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Note

Fractionation of proteins from single neurons of *Planorbis corneus* by micro-electrophoresis on SDS–gradient polyacrylamide gels

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The very large neurons in the central nervous system of gastropod molluscs can be isolated by free hand dissection[1], which allows their biochemistry to be studied. With the recent development of suitable micro-procedures, the amine, amino acid, RNA and enzyme contents of such neurons have been characterized[1–3]. Moreover, the incorporation of radioactive amino acids into various protein components of defined gastropod neurons has been reported[4–10], although the fractionation of the various components from a single neuron in a stained gel has not been demonstrated. As a major problem in neurobiology is that of discovering specific proteins in the nervous system, we decided in this initial study to see whether the sodium dodecyl sulphate (SDS)–polyacrylamide procedure as described by Rüchel *et al.*[11] (for details of microelectrophoretic techniques, see Rüchel *et al.*[12], Osborne[1] and Gainer[4]) can be adapted for the analysis of the proteins from single neurons isolated from the central nervous system of the water snail *Planorbis corneus*.

EXPERIMENTAL

The procedure used for moulding gradient gels (1–40%) in capillaries has been described elsewhere[12]. The gels formed in 2- or 5-μl capillaries were overlayed with a "sample solution" (see Fig. 1a) and were then ready for use. The circumoesophageal ganglia mass from a snail was removed and pinned on to the polythene base of a small chamber containing physiological solution[13]. Individual neurons ranging in size from 70 to 160 μm were then freed from surrounding cells using thin tungsten needles and single neurons were lifted free with a glass pipette[1] and placed directly on the meniscus of the "sample solution" above a gradient gel. Once a single cell had penetrated the "sample solution" due to gravity, the capillary, standing in a small amount of gel buffer, was covered with a beaker so as to create a moist atmosphere and placed in an oven for 2 h at 55° in the presence of nitrogen. Thereafter, any space at the top of the capillary due to slight evaporation of the sample solution was filled with a small amount of concentrated sucrose solution containing a small amount of bromophenol red as a marker dye, and then subjected to electrophoresis as shown in Fig. 1b. Electrophoresis was terminated after 30–45 min when the bromophenol red reached the lower end of the gradient gel; the gel was removed from the capillary using a

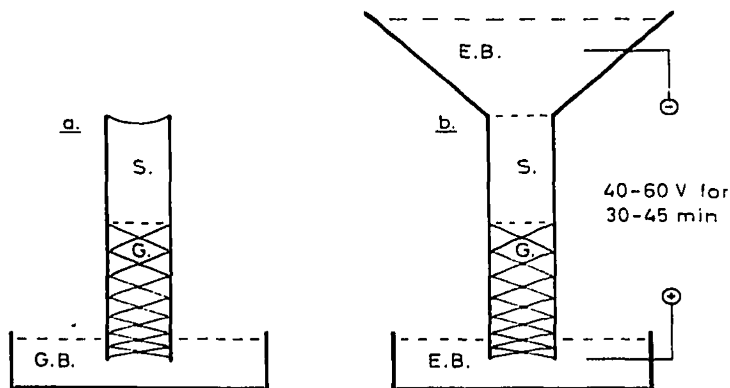

Fig. 1. Scheme of the procedure used. (a), Polymerized gradient gel (G) overlayed with the "sample solution" (S) in a small container containing gel buffer (G.B.). The dissected cell is placed directly on the meniscus of the "sample solution" and, following incubation (2 h at 55° in the presence of nitrogen) and the addition of a small amount of sucrose solution containing bromophenol red, is subjected to electrophoresis as shown in (b). Compositions: "sample solution" (S), 20 m*M* Tris–sulphate buffer (pH 8.4) + 1 % SDS + 1 % mercaptoethanol; electrophoresis buffer (E.B.), 50 m*M* Tris–glycine buffer (pH 8.4) + 0.1 % SDS; gel buffer (G.B.), 350 m*M* Tris–sulphate buffer (pH 8.4). The procedure for casting the micro-gradient polyacrylamide gels containing Tris–sulphate buffer is described elsewhere[11,12].

thin wire and stained in 0.5% coomassie blue[14] and examined after destaining by densitometry.

RESULTS AND DISCUSSION

A number of different neurons were fractionated using the sensitive micro-electrophoretic technique on SDS–gradient gels. The results showed clearly that the protein patterns from the different neurons were not constant (see Fig. 2), although no attempt was made to characterize the differences. Moreover, reproducible patterns were achieved when analyzing a single cell-type (see Fig. 2). As far as is known, the method used is unique in that it allows proteins from single neurons to be fractionated and localised by staining. As the dissected neuron is placed directly in the SDS–mercaptoethanol situated above the gel, the process of mechanical homogenization and transfer of extract is not necessary. This "single-step" procedure is not only simple but also results in minimal inaccuracies. Furthermore, in addition to fractionating total proteins from the cell, the procedure can be adapted so as to analyze only water-soluble proteins (see Fig. 3). In a previous study in which a micro-disc-electrophoretic method was also used, water-soluble proteins of more than six neurons each measuring about 160 μm in diameter were required to produce a single fractionated pattern in a 2-μl capillary[15].

The question thus arises as to whether the protein patterns observed in this study are real, as it is known that a number of artefacts can occur when using discontinuous buffer systems for electrophoresis, and special attention has to be paid to the incubation time of the tissue in the SDS–mercaptoethanol solution[11,12]. We have taken into account all of these possible sources of inaccuracy, and the consistency of the results argues in favour of the idea that artefacts are not produced by the proce-

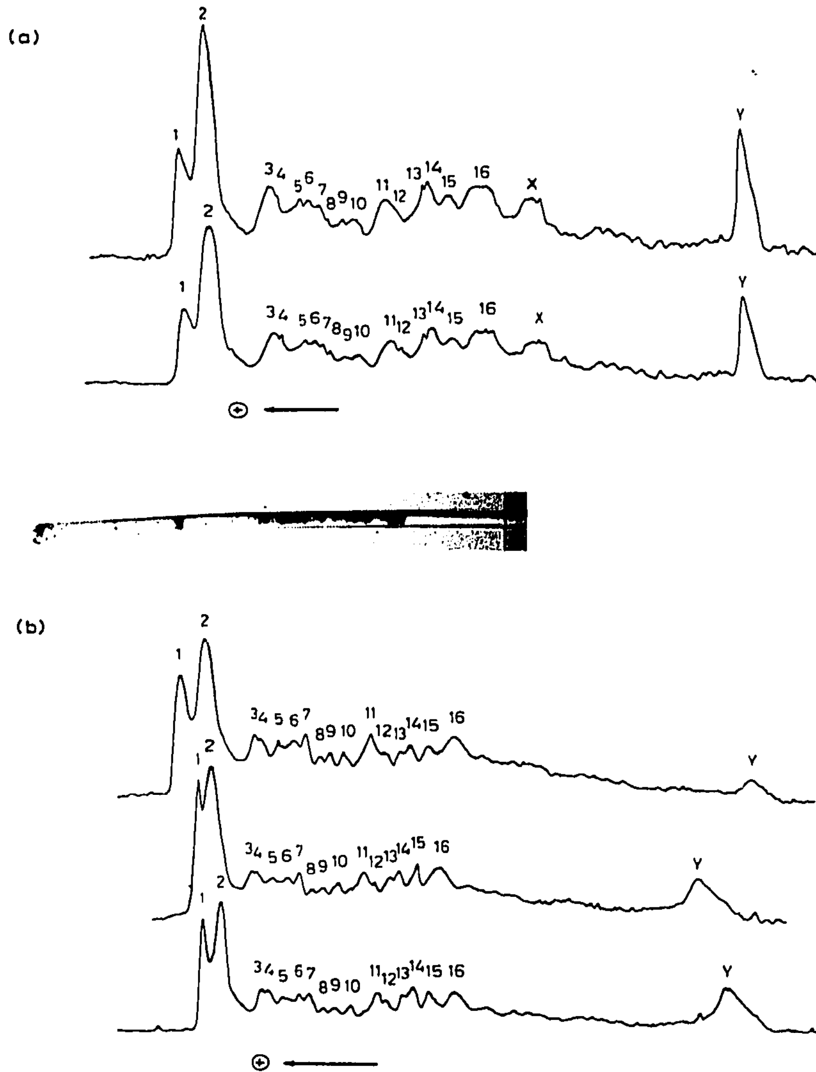

Fig. 2. Densitometric traces of micro-polyacrylamide gradient gels showing the separation of total proteins from single neurons of *Planorbis corneus* using the conditions of electrophoresis as shown in Fig. 1b and staining with coomassie blue. (a), Two electropherograms of different gels from the same cell type (together with an insert of a photograph of a stained micro-gel that was cast in a 2-μl capillary); (b), three electropherograms of different gels from the same cell type. It can be seen that a constant fractionation of protein patterns from a single cell type is achieved by the method used and that variations in the protein compositions between the different cell types (for example, protein band "X" occurs predominantly in one of the cell types) occur.

dure used. However, more experiments are required before an exact protein composition of individual cells can be determined. Special attention will still have to be paid to the nature of the gradient gels, the incubation time and the electrophoresis conditions.

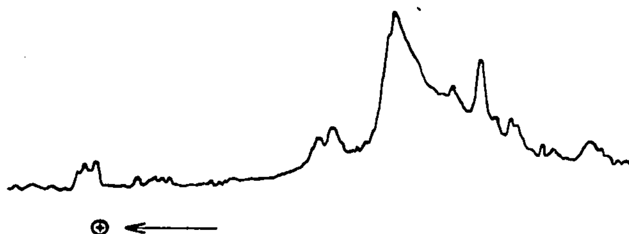

Fig. 3. Densitometric trace of a micro-polyacrylamide gradient gel showing the separation of water-soluble proteins from a single neuron using basically the same procedure as that described in Fig. 1 and staining with coomassie blue. However, in this instance, deionized, distilled water instead of the "sample solution" was placed above the gel (see Fig. 1). The dissected cell was placed directly on the meniscus of the water and left for 2 h at 4°. Thereafter, a small amount sucrose together with bromophenol red was added to the "free space" caused by slight evaporation of the water and the gel was then subjected to electrophoresis.

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