Journal of Chromatography, 134 (1977) 230–234
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 9783

Note

Chromatographic separation of melanosomes

JAN BOROVANSKÝ, PETR HACH and JIŘÍ DUCHOŇ

Department of Biochemistry and Department of Embryology, Faculty of Medicine, Charles University, 128 53 Prague (Czechoslovakia)

(Received November 2nd, 1976)

Melanosomes are specialized subcellular particles of pigment cells[1]. Their biochemical investigation is difficult as it is not easy to obtain isolated melanosomes that are free from contamination on the one hand and with preserved ultrastructural and biochemical integrity on the other[2].

Chromatographic techniques have only rarely been employed for the isolation of melanosomes. Riley and co-workers[3,4], using chromatography on diatomaceous earth, obtained melanosome samples with obvious markers of mitochondrial contamination, while the results of Dorner and Reich's experiments on Celite[5] and those of Pechan and Duchoň[6] on Sephadex G-200 columns are difficult to assess because no marker was followed and electron microscopic monitoring was not performed.

Gel filtration in columns of Sepharose, reported to be suitable for the separation of cell particles[7,8], was exploited in this work in an attempt to isolate melanosomes; in addition, material obtained according to Dorner and Reich[5] was evaluated from the point of view of present-day demands.

MATERIALS AND METHODS

Preliminary model runs were performed with isolated human hair melanosomes prepared by our own method[9]. The starting material used in the isolation studies was a large-granule fraction prepared in the classical way[10] from the iris and corpora ciliaria of cattle eyes and from Harding–Passey mouse melanoma. The large-granule fraction was suspended in a buffer solution containing 0.04 *M* Na_2HPO_4, 0.025 *M* NaH_2PO_4 and 0.069 *M* NaCl and then layered on the top of the column bed.

Gel chromatography was carried out in the above buffer in 300 × 15 mm water-cooled glass columns with a thermostat jacket (VEB Technisches Glass, Ilmenau, G.D.R.) on either Sepharose 4B (model run) or Sepharose 2B (proper isolation). The void volume (V_0) was determined by using Blue Dextran 2000 (Pharmacia, Uppsala, Sweden).

The fractions obtained were monitored at 280 and 400 nm with a Unicam SP 1800 instrument and the protein content was studied by the method of Lowry *et al.*[11].

From the darkest fractions, melanosomes were collected by a simple run on a table centrifuge and the pellet was subjected to an electron microscopic check.

Method No. 3 of Dorner and Reich[5], employed to isolate melanosomes from

Harding–Passey melanoma, had to be modified by decreasing the centrifugal force to 250 and 450 *g* in the introductory run and the subsequent one, respectively, otherwise no material was obtained.

For electron microscopy, the material was processed as described earlier[9].

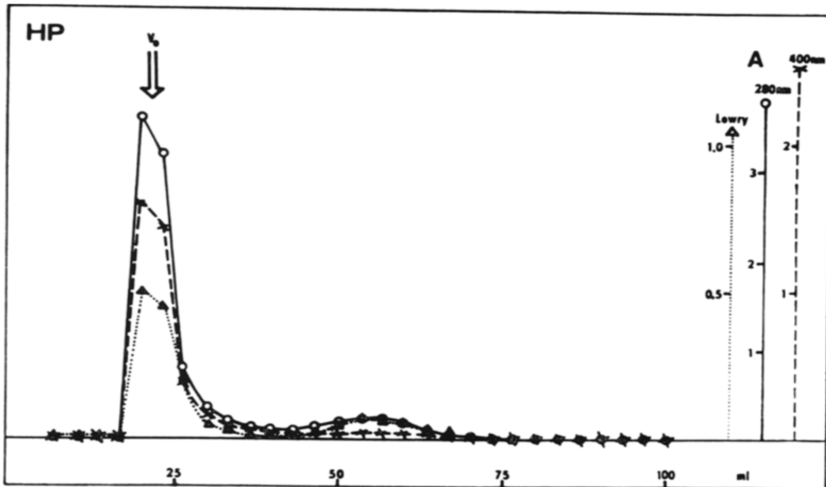

Fig. 1. Separation of Harding–Passey mouse melanoma melanosomes by gel chromatography on Sepharose 2B.

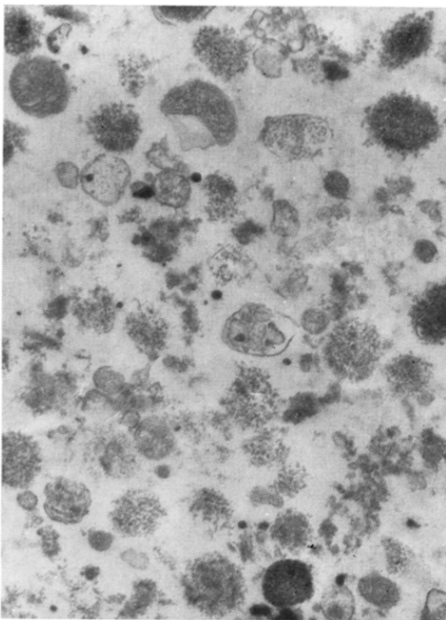

Fig. 2. Melanosome sample prepared from Harding–Passey melanoma by gel chromatography on Sepharose 2B (15,950:1).

RESULTS

As there is no information on the behaviour of melanosomes on a Sepharose column, we first carried out a model experiment with isolated human hair melanosomes. A melanosome suspension (1.5 ml, corresponding to 0.018 g of dry melanosomes) was layered on the Sepharose 4B column, and the melanosomes passed through the gel as a single, broad, brown band and were recovered in the void volume.

The suspended large-granule fraction of Harding–Passey melanoma was then layered on the Sepharose 2B column. The sample again passed through the gel as a brown, broad band that left the column in the void volume (Fig. 1). A dark pellet

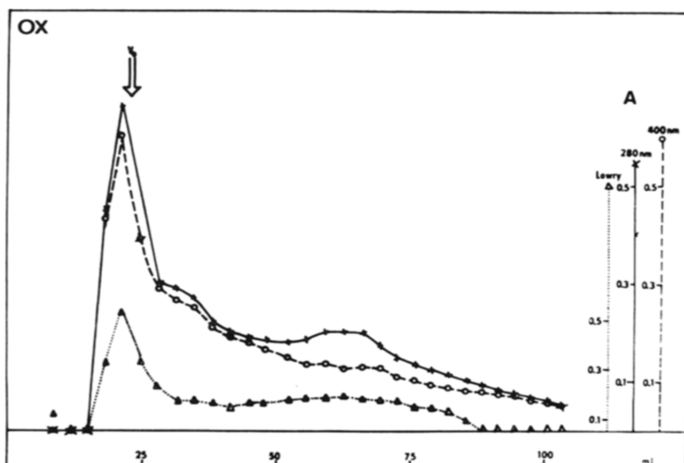

Fig. 3. Separation of cattle eye pigment tissue melanosomes by gel chromatography on Sepharose 2B.

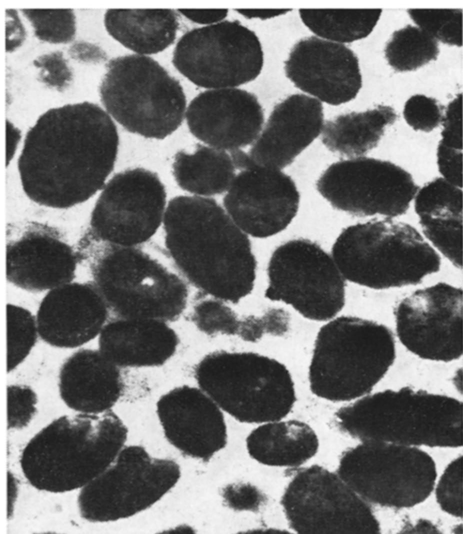

Fig. 4. Melanosome sample prepared from pigmented tissues of cattle eyes by gel chromatography on Sepharose 2B (17,000:1).

was prepared from the fraction with the highest protein content, which also had the highest $A_{400\ nm}$ value. As shown in Fig. 2, a melanosomal fraction of relatively well preserved ultrastructural integrity was obtained, slightly contaminated with fragments of endoplasmic reticulum.

The same procedure was performed with a large-granule fraction of cattle eye pigment tissue. The pattern of the gel chromatography was essentially the same (Fig. 3). The prepared melanosomal pellet, under the electron microscope, appeared as a homogeneous sample of melanosomes with a relatively well preserved ultrastructural integrity (Fig. 4).

Melanosomes prepared according to Dorner and Reich[5] were almost homogeneous without any particulate contamination, but their structure was modified and some particles fell into fine granules with an electron density typical of melanin (Fig. 5).

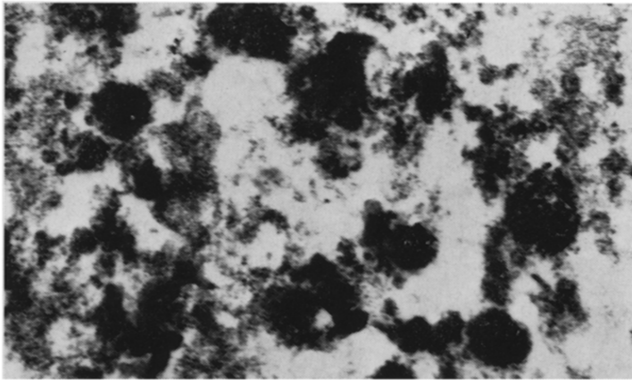

Fig. 5. Melanosome sample prepared from Harding–Passey mouse melanoma by the method of Dorner and Reich (14,810:1).

DISCUSSION AND CONCLUSIONS

Dorner and Reich's isolation procedure[5] did not yield satisfactory results, and the application of Sepharose seems to be more promising. The extremely high exclusion limits of Sepharose (agarose) gels and their very low levels of non-specific adsorption make them suitable for the separation and purification of subcellular particles and other multicomponent complexes[7,8,12]. Gel filtration procedures can be used to eliminate time-consuming centrifugation techniques, at the same time avoiding possible damage that could arise from high centrifugal forces, repeated resuspensions and the hypertonic influence of gradients[7,8].

This study demonstrated that gel chromatography could be applied to the separation of melanosomes. Any new approach to the isolation of melanosomes is welcome as there is no method that would meet all of the present demands concerning the homogeneity and integrity of subcellular fractions from various pigment tissues[2]. Melanosome samples prepared by gel chromatography on Sepharose columns were of a quality comparable to those prepared by other currently used methods. The possible use of gel chromatography in further purifying isolated melanosomes should also not be neglected.

REFERENCES

1 M. Seiji, T. B. Fitzpatrick, R. T. Simpson and M. S. C. Birbeck, *J. Invest. Dermatol.*, 36 (1961) 243.
2 J. Borovanský, J. Duchoň and P. Hach, *Abstr. Commun. 7th Meet. Eur. Biochem. Soc.*, (1971) 323.
3 V. T. Riley, M. L. Hesselbach, S. Fiala, M. W. Woods and D. Burk, *Science*, 109 (1949) 361.
4 V. Riley, G. Hobby and D. Burk, in M. Gordon (Editor), *Pigment Cell Growth*, Academic Press, New York, 1953, p. 231.
5 M. Dorner and E. Reich, *Biochim. Biophys. Acta*, 48 (1961) 534.
6 Z. Pechan and J. Duchoň, in G. Della Porta and O. Mühlbock (Editors), *Structure and Control of the Melanocyte*, Springer, Berlin, Heidelberg, New York, 1966, p. 78.
7 *Separation News*, Pharmacia, Uppsala, No. 11, 1972.
8 *Separation News*, Pharmacia, Uppsala, No. 1, 1975.
9 J. Borovanský and P. Hach, *Dermatologica*, 145 (1972) 37.
10 M. Seiji, K. Shimao, M. S. C. Birbeck and T. B. Fitzpatrick, *Ann. N.Y. Acad. Sci.*, 100 (1963) 497.
11 O. H. Lowry, N. J. Rosebrough, A. L. Faar and R. J. Randall, *J. Biol. Chem.*, 193 (1951) 265.
12 S. Hjertén, *Arch. Biochem. Biophys.*, 99 (1962) 466.