Further Characterization of a Protein Promoting Aggregation of Retina Cells

By E. H. CREASER and L. M. RUSSELL

Research School of Biological Sciences, Australian National University, Canberra, A.C.T., Australia

(Received 25 March 1971)

The present paper describes further work on a protein fraction isolated from the surface of chick-embryo retina cells that has been shown to cause dissociated cells to reaggregate (Daday & Creaser, 1970). Margoliash *et al.* (1965) isolated a polydisperse protein that caused the specific reaggregation of dissociated sponge cells; the smallest unit had molecular weight 15 000-20 000. Analogous proteins have been isolated from yeasts, where they are of importance in conjunction (Crandall & Brock, 1968). In addition, proteins have been isolated from serum that are the size of γ -globulins and cause aggregation of retina cells (Orr & Roseman, 1969).

Crude retina cell-surface protein was prepared as described by Daday & Creaser (1970), by washing cells with a buffered solution of EDTA, centrifugation of the cells and freeze-drying of the supernatant. The resulting solid was redissolved in water and passed through a column of Sephadex G-200 at 4°C. This column (5 cm $\times$ 50 cm) had been previously calibrated by passing Blue Dextran, Phenol Red and a range of standard proteins, all in Tyrode's solution, through the column. The retina extract was eluted from the column with Tyrode's solution, the eluate being monitored at 254 nm by an LKB u.v. detector and the separated material being recorded as peaks. Three peaks were observed when

Fig. 1. Chromatography of retina cell-surface material on Sephadex G-200, and (inset) polyacrylamide-gel electrophoresis of the total material and the separated fractions. The column (5 cm $\times$ 50 cm) of Sephadex G-200 was eluted with Tyrode's solution at 20 ml/h at 4°C. The position of markers is indicated from previous calibration. BD, Blue Dextran (corresponding to totally excluded molecules); SM, small molecules (mol.wt. 5000 or less, completely included in the gel); ϕ R, position of Phenol Red (retarded owing to adsorption by the Sephadex). Standard proteins used were: C, catalase (mol.wt. 250 000); YADH, yeast alcohol dehydrogenase (mol.wt. 150 000); Hb, haemoglobin (mol.wt. 68 000).

retina-cell extract was chromatographed. Peak 1 was opalescent and was eluted with the front in a position corresponding to the void volume of the column as estimated by Blue Dextran, indicating that its molecular weight was in excess of 800 000. Plots of $\log(\text{mol.wt.})$ against elution volume (Andrews, 1965) gave a value of approx. 5000–7000 for peak 2. Peak 3 was pink and corresponded to Phenol Red, which had been used for pH control during the preparation. The occurrence of these peaks was reproducible from one experiment to another, but the relative amounts of peaks 1 and 2 varied. On being freeze-dried and tested, peak 1 material was found to promote the reaggregation of dissociated retina cells in a fashion similar to that shown by the previously prepared crude extracts (Daday & Creaser, 1970). The second peak was also biologically active, but there was normally insufficient material for good measurements.

The fractions were further examined by the method of gel electrophoresis, the technique used being that of Hedrick & Smith (1968), where replicate samples are run in gels of different concentrations and a molecular-weight estimation can be made by using the relative mobilities of standard proteins run under the same conditions. When the total extract and the separated fractions were examined by this method the results shown in Fig. 1 were obtained. Separated fractions were freeze-dried and redissolved in the electrophoresis buffer (tris-glycine-HCl, pH 8.2) and subjected to electrophoresis at 250 V in gel tubes (0.5 cm $\times$ 10 cm). After electrophoresis the gels were extruded and stained with Amido Black. The total extract gave six major bands; peak 1 material gave a single band corresponding to the fastest-running band in the crude extract; that from peak 2 gave a similar pattern to peak 1, although weaker; that from peak 3 gave, as expected, no bands at all. Occasionally peak 2 material gave very faint bands

corresponding to those observed in the crude extract. When the mobility of the band from peak 1 was compared with the mobilities of known proteins in gels of 6%, 9%, 12% and 15% (w/v) acrylamide it was found that the molecular weight of this material was approx. 6000.

As the active protein appears to have molecular weight 6000 from the electrophoretic results and molecular weights in excess of 800 000 when run on Sephadex, it is suggested that the situation on Sephadex is the result of self-aggregation of the monomeric unit. The position of the second peak from Sephadex G-200 is consistent with a molecular weight of 6000. Other small proteins have been isolated from cell membranes in mitochondria, retina rods and erythrocytes by Laico, Ruoslahti, Papermaster & Dreyer (1970) and from *Neurospora* cell membranes by V. W. Woodward (personal communication). These 'miniproteins' also have molecular weights of approx. 6000, and it is possible that the aggregation protein described above and the miniprotein may represent members of a similar class of cell-surface proteins.

We thank Mr H. Daday of the Commonwealth Scientific and Industrial Research Organisation, Canberra, for providing crude retina extracts and checking the biological activities of the separated fractions.

- Andrews, P. (1965). *Biochem. J.* **96**, 595.
Crandall, M. A. & Brock, T. D. (1968). *Bact. Rev.* **32**, 139.
Daday, H. & Creaser, E. H. (1970). *Nature, Lond.*, **226**, 970.
Hedrick, J. S. & Smith, A. J. (1968). *Archs Biochem. Biophys.* **126**, 155.
Laico, M. T., Ruoslahti, E. I., Papermaster, D. S. & Dreyer, W. J. (1970). *Proc. natn. Acad. Sci. U.S.A.* **67**, 120.
Margoliash, E., Schenk, J. R., Hargie, M. P., Burokes, S., Richter, W. R., Barlow, G. H. & Moscona, A. A. (1965). *Biochem. biophys. Res. Commun.* **20**, 383.
Orr, C. W. & Roseman, S. (1969). *J. Membrane Biol.* **1**, 125.