipment as shown in Fig. 2. The $P_{O_2}$ value of the mixture solution was measured with an Oxi3310 dissolved oxygen meter (WTW Company, Germany) (measurement range = 0–199.9 mbar). The data could be read automatically, which made it easier to
read the \( \text{PO}_2 \) value when the \( \text{O}_2 \) of the mixture changed. After the readings of the Oxi3310 was 0, turn off the fluid flow of \( \text{N}_2 \), and put the mixture in the state of nature to restore to oxygenation. Collected mixture solution samples under different \( \text{PO}_2 \) levels, and sealed them in glass capillary. Glass capillary path length and Hb concentration did not affect measurements from Raman spectrometer. The experimental temperature was controlled at about 37°C.

2.4. Raman spectra collection and data processing

The micro-Raman spectra were obtained by applying the InVia+Plus confocal micro-Raman spectrometer of Renishaw Inc. with 514.5 nm excitation line from an Ar+ laser. The system is equipped with 20× Leica microscope objective times (NA = 0.35) and a spectrum resolution of 1 cm\(^{-1}\). The spectra were collected in back-scattered geometry with a detection range from 500 to 1800 cm\(^{-1}\). The laser power focused on sample was \(~ 10\) mW and the acquisition time of each spectrum was 10s by 3 times. All the data were collected under the same conditions. The 520.5 cm\(^{-1}\) band of a silicon wafer was used to calibrate the instrument on a daily basis. Cosmic rays were removed during the postprocessing of the spectra.

Five spectra were obtained from every sample. Then the averaged spectra were used as representative spectra for spectral analysis. All spectral pre-treatments, analyses, and baseline correction were performed by using the baseline Wavelet library of R version 2.8.1 software (The R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org/). In order to obtain sharp contrast, eliminate errors by instrument operation and get better analysis results, the intensity of 1004 cm\(^{-1}\) was chosen to make normalization. Normalization and smoothing were conducted through Originpro 8.5 software. Spectra were baseline corrected and cosmic ray signals removed in WIRE3.2 Spectroscopic Software.

3. Results and Discussion

3.1 The Raman spectra of oxygenated and deoxygenated Hb

Fig. 3 depicts spectra recorded of the Hb in the oxygenated and deoxygenated states by using the InVia+Plus confocal micro-Raman spectrometer of Renishaw Inc. with 514.5 nm excitation line from an Ar+ laser.

![Fig. 3 Raman spectra of the Hb in the oxygenated and deoxygenated states.](image)

According to Fig. 3, the band of 679 cm\(^{-1}\) is assigned to \( \nu_7 \) and \( \tilde{A}_{1g} \), which is sensitive to the deformation of pyrrole ring. The 756 (\( \nu_{15} \sim \tilde{A}_{1g} \)) cm\(^{-1}\) and 799 (\( \nu_{6} \sim \tilde{A}_{1g} \)) cm\(^{-1}\) are represented the breathing of the pyrrole ring. The
methane deformation region lies between 1200 and 1300 cm⁻¹. The core-size or spin state marker band region lies between 1500 and 1650 cm⁻¹. 1357 (v₁₁~A₁g) cm⁻¹, 1545 (v₁₁~A₁g) cm⁻¹, and 1606 (v₁₁~A₂g) cm⁻¹ are the maker bands of deoxygenated Hb and 1377 (v₁₁~E₁u) cm⁻¹ and 1640 (v₁₁~B₁g) cm⁻¹ are the maker bands of oxygenated Hb. With the increase of the O₂ concentration, 1357 cm⁻¹ decreased and the 1377 cm⁻¹ appeared. In deoxygenated state, the 1545 (v₁₁~A₁g) cm⁻¹ and 1606 (v₁₁~A₂g) cm⁻¹ are very strong while the 1640 (v₁₁~B₁g) cm⁻¹ disappeared. The band position, assignments symmetry terms and local coordinates of the oxygenated Hb and deoxygenated Hb when irradiated by the 514.5 nm laser light were detailed in Table 1.

Table 1. Band position, assignments, symmetry terms and local coordinates for oxygenated and deoxygenated Hb by using 514.5 nm excitation

<table>
<thead>
<tr>
<th>Oxy514</th>
<th>Deoxy514</th>
<th>Assignment</th>
<th>Symmetry</th>
<th>Local coordinate</th>
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<tr>
<td>679</td>
<td>679</td>
<td>v₄</td>
<td>A₁g</td>
<td>δ(pyr deform)ₚₘₚₚ</td>
</tr>
<tr>
<td>756</td>
<td>756</td>
<td>v₁₅</td>
<td>B₁g</td>
<td>ν(pyr breathing)</td>
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<td>799</td>
<td>799</td>
<td>v₆</td>
<td>A₁g</td>
<td>ν(pyr breathing)</td>
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<tr>
<td>829</td>
<td>829</td>
<td>v₁₀</td>
<td>B₁u</td>
<td>γ(CαH)</td>
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<tr>
<td>975</td>
<td>975</td>
<td>v₄₆</td>
<td>Eₜ</td>
<td>δ(pyr deform)ₚₚₚ</td>
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<td>v₂₃</td>
<td>A₂g</td>
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<td>1587</td>
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</tr>
<tr>
<td>1606</td>
<td>1606</td>
<td>v₁₉</td>
<td>A₂g</td>
<td>ν(C=C)ₚₚₚ</td>
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<tr>
<td>1640</td>
<td>absent</td>
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<td>B₁g</td>
<td>ν(C₇C₇)ₚₚₚ</td>
</tr>
</tbody>
</table>

ν, stretch; δ, in-plane deformation; γ, out-of-plane deformation; sym, symmetric; asym, asymmetric; pyr, pyrrole; Phe, phenylanaline; deform, deformation.

3.2 Raman spectra of Hb by IHP sodium salt of different molar ratios under different PO₂ levels
Fig. 4 Raman spectra of Hb by IHP sodium salt of 1:0 (a), 1:0.2 (b), 1:0.4 (c), 1:0.6 (d), 1:0.8 (e), and 1:1 (f) molar ratios under different PO$_2$ levels.

Raman spectra of Hb by IHP sodium salt of different molar ratios under different PO$_2$ levels were presented at Fig. 4. From Fig. 4(a) to (f), the molar ratios of Hb and IHP sodium salt were 1:0, 1:0.2, 1:0.4, 1:0.6, 1:0.8, and 1:1, respectively.

The bands of the low wavenumber region (600-1100 cm$^{-1}$) are mainly the deformation and breathing of
pyrrole ring. Compared with the central region and high wavenumber region, the change of the bands in 600-1100 cm\(^{-1}\) is not distinct when the molar ratios of the Hb and IHP sodium salt changed, so we didn’t give an analysis in the research.

In the central region (1200-1400 cm\(^{-1}\)), the bands of the Raman spectra presented are reported to be sensitive to the oxidation and spin state of the central metal atom within the porphyrin macrocycle. Bands in 1200-1300 cm\(^{-1}\) are the methane deformation region. Bands in 1356-1361 cm\(^{-1}\) mean the ferrous ion in high spin state while the bands in 1370-1378 cm\(^{-1}\) mean the hemoglobin in oxidation state. The band of 1357 (\(\nu_4\)) cm\(^{-1}\) and 1377 (\(\nu_4\)) cm\(^{-1}\) are impressive to the electron distribution in the \(\pi\)-orbitals of the porphyrin macrocycle. When the PO\(_2\) levels increased from 0 to 100 mmHg, the intensity of the 1357 (\(\nu_4\)) cm\(^{-1}\) decreased and disappeared finally while the 1377 (\(\nu_4\)) cm\(^{-1}\) appeared and intensity increased, which indicated that the electron population in the \(\pi\)-orbitals decreased. With the increase of the molar ratios of Hb and IHP sodium salt, the band of 1377 (\(\nu_4\)) cm\(^{-1}\) appeared at a lower PO\(_2\) levels. The band of 1377 (\(\nu_4\)) cm\(^{-1}\) appeared at 30 mmHg when the molar ratio of Hb and IHP sodium salt is 1:0, while it appeared at 10 mmHg when the ratio is 1:1. The disappearing speed of 1357 (\(\nu_4\)) cm\(^{-1}\) is also related to the ratios of the Hb and IHP sodium salt. It still existed when the PO\(_2\) is 100 mmHg at low molecular ratio. However, it vanished at 10 mmHg when the ratio is 1:1.

Bands of the high wavenumber region (1500-1650 cm\(^{-1}\)) are sensitive to porphyrin in-plane vibrational modes and can reflect the size of center aperture of porphyrin ring. The band at about 1545 (\(\nu_{11}\)) cm\(^{-1}\) means the iron ion in high spin state while the band at about 1587 (\(\nu_{37}\)) cm\(^{-1}\) means the iron ion in low spin state. The 1606 (\(\nu_{10}\)) cm\(^{-1}\) band is sensitive to the size of center aperture of porphyrin ring while the 1640 (\(\nu_{10}\)) cm\(^{-1}\) is sensitive to the oxygen concentration. When the PO\(_2\) levels increased from 0 mmHg to 100 mmHg, the band of 1545 (\(\nu_{11}\)) cm\(^{-1}\) disappeared while the band of 1587 (\(\nu_{37}\)) cm\(^{-1}\) appeared, the band of 1606 (\(\nu_{10}\)) cm\(^{-1}\) disappeared while the band of 1640 (\(\nu_{10}\)) cm\(^{-1}\) appeared gradually at every molar ratio, which indicated that the iron ion is changed from high spin state to low spin state and is closed to the plane of porphyrin ring. With the molar ratio of IHP sodium salt to Hb increase, the band of 1545 (\(\nu_{11}\)) cm\(^{-1}\) and the band of 1606 (\(\nu_{10}\)) cm\(^{-1}\) disappeared more rapidly while the intensity of 1587 (\(\nu_{37}\)) cm\(^{-1}\) and 1640 (\(\nu_{10}\)) cm\(^{-1}\) increased more rapidly.

### 3.3 Raman intensity quantitative analysis of Hb / IHP sodium salt of different ratios under low PO\(_2\) levels
Fig. 5 Linear fits for Raman intensity of Hb / IHP sodium salt of different ratios at 1357 cm$^{-1}$, 1377 cm$^{-1}$, 1545 cm$^{-1}$, 1585 cm$^{-1}$, 1606 cm$^{-1}$ and 1640 cm$^{-1}$. (a), (c) and (e) are the linear fit for Raman intensity of deoxygenated Hb bands (1357, 1545, and 1606 cm$^{-1}$) at 10, 20 and 30 mmHg, respectively; (b), (d) and (f) are the linear fit for Raman intensity of oxygenated Hb bands (1377, 1585, and 1640 cm$^{-1}$) at 10, 20 and 30 mmHg, respectively.

The Raman intensities at 1357 cm$^{-1}$, 1377 cm$^{-1}$, 1545 cm$^{-1}$, 1585 cm$^{-1}$, 1606 cm$^{-1}$ and 1640 cm$^{-1}$ of Hb / IHP...
sodium salt whose molar ratios were 1:0, 1:0.2, 1:0.4, 1:0.6, 1:0.8 and 1:1 were linear fitted respectively under low PO$_2$ levels (10, 20, and 30 mmHg), as shown in Fig. 5. The fitted intercepts and slopes were presented at Table 2. The $R^2$ of the deoxygenated Hb bands (1357 cm$^{-1}$, 1545 cm$^{-1}$, 1606 cm$^{-1}$) and oxygenated Hb bands (1377 cm$^{-1}$, 1585 cm$^{-1}$, 1640 cm$^{-1}$) under low PO$_2$ levels were all greater than 0.910, demonstrated that there is a good linear relationship between the proportions of IHP sodium salt and its function of promoting the release of oxygen in oxygenated hemoglobin under low PO$_2$ levels. This confirmed that the IHP could decrease Hb-O$_2$ affinity and regulate the arterial / interstitial tissue oxygen pressure, especially under low PO$_2$ levels.

Table 2. The fitted intercepts and slopes of 1357 cm$^{-1}$, 1377 cm$^{-1}$, 1545 cm$^{-1}$, 1585 cm$^{-1}$, 1606 cm$^{-1}$ and 1640 cm$^{-1}$ under low PO$_2$ levels (X ± S).

<table>
<thead>
<tr>
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<th>Deoxygenated Hb Bands</th>
<th>Oxygenated Hb Bands</th>
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<td>1545 cm$^{-1}$</td>
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<tr>
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<tr>
<td>intercept</td>
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<td>17.093</td>
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<tr>
<td>slope</td>
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<td>-4.114</td>
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<tr>
<td></td>
<td>± 0.285</td>
<td>± 0.319</td>
</tr>
<tr>
<td>20 mmHg</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>± 1.527</td>
<td>± 0.040</td>
</tr>
<tr>
<td>30 mmHg</td>
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<td></td>
</tr>
<tr>
<td>slope</td>
<td>-6.261</td>
<td>-5.486</td>
</tr>
<tr>
<td></td>
<td>± 0.012</td>
<td>± 0.448</td>
</tr>
</tbody>
</table>

3.4 Peak ratios of Hb by IHP sodium salt of different molar ratios under different PO$_2$ levels

![Fig. 6 PR values of oxygenated Hb (a) and deoxygenated Hb (b) by IHP sodium salt of different molar ratios under different PO$_2$ levels.](image-url)
Torres et al.\(^4\) used the general formula 
\[
PR = \left[ \frac{I_{\text{oxy}}}{I_{\text{deoxy}} + I_{\text{oxy}}} \right] \times 100
\]

where \(I_{\text{oxy}}\) and \(I_{\text{deoxy}}\) are the intensity at each \(\nu\) Raman band, found that the PR values have a good linear relation with the hemoglobin oxygen saturation. In Fig. 3, the band of 1357 (\(\nu_2\) cm\(^{-1}\)), 1377 (\(\nu_4\) cm\(^{-1}\)), 1545 (\(\nu_{11}\) cm\(^{-1}\)), 1587 (\(\nu_{13}\) cm\(^{-1}\)), 1606 (\(\nu_{19}\) cm\(^{-1}\)) and 1640 (\(\nu_{16}\) cm\(^{-1}\)) presented apparent change when the PO\(_2\) levels changed from 100 mmHg to 0 mmHg. The 1357 (\(\nu_2\) cm\(^{-1}\)), 1545 (\(\nu_{11}\) cm\(^{-1}\)), and 1606 (\(\nu_{19}\) cm\(^{-1}\)) are the maker bands of deoxygenated Hb while 1377 (\(\nu_4\) cm\(^{-1}\)), 1587 (\(\nu_{13}\) cm\(^{-1}\)) and 1640 (\(\nu_{16}\) cm\(^{-1}\)) are the maker bands of oxygenated Hb. In this study, these six bands were used to calculate the PR values of oxygenated Hb and deoxygenated Hb, the results were shown in Fig. 6.

Fig. 6(a) is the variation tendencies of the Raman PR values of oxygenated Hb under different PO\(_2\) levels. The Raman PR values of the oxygenated Hb increased rapidly when the PO\(_2\) increased from 0 to 20 mmHg, but they changed a little when the PO\(_2\) increased from 30 to 100 mmHg. When the molar ratio of the Hb and IHP sodium salt is 1:1, the Raman PR value of the oxygenated Hb is approximately 75% at 20 mmHg, while the Raman PR value of the oxygenated Hb is just 35% at 20 mmHg when the molar ratio of IHP sodium salt to Hb is 1:0. When the PO\(_2\) reach 100 mmHg, the Raman PR values of the oxygenated Hb was 77% (1:1), 75% (1:0.8), 73% (1:0.6), 69% (1:0.4), 58% (1:0.2), 56% (1:0).

Fig. 6(b) showed the variation tendencies of the Raman PR values of deoxygened Hb under different PO\(_2\) levels. They decreased quickly when the PO\(_2\) increased from 0 mmHg to 20 mmHg. And the decreased tendency is weaker when the PO\(_2\) increased from 20 to 100 mmHg. The Raman PR values of deoxygenated Hb which were respectively 43% (1:0), 39% (1:0.2), 35% (1:0.4), 29% (1:0.6), 24% (1:0.8) and 21% (1:1), reached the minimum when the PO\(_2\) increased to 100 mmHg.

In a word, PR values of oxygenated Hb and deoxygenated Hb by IHP sodium salt of different molar ratios under different PO\(_2\) levels reflect that the IHP can accelerate the release of oxygen in oxygenated Hb and affect the quaternary conformational equilibrium of hemoglobin toward the T state.

4. Conclusions

In this research, we collected and assigned the Raman spectra of Hb with IHP sodium salt of different molar ratios under different PO\(_2\) levels by the InVia+Plus confocal micro-Raman spectrometer. The maker bands of deoxygenated Hb (1357 cm\(^{-1}\), 1545 cm\(^{-1}\), and 1606 cm\(^{-1}\)) and oxygenated Hb (1377 cm\(^{-1}\), 1587 cm\(^{-1}\) and 1640 cm\(^{-1}\)), and the peak ratio values of both deoxygenated Hb bands and oxygenated Hb bands changed at the different PO\(_2\) levels, and the linear fits for Raman intensity of Hb / IHP sodium salt of different ratios at these maker bands under low PO\(_2\) levels were good, which confirmed that the IHP has the property of promoting the release of oxygen in oxygenated Hb, and IHP could guarantee the oxygen supply to the tissue under low PO\(_2\) levels. Raman spectroscopy is a reliable method for the research of hemoglobin allosteric regulation. It is promising that the results could provide reference for allosteric regulation of IHP and other allosteric factors on hemoglobin by Raman spectrum.

Acknowledgment

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Notes and references

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Hb by IHP sodium salt of different molar ratios under different oxygen pressures by Raman spectroscopy.