HEMOGLOBIN IN MITOCHONDRION-LIKE ORGANELLES OF IMMATURE CHICK EMBRYO ERYTHROCYTES

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SUMMARY — Thin sections of chick proerythrocytes show in their interior the presence of mitochondrion-like organelles (MLO) containing a considerable number of dense granules identical to the particles within the hemoglobinized cytoplasm, suggesting that the content of these organelles are hemoglobin molecules.

Electrophoresis of the supernatant from the lysed MLO fraction in polyacrilamide gel confirmed the presence of hemoglobin, however with a higher migration rate than that of the cytoplasm from proerythrocytes. The authors propose the term “hemosome” for these organelles.

UNITERMS — Hemoglobin in mitochondrion-like organelles.

INTRODUCTION

Hemoglobin biosynthesis in immature chick erythrocytes is not related to the nucleus but to the basophilic cytoplasmic reticulum (9). Furthermore, hemoglobin biosynthesis was found to be proportional to the amount of the basophilic reticulum or “Substantia granulo-filamentosa” (7). Through thin sections and in hemolysed smears of supravitally stained mammalian reticulocytes, it was shown that the filamentous mitochondria are one of the constituents of the “Substantia granulo-filamentosa” (1). Besides mitochondria, however, reticulocytes contain other organelles. Since neither DNA nor aspects suggesting division have been detected yet, the term mitochondrion-like organelles (MLO) was proposed on account of their structural similarity to mitochondria. Recently, the term hemosome was suggested for these organelles because very probably the final hemoglobin biosynthesis takes place in the MLO (3).

This report is meant to show that immature chick erythrocytes, or proerythrocytes, contain the same MLO as found in mammalian immature erythrocytes.

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MATERIALS AND METHODS

Blood was obtained from 16-day-old chick embryos by cardiac puncture. Supravital staining with brilliant cresyl blue showed that the peripheral blood contains mostly erythroblasts and proerythroblasts. For electron microscopic examination, blood was fixed according to the following procedures: a.) directly in 1.0% osmium tetroxide in phosphate buffer, pH 7.3, for 30 min; b.) directly in 1.5% glutaraldehyde in phosphate buffer, pH 7.3, for 1 hr, followed by osmium tetroxide fixation (15 min), after three washings in the buffer, uranyl acetate staining (30 min), dehydration, and embedding, in Poly-lite 8001 (4.5). Thin sections were obtained in a Porter-Blum MT-1 microtome, stained by lead citrate (10), and photographed in an Elmiskop I electron microscope at 60 and 80 Kv with magnifications from X 8,000 to X 40,000.

The presence of hemoglobin within the MLO has been demonstrated by electrophoresis. Blood cells from 50 16-day-old chick embryos as well as from 6 newborn chicks were submitted to fractionation, the MLO isolated and lysed according to the following procedure: 1. Blood is poured on 18 ml of a 0.153M NaCl, 0.005M KCl, 0.005M MgCl₂, 0.006M NaHCO₃, 0.0014M EDTA and 0.05M phosphate buffer (pH 7.2). 2. Centrifugation of the cell suspension for 15 min at 200Xg, discarding of the supernatant, and resuspension of the sedimented cells (1.0 - 1.3 ml) in ten-fold their volume of a 0.32M sucrose and 0.04M phosphate buffer. 3. Homogenization in a Potter-Elvehjem tube at 1,000 rpm for 4 min at 4°C. 4. Centrifugation of the homogenate at 1,350Xg for 10 min. 5. Centrifugation of the 1,350Xg supernatant at 26,360Xg for 10 min at 4°C. 6. Resuspension of the nuclei-free MLO sediment in 0.32M buffered sucrose and five washings of the fraction by successive resuspensions and centrifugations at 26,360Xg for 10 min. The sediment was lysed by resuspension in 3.0 ml distilled water. 7. The suspension was centrifuged at 30,000Xg, for 20 min., after the lysis was completed, and the supernatant was used for electrophoresis.

Supernatants of the lysed MLO and of the last washing medium were concentrated about 10-fold in a vacuum chamber, and then submitted to electrophoresis. Diluted hemoglobin obtained from the supernatant of the 26,360Xg centrifugation (step 5) was used for comparison through this method.

Samples were run on disc electrophoresis in polyacrylamide gel, according to Dietz and Lubrano (6). A 2.5mA current per tube was applied for 40 min at 5°C, and the hemoglobin band was identified by benzidine or orto-dianizidine reagents.

RESULTS AND DISCUSSION

The general aspect of chik proerythrocyte cytoplasm, with regard to its high electron density due to hemoglobin molecules, is similar to that of mamma-
lian reticulocytes. Polyribosomes, responsible for globin synthesis, are present from the monomer to the heptamer forms, as found in mammalian reticulocytes (11). The MLO, constituted by lamellae in transversal, oblique or longitudinal disposition, are highly electron dense, generally more than the hemoglobinized cytoplasm. Those three types of lamellae disposition may be found in one and the same organelle (Fig. 1a).

At higher magnifications, MLO show dense particles dispersed within the interlamellar space, presenting 90 to 100 A in diameter. These particles are identical to the particles in the cytoplasm; they are, howevver, more agglomerated in the MLO, thus conferring to the organelles a higher electron density at some regions (Figs. 1b).

Through disc electrophoresis on polyacrylamide gel of the supernatant from the lysed MLO fraction, at least two hemoglobin bands were obtained, resembling those from the supernatant of the 26:300Xg centrifugation, which contain the cytoplasmic hemoglobin of proerythrocytes. The former showed, however, a higher migration rate than the latter (Fig. 2). The second MLO hemoglobin band has a migration rate corresponding to that of the first cytoplasmic hemoglobin band. One of the three hemoglobin types thus found is common to the cytoplasm and the MLO. The last washing supernatant showed no visible band at all, indicating that no contamination of the MLO by the cytoplasmic hemoglobin occurred.

These results suggest that the final hemoglobin biosynthesis, or combination of globin with heme, occurs within the MLO, in the same way as it might happen within the MLO of mammalian reticulocytes (8). Iron-containing material is incorporated by the immature erythocytes through pinocytosis, or in erythroblasts by rhopheocytosis (8), and is then enveloped by membranes resembling the smooth endoplasmic reticulum probably with the presence of globin while the ferruginous material is transformed for heme synthesis (Fig. 3a); this whole gives rise to a pro-MLO which originates the MLO (2) (Fig. 3b). Since the MLO of chick proerythrocytes seem to play a role in hemoglobin biosynthesis, they may be termed hemosome, as suggested for the MLO of mammalian immature erythrocytes (8).

RESUMO — Cortes finos de proeritrócitos de ave mostram a presença de organelos semelhantes a mitocôndrios (OSM) contendo um considerável número de grânulos densos, idênticos às partículas encontradas no citoplasma hemoglobinizado, sugerindo serem as partículas no interior dos organelos, moléculas de hemoglobina.

A eletroforese do sobrenadante do lisado da fração OSM, em gel de poliacrilamida, confirmou a presença de hemoglobina, porém, com velocidade de migração superior a da hemoglobina citoplasmática dos proeritrócitos. Os autores propõem o termo hemosomo para os OSM.

UNITERMOS — Hemoglobina em organelos semelhantes a mitocôndrios.

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REFERENCES


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Fig. 1 — Thin sections of chick-embryo proerythrocytes. The nuclei (N) are retracted due to the fixation.

a) With a mitochondrion-like organelle (m) constituted by lamellae in oblique or longitudinal dispositions.

b) Containing an electron-dense mitochondrion-like organelle (m) completely filled with particles identical to the ones of the hemoglobinized cytoplasm; the transverse or oblique lamellae are hardly visible. V — vesicle.
Fig. 2 — Electrophoresis patterns of hemoglobin from the supernatant of 26,350Xg centrifugation (cytoplasmic hemoglobin (I) and from mitochondrion-like organelle content (II); o — origin; Hb — hemoglobin bands; the last washing supernatant shows no visible bands (III).
Fig. 3 — Thin sections of proerythrocytes.

a) An electron-dense material is enveloped by the smooth endoplasmic reticulum (arrow). Remnants of the Golgi complex (G) and a vesicle (V) are seen. N — nucleus.

b) Organelles identical to pro-hemosome (ph), and hemosome in development (h), of mammalian reticulocytes are observed. V — empty vesicle.