HEMOGLOBIN AS THE RED PIGMENT OF MICROSOMES

by

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INTRODUCTION

The characteristic reddish color of microsomes was originally ascribed by BENSLEY to a lipid-like material[1]. In a later report[2] BENSLEY suggested that the color of microsomes was due to the presence of blood pigments. STRITTMATTER AND BALL[3] however, have demonstrated by spectrophotometric methods that the principal visible pigment is a hemochromogen. Results obtained in this laboratory indicate that the red color of microsomes is in fact due to the presence of hemoglobin, which, however, is present in too low concentration relative to the brownish hemochromogen to affect significantly the absorption spectrum in the visible region.

MATERIALS AND METHODS

Microsomes were isolated from the livers of mice which were given no food for 18 hours prior to sacrifice. The procedure of SCHNEIDER AND HOGEBOOM[4] employing 0.25 *M* sucrose was followed throughout. A solution of authentic mouse hemoglobin was obtained from heparinized mouse blood. The erythrocytes were centrifuged off, washed three times with isotonic saline, lysed by the addition of 3 volumes of distilled water, and the ghosts centrifuged down.

Zone electrophoresis was carried out in the cold employing 0.10 *M* glycylglycine buffer in 0.25 *M* sucrose, pH 7.6, with starch as the supporting medium. A description of the apparatus used is published elsewhere[5]. Sedimentation measurements were made in a Spinco model E ultracentrifuge at concentrations of 0.009% protein, by using absorption optics[6]. For this purpose essentially monochromatic light of wavelength 415 mμ was employed. This was obtained with an interference filter having a half band width of 2 mμ. Absorption spectra were obtained with a Cary recording spectrophotometer.

RESULTS

When a suspension of microsomes was subjected to zone electrophorises at pH 7.6 in the cold, a pink band appeared which moved rapidly along the starch column tc ward the cathode. The microsome zone, remained at the origin, and became brown in color. The pink zone, when eluted from the starch geve the absorption spectrum shown on Fig. 1a. Fig. 1b shows the spectrum of mouse hemoglobin. Both materials had absorption maxima at 415, 540 and 575 mμ, and in both cases the visible absorption bands *disappeared* upon reduction while the intense band at 415 mμ shifted to 420 mμ. This loss of the visible absorption bands upon reduction is in contrast to the behavior of any known cytochrome. For a cytochrome the two visible absorption bands *appear* on reduction, rather than the reverse.

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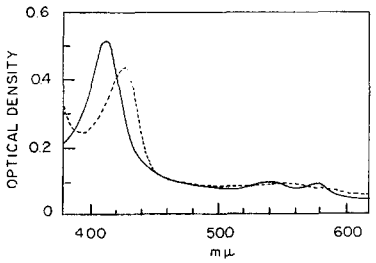

Fig. 1 (a)

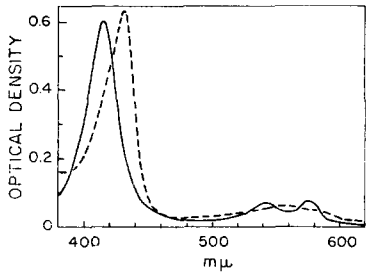

Fig. 1 (b)

Fig. 1. (a) microsome pigment. (b) Mouse hemoglobin. Spectra were obtained in 0.04 *M* glycylglycine buffer, pH 7.6, containing 0.25 *M* sucrose. —— oxidized; -----, reduced with a trace of sodium hydrosulfite.

Fig. 2 gives the absorption spectra of oxidized and reduced microsomes. The absorption bands of hemoglobin were not discernible.

The following experiments supplied confirmatory evidence for the identification of the red pigment as hemoglobin. Hemoglobin and the red pigment were found to have the same electrophoretic mobility on starch at pH 7.6 (0.76 mm/h corrected for electro-osmosis at $\mu = 0.025$ *M*, and a potential of 10 V/cm) and a mixture of the two showed no resolution into two spots. In the ultracentrifuge both yielded the same sedimentation coefficient, 4.6 *S*, and the extent of boundary spreading was the same for both.

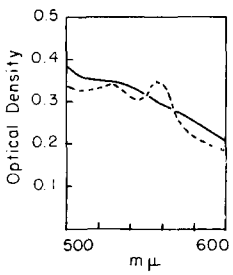

Fig. 2. A 1% sodium desoxycholate extract of microsomes. Conditions are the same as Fig. 1. ——, oxidized; -----, reduced with a trace of sodium hydrosulfite.

The ease with which the pigment was released form the particles (0.1 *M* glycylglycine, pH 7.6 at 0°C) suggested that it was bound by surface adsorption. That the binding was, in fact, due to adsorption was supported by the finding that hemoglobin could be removed and again attached to the microsomes by appropriate adjustment of the ionic strength. Desorption was complete at an ionic strength of 0.1 *M*, and was still appreciable at 0.01 *M*. No specific salt effects were observed.

Mitochondria show no affinity for the pigment under conditions where microsomes adsorb large amounts of hemoglobin. Hemoglobin can, in fact, be used as a differential stain to distinguish microsomal from mitochondrial layers in centrifuge pellets. The addition of 0.02% hemoglobin to sucrose homogenates results in an intense staining of microsomal material, with no change in the color of the mitochondrial pellet.

DISCUSSION

The results obtained in the present work indicate that the red color of microsomes is due to hemoglobin, although the principal pigment is actually the brownish hemochromogen described by STRITTMATTER AND BALL[3].

The physiological significance of the presence of hemoglobin on the microsomes is obscure. It is doubtful whether hemoglobin exists on the surface of the microsomes

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intracellularly. The most likely source of the hemoglobin is an occasional red cell which is ruptured during the original preparation of the homogenate.

The existence of hemoglobin binding by microsomes does serve to demonstrate a fundamental difference in the surface properties of the two types of particles.

The ability of microsomes to adsorb protein is not limited to hemoglobin. The adsorption of cytochrome *c*[7] and nucleases[8] has been reported previously, and adsorption of lysozyme has also been observed in this laboratory. It is possible that the adsorption of basic proteins by microsomes, as contrasted with the failure of these proteins to adsorb to mitochondria, is related to the high nucleic acid content of the microsomes.

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SUMMARY

The red color of isolated microsomes results from the presence of adsorbed hemoglobin. Adsorption occurs only at very low ionic strengths. In contrast to microsomes, mitochondria do not adsorb hemoglobin. Hemoglobin added to tissue homogenates is useful as a specific stain to differentiate microsomal from mitochondrial material in mixed centrifuge pellets.

RÉSUMÉ

La coloration rouge des microsomes isolés résulte de la présence d'hémoglobine adsorbée. L'adsorption ne se produit qu'à des forces ioniques très basses. A l'inverse des microsomes, les mitochondries n'adsorbent pas l'hémoglobine. De l'hémoglobine, ajoutée à des tissus homogénéisés, peut servir de colorant spécifique pour différencier les microsomes des mitochondries dans des culots de centrifugations mixtes.

ZUSAMMENFASSUNG

Adsorbiertes Hemoglobin verursacht die rote Farbe von isolierten Mikrosomen. Adsorption kommt nur bei sehr geringen Ionenstärken vor. Im Gegensatz zu Mikrosomen adsorbieren Mitochondrien kein Hemoglobin. Zu Gewebshomogenaten hinzugefügtes Hemoglobin kann als spezifischer Farbstoff benützt werden, um in gemischten Zentrifugenrückständen Mikrosomen von Mitochondrien zu unterscheiden.

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