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High Pressure NMR Studies of Hemoproteins. The Effect of Pressure on the Quaternary Structure of Hemoglobin

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SUMMARY: Proton NMR spectra for nitrosyl- , aquomet- and deoxy des-Arg(α141)-hemoglobin in H_2O were studied at high pressures up to 1400 atm with attention to the exchangeable proton resonances due to the intra- and intersubunit hydrogen bonds. For aquomethemoglboin, the T state marker signal at 6.4 ppm is insensitive to pressure while the R state marker signal at 6.0 ppm exhibits progressive upfield shift upon pressurization. For nitrosylhemoglobin, the T state signals at 9.6 and 6.5 ppm decrease their intensities upon pressurization while the R state marker signal at 6.0ppm remains unchanged. Pressure-induced spectral changes for some of exchangeable resonances are also encountered for deoxy des-Arg(α141)-hemoglobin while the R and T quaternary structural indicators at 6.0 and 9.4 ppm are insensitive to pressure. These pressure-induced spectral changes for these hemoglobin derivatives are significantly distinguished from those associated with the R-T transition induced by addition of IHP or by variatiuon of pH. It is therefore concluded that pressure induces subtle quaternary structural changes in these hemoglobin derivatives without causing the R-T transition.

The structure of hemoglobin(Hb) is often represented by a simple model based on the T(tensed) and R(relaxed) quaternary structures which are in equilibrium depending on whether the heme iron is ligated(R) or unligated(T), presence or absence of the allosteric effector such as inositol hexaphosphate(IHP) and on pH etc. We have been interested in the effect of pressure on the quaternary structure of Hb with the idea that pressure might affect the R-T equilibrium if the volumes of these two states are different.

By the use of high pressure NMR spectroscopy (1), we have previously studied the pressure effect on the tertiary and quaternary structures of oxy-, carbonmonoxy- and deoxyhemoglobin

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(HbO_2, HbCO and deoxyHb) where either R or T state predominates and revealed that the tertiary structure of these Hb's is substantially changed in their heme environments upon pressurization, while the quaternary structure is pressure-independent (2). For these Hb derivatives, the exchangeable proton resonances due to the intra- and intersubunit hydrogen bonds which have been used as the R and T structural probes were not changed upon pressurization. On the other hand, β E11 valine γ_1-methyl signal for HbO_2 and HbCO and the hyperfine-shifted heme and proximal histidyl NH resonances for the β subunit of deoxyHb are much affected by pressure.

To gain further insight into the relation between pressure-induced structural changes and the quaternary structural (R-T) transition, we have extended the high pressure NMR studies on hemoglobin derivatives which are in slightly different quaternary conformation from HbO_2, HbCO and deoxyHb. We wish to report here the NMR evidence that pressure affects the quaternary structures of nitrosyl-, aquomet- and deoxy des-Arg(α141)-hemoglobin (HbNO, HbH_2O and deoxy des-Arg(α141)-Hb) where in contast to HbO_2, HbCO and deoxyHb both R and T forms are populated at an appropriate pH and R to T quaternary transition is readily induced by addition of IHP or by variation of pH (3-17).

MATERIALS AND METHODS Human adult hemoglobin was prepared in the usual manner from fresh whole blood obtained from the normal individual. Organic phosphate was removed according to the method of Berman et al.(18). Methemoglobin was prepared by mixing oxyhemoglobin with 1.2 mol ratio of pottasium ferricyanide. The mixture was passed through a sephadex G-25 column. Nitrosylhemoglobin was prepared by adding sodium dithionite to the mixture of deoxyhemoglobin and sodium nitrite. Excess NO was removed by flushing with dry nitrogen. Des-Arg(α141)-Hb was prepared as previously reported (15).

We used 0.1M Tris-HCl and bis-Tris buffer throughout the present high pressure NMR study, because the pH of these buffers has been shown to be independent of pressure (19,20). The pH value was a direct reading of the pH mater(Radiometer) equipped with a microelectrode(Ingold).

A simple device for the high pressure NMR measurements and experimental details are described in our previous reports(1). Proton NMR spectra at 300MHz were recorded at 30°C on a Nicolet NT-300 spectrometer equipped with a 1280 computer system. We used a Redfield 2-1-X pulse sequence with 28.9μs pulse and 4K data points over 6KHz spectral width. The carrier frequency was placed at 3KHz downfield from the water proton resonance. The spectra at 1 atm were obtained with a usual 5-mm sample tube with spinning. Proton shifts were referenced with respect to the water proton signal, which was insensitive to pressure in its chemical shift from an internal reference, DSS (sodium 2,2-dimethyl-2-silapentane-5-sulfonate).

RESULTS AND DISCUSSION We studied proton NMR of HbNO, HbH_2O and deoxy des-Arg(α141)-Hb in H_2O under pressure with attention to the resonances of the exchangeable protons associated with hydrogen bonds in the subunit interface. These resonances have been established to serve as the sensitive probe for quaternary structures of Hb (8-10).

Fig. 1a shows the pressure-dependence of the exchangeable proton resonances for HbH_2O at pH 6.5 in 0.1M bis-Tris buffer.

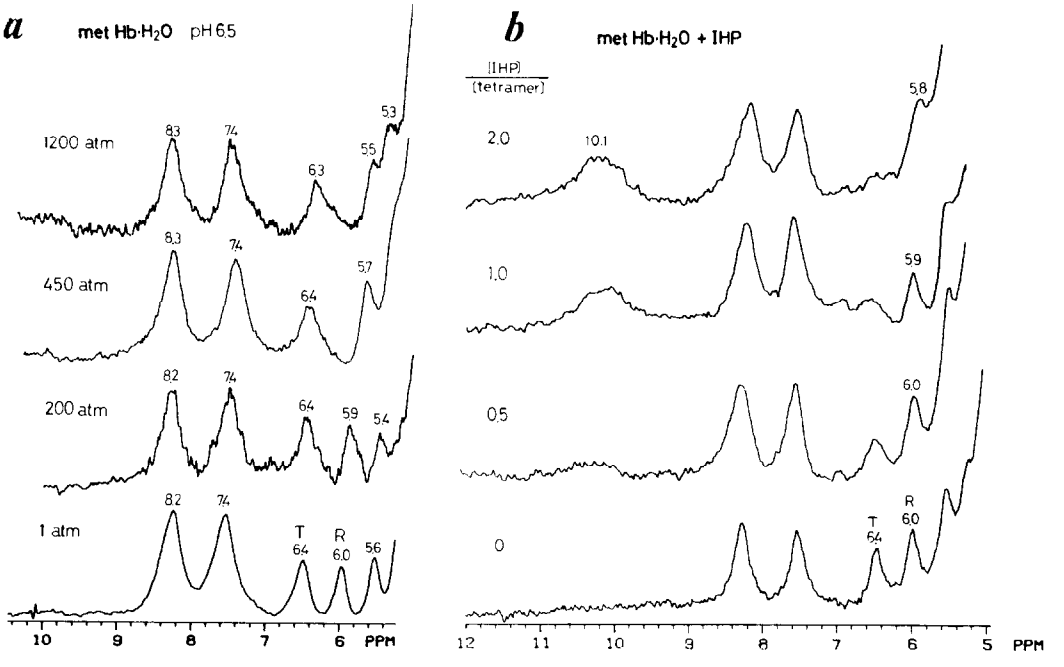

Figure 1 NMR spectra of aquomethemoglobin at various pressures in the absence of IHP(a) and at 1 atm with various amounts of IHP (b). All spectra were taken at 30°C. Samples were buffered at pH 6.5 in 0.1M bis-Tris.

The signal at 6.4 ppm, which arises from the intrasubunit hydrogen bond between β98(FG5) valine and β145(HC2) tyrosine and has been assigned as a deoxy-like(T) quaternary state marker (21), is insensitive to pressure. However, the resonance at 6.0 ppm, which has been utilized as an indicator for the oxy-like(R) quaternary state, exhibits progressive upfield shift with increasing pressure. It is therefore likely that pressure-induced quaternary structural changes occur in such a way that the hydrogen bond involved in the R state of HbH_2O is weakened, but not completely ruptured. The exchangeable resonance at 5.6 ppm, which experiences similar pressure-induced upfield shift, may reflect such a quaternary structural change upon pressurization.

It seems interesting to compare pressure-induced spectral changes with IHP-induced ones for HbH_2O. As Fig.1b shows, the T state marker signal at 6.4 ppm gradually decreases its signal intensity with successive addition of IHP and concomitantly a new exchangeable proton peak grows up at 10.1 ppm (10,11), which may correspond to the T state marker signal observed at 9.4 ppm for deoxyHb (11). This is in accord with the well established result that the binding of IHP to HbH_2O shifts the R-T equilibrium toward the T state (10-14). It is also suggested that IHP perturbs the hydrogen bond involved in the R structure, as is evidenced by the IHP-induced upfield shift for the R state marker signal at 6.0 ppm. Since pressure did not induce the T state marker signal which should be observed at 10.1 ppm, but rather affected the R state signal at 6.0 ppm as did IHP, it is then concluded that pressure induces subtle quaternary structural changes in HbH_2O without switching its quaternary conformation to the T state.

In Fig. 2 are shown the exchangeable proton spectra for HbNO in 0.1M bis-Tris buffer at various pH and pressures. At 1 atm and

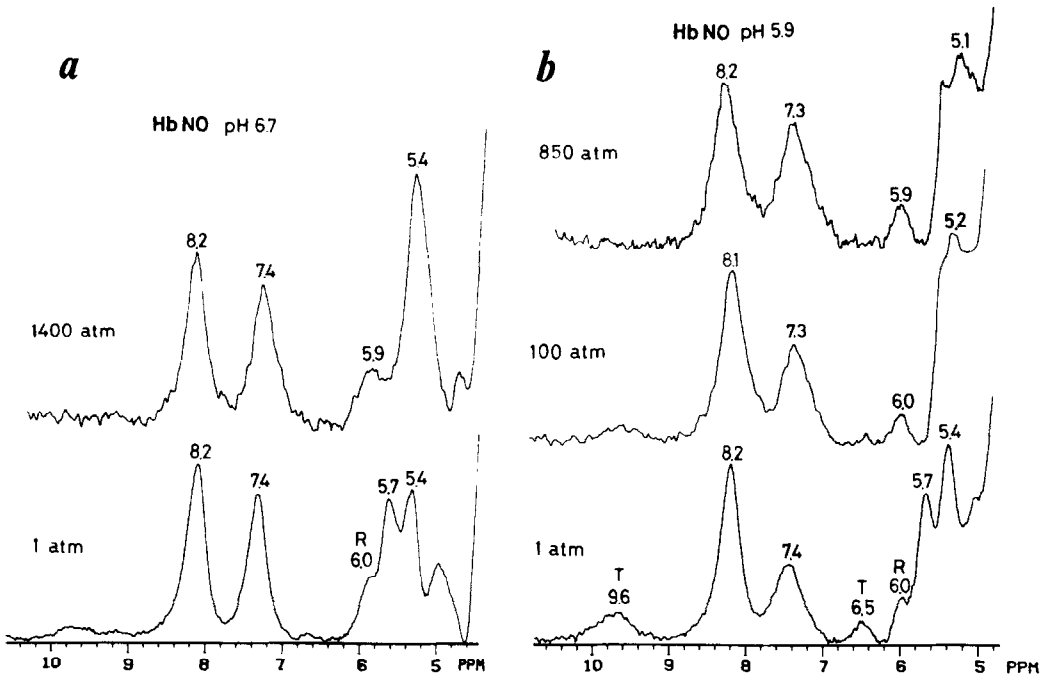

Figure 2 Pressure-dependence of the proton NMR spectra for nitrosylhemoglobin at 30°C, pH 6.7(a) and 5.9(b) in 0.1M bis-Tris.

pH 6.7, the R state marker signal is observed at 6.0 ppm. With lowering pH to 5.9, the T state marker signals at 9.6 and 6.5 ppm grow up (10) and the R state resonance at 6.0 ppm is reduced, giving two state marker signals simultaneously at pH 5.9. Upon pressurization to 100 atm at pH 5.9, the T state signals at 9.6 and 6.5 ppm are reduced and eventually disappear at 850 atm[1], while the R state marker at 6.0 ppm remains unchanged under high pressure. This pressure-induced spectral change for the hydrogen bonded protons in HbNO could be interpreted in terms of pressure-induced structural alterations characterized by the rupture of the hydrogen bond involved in the T conformation. This rupture of the hydrogen bond could be responsible for

[1] This spectral change was irreversible with respect to the application and the release of pressure. All of other pressure-induced spectral changes described in this report were reversible.

disappearance of the exchangeable proton signal due to fast proton exchange with the solvent water proton. These results may allow us to suggest that for HbNO the R-T quaternary transition is not induced by pressure, but rather pressure affects the quaternary structure in such a fashion that the intersubunit hydrogen bond involved in the T structure is broken with the R structure remaining unperturbed.

Fig.3 illustrates the pressure-dependence of the exchangeable proton resonances for deoxy des-Arg(α141)-Hb at pH 7.0 and 8.5 in 0.1M Tris-HCl buffer. Consistent with its T structure at neutral pH (15-17), two T state marker signals at 9.4 and 6.2 ppm are observed at pH 7.0 and 1 atm. These two signals remain unchanged under high pressure, while the exchangeable proton resonance at 7.4 ppm reduces its signal intensity. These observations indicate that pressure may perturb the subunit boundary structure with the T quaternary structure

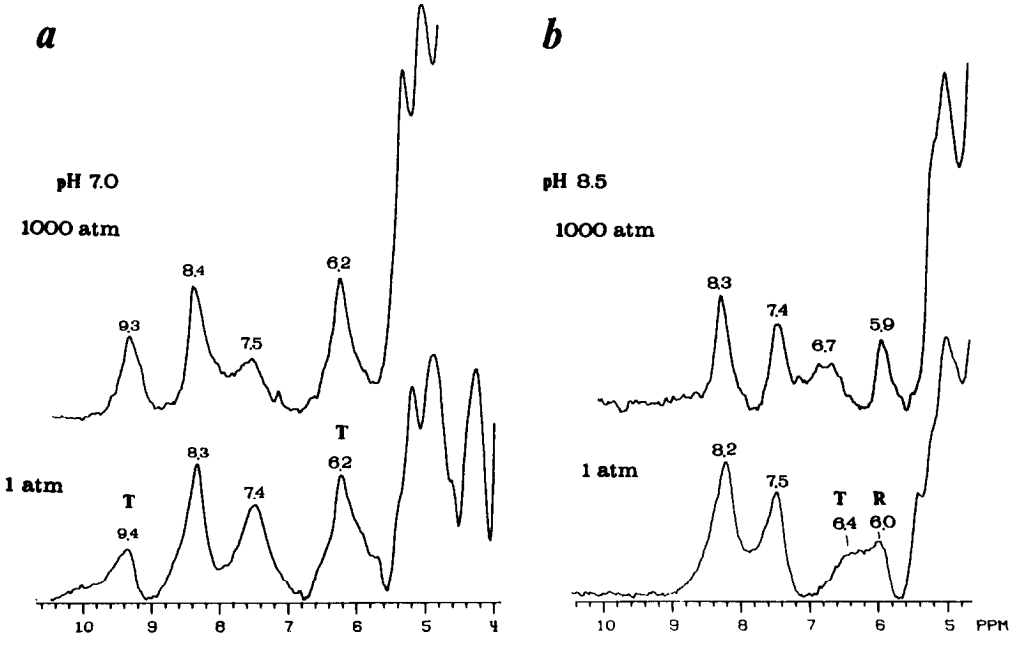

Figure 3 Pressure-dependence of the proton NMR spectra for deoxy des-Arg(α141)-hemoglobin at 30° C, pH 7.0(a) and 8.5(b) in 0.1M Tris-HCl.

retained. At pH 8.5 and 1 atm, the R state marker signal at 6.0 ppm is observed, in agreement with the well established results of the conversion of the protein to the R structure at alkaline pH (15-17). Upon pressurization at pH 8.5, the R state signal at 6.0 ppm remains unchanged, while the T tertiary structural marker at 6.4 ppm exhibits slight downfield shift to 6.7 ppm[2]. From above observations, pressure appears to perturb the tertiary structure of the β subunit in des-Arg(α141)-Hb, as is manifested as a spectral shift of the T state marker signal at 6.4 ppm, but does not cause the R to T quaternary structural transition.

The present finding of pressure-induced quaternary structural alterations without accompanying the R to T transition for HbH_2O , HbNO and deoxy des-Arg(α141)-Hb is in sharp contrast to the case of HbO_2, HbCO and deoxyHb in which R or T state predominates and each quaternary structure is pressure-resistant. In other words, the quaternary structure of Hb derivatives with R and T states in equilibrium is more susceptible to high pressure than those with R or T state predominated.

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[2] We have also examined the effect of pressure on the tertiary structure of deoxy des-Arg(α141)-Hb at pH 7.0 and 8.5. The hyperfine-shifted heme and proximal hystidyl NH resonances were almost pressure independent.

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