EFFECT OF PRESSURE ON THE ABSORPTION SPECTRUM OF SOME HEME COMPOUNDS[1]

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SUMMARY: Increased hydrostatic pressure shifts the spectra of several derivatives of menhaden hemoglobin towards longer wavelengths, producing a difference spectrum similar to that described by Adams and Schuster (Biochem. Biophys. Res. Commun. 58, 525). The effect requires the protein, but is similar for the R and T-forms of both liganded and deoxyhemoglobins.

Although there is a large body of work on the effects of hydrostatic pressure on proteins, there are relatively few reports describing changes in the absorption spectra of hemoglobin derivatives. The detailed studies of Zipp and Kauzmann (1) on the pressure denaturation of myoglobin have been concerned with a pressure range considerably higher than that used here, and were confined to metmyoglobin, while a wider range of derivatives was examined in much less detail by Fabry and Hunt (2). Our experiments were undertaken because of the recent observation by Knowles et al. (3) that the difference spectrum described by Adams and Schuster (4) may be obtained not only by the addition of inositol hexaphosphate to oxyhemoglobin, but by changes in temperature, addition of inorganic phosphate to stripped oxyhemoglobin, and by differentiation of the oxyhemoglobin spectrum. It seemed likely that it might also be obtained as the result of change in hydrostatic pressure.

The experiments were performed using a pressure cell with a range up to 1,000 atm. having a path length of 1 cm. This was introduced into one beam of a Cary 118C spectrophotometer, and an ordinary cuvette was placed in the other. Difference spectra were recorded with pressures of 1, 100, 330, 660 and 990 atm.

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The change on passing from 1 to 100 atm. was chiefly due to settling of the windows of the cell on their sealing rings and to induced birefringence in the cell windows. The absolute spectrum at 1 atm. was also recorded in all cases and was used to allow correction for the change in concentration of the solute due to compression of water. In every case, the results have been corrected both for cell artifact and for concentration change.

The blood used was drawn from live menhaden (Brevoortia tyrannus), the cells hemolyzed, and the hemoglobin solution cleared by centrifugation and filtration through cotton. The advantage of using menhaden blood is that, as shown in unpublished experiments (Carey and Gibson), both liganded and unliganded forms may be obtained in the R and T states by moderate changes in pH without need to resort to the extremes of pH required to bring about these changes in the hemoglobin of the carp. In addition, some experiments were carried out with sperm whale myoglobin (Calbiochem) and with several hematin derivatives.

The pressure difference spectrum for oxyhemoglobin in borate buffer pH 9.1, 20°, 250μM O_2 and 660 atm. is shown in Figure 1 for the Soret region. This result is compatible with the report of Fabry and Hunt (2) that increase in pressure moves the Soret maximum towards the red. Unlike (2), however, who were unable to observe any change in the position of the α and β bands of oxyhemoglobin, a corresponding difference spectrum was observed in the visible region (Figure 2). This spectrum is closely similar in form to that reported by Adams and Schuster (4) on the addition of inositol hexaphosphate to oxyhemoglobin. The changes in the absorption spectrum shown in these figures are considerable, amounting in most cases to more than 5% of peak absorbance in the region studied at pressures of about 1,000 atm., and appear to be proportional to pressure within that range. Similar results with oxyhemoglobin were also obtained at pH 7.4 using phosphate buffer. The shifts cannot be attributed to change in pH due to the effect of pressure on the buffer since these pH changes are small compared with those required to shift the hemo-

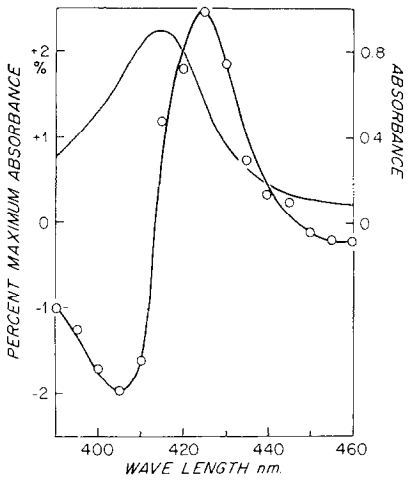

Figure 1. Pressure difference spectrum. Deoxy menhaden hemoglobin, 990 atm. borate buffer 0.05 M pH 9. Continuous line: absolute absorpotion spectrum 1 atm.

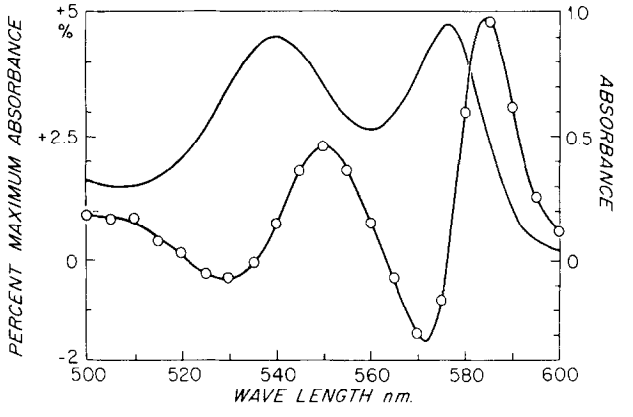

Figure 2. Pressure difference spectrum of oxy menhaden hemoglobin. Borate buffer 0.05 M pH 9, 990 atm. Continuous line: absolute spectrum 1 atm.

globin spectra at a constant pressure of 1 atm. At more acid pH difficulty is met with in recording the spectra since the affinity of the hemoglobin is so low that it is not fully saturated even with a partial pressure of oxygen of 1 atm., and pressure effects on the oxyhemoglobin spectrum are complicated by changes in saturation of the hemoglobin with oxygen.

The experiments with oxyhemoglobin shown in Figures 1 and 2 were carried out with R-state hemoglobin, as judged from kinetic experiments on the rate of

dissociation of oxygen in the presence of dithionite, and from oxygen equilibrium curves. In order to compare the effects of pressure on R and T liganded forms, the CO form was examined at pH 9 and at pH 6. The results show that carbon monoxide hemoglobin is affected in much the same way as oxyhemoglobin is, and that both R (pH 9) and T (pH 6) forms are similarly sensitive to pressure.

Experiments with deoxyhemoglobin prepared by the action of dithionite showed that the Soret band was displaced towards the red by about the same amount as for oxyhemoglobin, and that the effect was independent of pH in the range where an R to T transition was expected. No shift in the visible band of deoxyhemoglobin was demonstrated, but the very diffuse nature of this band makes this particularly difficult. The results for the Soret band are illustrated in Figure 3.

Experiments with myoglobin showed that the carbon monoxide compound resembles that of hemoglobin. With metmyoglobin we saw slow changes as well as immediate ones, and the return to the initial spectrum did not seem to follow immediately on the release of pressure. In all the other experiments so far

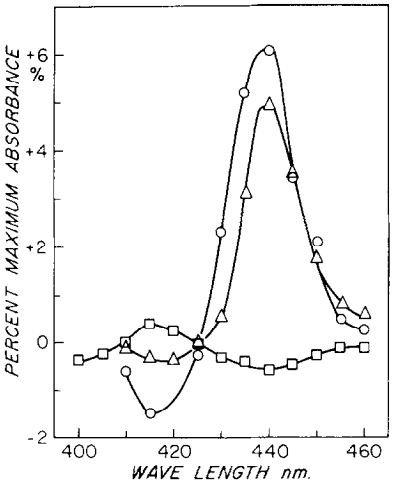

Figure 3. Pressure difference spectra. O deoxy menhaden hemoglobin pH 9, 0.05 M borate buffer, Δ pH 6.0 phosphate buffer 0.05 M. □ Dicyanide ferriheme pH 9, borate, 0.05 M cyanide.

quoted, the effect of application and of release of pressure was immediate within the time resolution of the spectrophotometer and pressure cell (about 5 sec.).

In a few experiments with hematin derivatives it was found that hematin itself in borate buffer pH 9 showed large changes with pressure, in part slow, as did carbon monoxide heme at pH 9 with 1 atm. CO. With dicyanide ferriheme absorbance changes were less than 10% of those seen with the hemoglobin derivatives in the Soret region (Figure 3), while with dicyanide ferroheme the changes were slightly larger, but in a direction to be explained by a shift in the equilibrium between mono and dicyanide heme. It seems plausible to attribute the large changes seen with hematin and CO-heme to changes in the degree of aggregation of the solute with pressure in the case of hematin alone, and to the combined effects of polymerization and change in activity of CO with CO-heme. Photochemical experiments (Gibson, unpublished) have suggested that CO-heme in solution is a mixture of photosensitive and photo-insensitive forms which may reasonably be identified with monomers and polymers respectively.

The experiments reported here seem to permit the conclusion that pressure alters the spectrum of all the hemoglobin derivatives examined in the same way as temperature, pH, and inositol hexaphosphate do, and that these changes do not depend significantly on the distribution of the hemoglobin between the R and T states. Their development, however, does seem to require that the heme be incorporated in a protein, and they must reflect a change in the environment of the heme which may, or may not be the same when the agent altering the spectra is different.

REFERENCES:

1. Zipp, A. and Kauzmann, W. (1973) Biochemistry 12, 4217-4228.
2. Fabry, T. L. and Hunt, J. W., Jr. (1968) Arch. Biochem. Biophys. 123, 428-429.
3. Knowles, F. C., McDonald, M. J. and Gibson, W. H. (1975) Biochem. Biophys. Res. Commun. (Adjacent paper).
4. Adams, M. L. and Schuster, T. M. (1974) Biochem. Biophys. Res. Commun. 58, 525-533.