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Analysis of crystalline hemoglobin of the nematode

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Abstract
Crystalline and dissolved hemoglobin in the optical part could affect the interpretation of the absorption spectra if the hemoglobin in the two states were different. However, there was no evidence of a difference in spectral properties except for the effect of the orientation of the chromophore in the crystal. This paper presents the unusual absorption spectrum due to contamination with ferric hemoglobin. Are the crystals composed of hemoglobin as is suggested by high hemoglobin concentration and high electron density. We show in fresh preparations that the native hemoglobin spectrum is the same as in extracts and entirely due HbO_2

Keywords: Crystalline Hemoglobin, Nematode

Introduction
A bright red region develops in the head of the adult female Mermis during a dormant period in the soil, several months before the nematode emerges to lay eggs on vegetation. Because it seemed to be associated with photobehavior, Cobb (1929) [1] called it a "chromatrope." It has been suggested that the pigment may be the photoreceptor pigment (Cobb, 1929; Croll, 1966) [1, 5] or an ocellar shading pigment (Burr *et al.*, 1975) [3]. Ellenby (1964) [4] identified the pigment as a hemoglobin, showed that it was located in the hypodermis (Ellenby and Smith, 1966) [5], and proposed that it could have a respiratory function. Working with a limited amount of extracted hemoglobin, Burr *et al.* (1975) [3] found that the hemoglobin binds oxygen and carbon monoxide reversibly and determined the intracellular concentration to be very high, on the order of 10 mM in heme. A large fraction of the cytoplasm of the hypodermal cells is observed to contain microscopic crystals, 0.3-0.9 μm in diameter and up to 20μm long, whose long axis is oriented roughly parallel to the body axis (Burr, A. H., manuscript in preparation). The cytoplasm has a high electron density similar to that of the crystals.
This paper addresses several questions arising from these studies. Is the unusual absorption spectrum due to contamination with ferric hemoglobin? Is the high oxygen affinity of extracted Mermis hemoglobin present also in vivo, where the concentration is much higher and allosteric modifiers could be present? Are the crystals present in vivo and not an artifact of fixation and embedding? Are the crystals composed of hemoglobin as is suggested by the high hemoglobin concentration and high electron density?

Methods
Human erythrocytes were prepared by diluting a drop of blood in a mammalian Ringer's solution (130 mM NaCl, 5.4 mM KCl, 2 mM $CaC1_2$, 1 mM $MgSO_4$, 1 mM NaH_2PO_4, 30 mM $NaHCO_3$, 6 mM d-glucose, 0.1 mM each of absorbic acid, d-α-tocopherol acetate, and EGTA, pH 7.8). The suspension was drawn between the coverslips of the perfusion chamber by absorbing solution from the opposite side with filter paper.
To prevent the erythrocytes from being dislodged during perfusion, the lower coverslip was coated beforehand with polylysine (Mazia *et al.*, 1975) [6] to which the erythrocytes adhered tightly. A more uniform coating was obtained after cleaning the coverslip with the flame of an oxygen torch (E. F. Mac Nichol, Jr., personal communication). Draining-off the solution before drying the slide gave better adherence. Pigment absorbance was assumed to be equal to the optical density of the pigmented area minus that of an equivalent nonpigmented reference area.
The average pigment transmittance, T, was computed as the ratio of the two stored DC signals corresponding to average light fluxes transmitted through sample and reference. The average absorbance, A, was computed as log (1 /T).

Linear dichroism, LD, was obtained by dividing the AC signal by the DC signal (Jasperson and Schnatterly, 1969) [7]. With appropriate adjustment of the photoelastic modulator (Goode and Buchanan, 1980), LD is proportional to the polarization of the transmitted light, defined as $p = (T_1-T_\perp/(T_I + T_\perp)$. Following Land and West (1946), the dichroic ratio ($DR = A_\perp/A_l = \varepsilon_\perp/\varepsilon_l$) was calculated as

$$PR = DR = \frac{2A + \log k}{2A - \log k}, where\ k = \frac{1+p}{1-p} = \frac{T_1}{T_\perp}$$

Details of these relationships will be given elsewhere (Harosi, F. I., manuscript in preparation). In the present paper, the DR will be called polarization ratio (PR) to conform with the hemoglobin literature. Automatic baseline correction was used routinely. This is achieved by sampling the dark values of the AC and DC channels before each scan and subtracting their averages from the subsequent sample values of that scan. For reference measurements, the LD was zero and the PR was one at all wavelengths.

Conditions were selected to minimize the errors that are always present in absorbance measurements of tissue. Experiments with blocking filters indicated that there was no error due to the presence of light at other than the nominal wavelength for absorbances up to 2. The error due to stray light that is scattered around the pigmented zone could not be determined. It probably becomes significant at absorbances >2.

On the other hand, errors due to inhomogeneity in the measured area is more likely in regions of low average pigment density. Therefore, regions were chosen where the maximum observed absorbance was between 1.0 and 1.5 units for recording the Soret band, and the densest streaks were chosen for recording the visible peaks (absorbances between 0.3 and 0.5).

Discussion

The technique of the present study had several inherent advantages over that of the previous study done on extracts (Burr *et al.*, 1975) [3]. The microspectrophotometer enabled the measurement of the spectra of hemoglobin in a single specimen, containing -5 pmol heme (Burr *et al.*, 1975) [3], at a higher concentration than in the extracts and under nearly in vivo conditions. Secondly, the perfusion slide facilitated the changing of hemoglobin ligands and oxidation state, the intact tissue acting as a micro-dialysis bag. Thirdly, anaerobic conditions could be maintained. Finally, the dichroic ratio spectra of the native hemoglobin crystals and derivatives were obtainable with this microspectrophotometer.

The presence of both crystalline and dissolved hemoglobin in the optical path could affect the interpretation of the absorption spectra if the hemoglobin in the two states were different. However, there was no evidence of a difference in spectral properties except for the effect of the orientation of the chromophore in the crystal. Under conditions where the hemoglobin spectra indicated a partially liganded state, the spectra did not vary with location in the chromotrope region even though the fraction that was crystalline would be expected to vary. Further, the spectra were not different in specimens containing only noncrystalline hemoglobin.

Conclusion

Our finding that Mermis hemoglobin has a very high oxygen affinity, probably on the order of that of Ascaris hemoglobins, raises the question of whether the hemoglobin can give up its oxygen at an oxygen tension that is useful in respiration.

Ascaris body wall hemoglobin is nearly as capable as leghemoglobin in augmenting the oxygen uptake by nitrogen-fixing bacteriods of legume root knots. It is possible that the high oxygen affinity is an adaptation to the low environmental oxygen pressures, which can occur in the water-saturated soil in which Mermis is active in the late spring. The findings of this study confirm that the crystals, seen in electron micrographs to fill the hypodermal chords, are composed of HbO_2. The biological purpose of intracellular crystalline hemoglobin is an interesting question for future studies.

References

1. Cobb NA. The chromatropism of Mermis subnigrescens, a nemic parasite of grasshoppers. J Wash Acad Sci 1929;19:159-166.
2. Croll NA. A contribution to the light sensitivity of the "chromatrope" of Mermis subnigrescens. J Helminthol 1966;40:33-38.
3. Burr AH, Schiefke R, Bollerup G. Properties of a hemoglobin from the chromatrope of the nematode Mermis nigrescens. Biochim Biophys Acta 1975;405:404-411.
4. Ellenby C. Haemoglobin in the 'chromatrope' of an insect parasitic nematode. Nature (Lond.) 1964;202:615-616.
5. Ellenby C, Smith L. Haemoglobin in Mermis subnigrescens (Cobb) Enoplus brevis (Bastian) and E. communis (Bastian). Comp Biochem Physiol 1966;19:871-877.
6. Mazia D, Schatten G, Sale W. Adhesion of cells to surfaces coated with polylysine. J Cell Bio 1975;66:198-200.
7. Jasperson SN, Schnatterly SE. An improved method for high reflectivity ellipsometry based on a new polarization modulation technique. Rev Sci Instrum 1969;40:761-767.
8. Land EH, West CD. Dichroism and dichroic polarizers. In Colloid Chemistry, J Alexander, editor. Van Nostrand Reinhold Company, Inc., New York 1946;6:160-190.