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Protein analysis of an individual *Caenorhabditis elegans* single-cell embryo by capillary electrophoresis

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Abstract

We present a simple one-dimensional electrophoretic map of the expressed proteins in a *Caenorhabditis elegans* embryo. The embryo was taken from an adult nematode, injected into a 50-μm I.D. capillary, and lysed. The proteins were fluorescently labeled and then separated by capillary electrophoresis and detected by laser-induced fluorescence. Over 20 components were resolved in the 22-min separation. The dynamic range was outstanding for this separation, noise in the baseline was less than 0.01% the amplitude of the largest component.

Keywords: *Caenorhabditis elegans*; Proteins

1. Introduction

The development of a fertilized egg into a mature organism is one of the great research topics of biology [1]. In most organisms, the first few cell divisions create identical cells; in all organisms, a point is reached in the development of the embryo where a cell division creates differentiated cells. These differentiated cells are morphologically distinct and grow into different tissues in the mature organism.

The nematode *Caenorhabditis elegans* has been a favorite organism in developmental biology [2]. During development, the organism creates 1090 cells, of which 131 undergo apoptosis, leaving 959 cells in the adult [3]. Most cells arise through invariant cell lineages; their origins can be traced unambiguously to founder cells. Although they contain identical genetic information, the cells differ because they express different proteins. The 97 Mbase genome of *C. elegans* has been sequenced [4]. There are roughly 20 000 genes expressed in the organism; only a fraction of these genes are expressed in any given cell.

We have a long-term goal of monitoring the evolution of protein expression as a founder cell divides and differentiates into its progeny. This study will provide exquisitely detailed information on differentiation and development. In this paper, we present preliminary results for the protein expression in the earliest stage of development: this is the first description of the protein expression of an embryo at the single-cell stage.

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The proteome analysis of a single cell does not require extraordinary analytical sensitivity. A typical *C. elegans* embryo is ~50 μm in diameter and has a volume of about 60 pl. Assuming that the embryo is 10% protein by mass and that the average protein is about 25 000 in molecular mass, an embryo contains a total of about 250 fmol of protein. If 4000 proteins are expressed in a single cell, the average protein is present at the 5 amol level. We reported earlier the protein analysis of a single human cancer cell, which contains roughly an order of magnitude less protein than a *C. elegans* embryo [5,6].

2. Experimental

2.1. Reagents

Unless noted, all reagents were purchased from Sigma (St. Louis, MO, USA). Solutions were prepared in deionized water (Millipore, Bedford, MA, USA).

2.2. C. elegans culture

To culture *Escherichia coli*, LB broth was prepared by adding 6.25 g of LB broth powder to 250 ml of distilled water. This mixture was autoclaved and inoculated with a loopful of *E. coli*. This mixture was incubated overnight at room temperature with shaking.

A nematode medium was prepared by mixing 3.44 g Tris–HCl, 1.5 g Tris, 19.4 g peptone, 12.5 g NaCl, and 50 mg cholesterol. A 5.9-g aliquot of this mixture was added to 20 g of agar and diluted to 1 l with distilled water. This medium was sufficient for 80 plates. Each plate was inoculated with a few drops of the *E. coli* culture placed onto the medium and spread evenly in the center of the plate.

The *C. elegans* culture was propagated by transferring a few nematodes to a plate containing freshly prepared growth medium. The original wild-type *C. elegans* culture was a kind gift from Professor David Pilgrim of the Biological Sciences Department of the University of Alberta.

2.3. C. elegans embryo reagents

An egg salt solution was prepared by mixing 3.445 g NaCl, 1.8 g KCl, 0.25 g $CaCl_2$, 0.46 g $MgCl_2$ and 10 ml of 0.25 *M* 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) buffer, pH 7.4 with sufficient distilled water to make 500 ml.

A simplified *C. elegans* early blastomere growth medium was prepared by mixing 9 ml of Drosophila Schneiders's medium (Gibco, Edmonton Canada), 1 ml of 5 mg/ml inulin, 50 mg tissue culture-grade PVP powder, 100 μl basal medium Eagle vitamin solution (Gibco), 100 μl chemically defined lipid concentrate (Gibco) and 100 μl penicillin–streptomycin solution (Gibco) [7]. This solution was stored at 4°C. Before use, it was mixed with bovine fetal calf serum (Gibco) that had been heat treated for 30 min at 56°C to 35% (v/v).

Chitinase/α-chymotrypsin digestion solution was prepared by adding 5 units of chitinase and 20 mg α-chymotrypsin to 1 ml egg salt solution.

2.4. Single embryo analysis

Single cell proteome analysis has been described elsewhere [5,6]. The procedure was modified slightly for analysis of a single embryo.

Briefly, a suspension of adult nematodes was placed on a microscope slide and viewed with an inverted microscope. A mature hermaphrodite nematode was dissected to free its embryos. The eggs were mouth-pipetted into a siliconized slide containing a 10% (v/v), freshly diluted solution of 4–6% sodium hypochlorite and incubated for 2 min.

The eggs were then rinsed with egg salt solution. They were pipetted into a chitinase/chymotrypsin solution and incubated for 8 min to remove the eggshell and outer membrane. Simplified *C. elegans* early blastomere growth medium was added to the eggs.

Just before injection onto the capillary for analysis, the eggs were washed with the egg salts to remove the enzymes and growth medium. The wash buffer contained 2.5 m*M* NaCN; the cyanide takes part in the fluorescent labeling reaction. The procedure must be performed rapidly. Cell division requires about 10 min in *C. elegans*, so the procedure

from dissection to lysis must be completed during this period to capture the proteome map of an embryo at the earliest, single-cell stage.

2.5. Cell lysis

A 40 cm×50 μm I.D.×144 μm O.D. fused-silica capillary was used for analysis. The capillary was filled with a 10 m*M* Na_2HPO_4–10 m*M* NaH_2PO_4 with 25 m*M* sodium pentasulfate (SPS) (pH 6.8) separation buffer. A plug of 10-m*M* sodium dodecyl sulfate (SDS) solution was injected to the capillary. The capillary was then placed over the cell of interest by means of a micromanipulator. The cell was injected into the capillary by applying a computer-controlled pulse of vacuum to the distal end of the capillary.

2.6. Fluorescent labeling

To fluorescently label the proteins, a 5 m*M* aqueous solution of 3-(2-furoyl)quinoline-2-carboxaldehyde (FQ Molecular Probes, Eugene, OR, USA) with 10 m*M* SDS was injected for 1 s. Following addition of the labeling reagent, the capillary was incubated at room temperature for 1 min; during this period, the cell was lysed by the SDS solution. The capillary tip was then heated at 65°C for 1 min to accelerate the labeling reaction.

2.7. Capillary electrophoresis

Capillary electrophoresis was performed at an electric field of 250 V/cm. The locally constructed laser induced fluorescence detector was based on a sheath flow cuvette [8,9]. Fluorescence was excited with a 10-mW argon ion laser operating at 488 nm and detected through a 630DF30 bandpass filter from Omega Optical (Burlington, VA, USA).

2.8. Data analysis

Data were smoothed and regression analysis was performed using routines written in Matlab (Mathworks, Natick, MA, USA) and run on a G4 Macintosh computer (Apple, Cupertino, CA, USA).

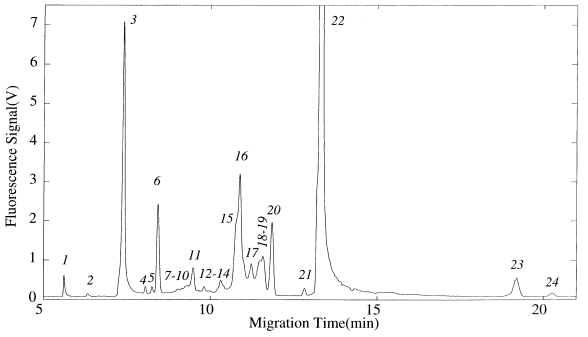

Fig. 1. Submicellar capillary electrophoresis analysis of the proteins from a single *C. elegans* embryo before the first cell division. The data have been treated with a nine-point median filter to remove bubbles and smoothed with a 0.6 s Gaussian filter.

3. Results

The submicellar electropherogram from a single *C. elegans* embryo is shown in Fig. 1. A few dozen peaks are observed in a 21-min separation. The major component migrates at 14 min and generates a peak that is off-scale with this instrument.

Peaks 1, 3, and particularly 22 generated noticeable tailing. This tailing may be due to the migration of closely related components with slightly longer migration time, or the tailing may reflect interaction of hydrophobic components with the capillary wall. This tailing is not due to extra-column dispersion because other components, such as peaks 6 and 20, are quite symmetric. Fig. 2 presents the results of a

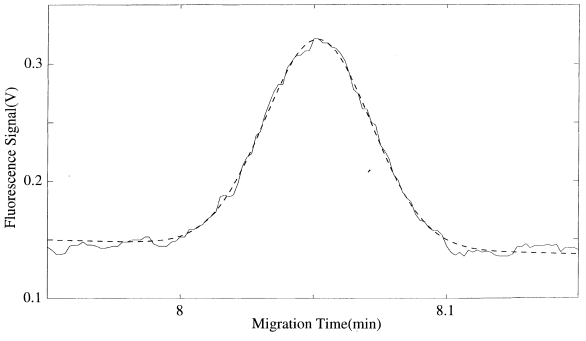

Fig. 2. Close-up of peak 4. The data were treated with a three-point median filter. The dashed curve is the result of a nonlinear regression analysis of the data with a Gaussian function with sloping baseline.

nonlinear regression fit of the data of peak 4 with a Gaussian function with sloping baseline. The theoretical plate count for this peak is 80 000, which is similar to plate counts that we have observed for separation of standard proteins. Single embryo protein analysis can provide exquisite separation efficiency.

Fig. 3 presents an expanded view of Fig. 1 to highlight the low-intensity components. These components generate peaks with heights that are roughly 0.1% as intense as the major component. This separation produces a 10^4 dynamic range. A high dynamic range is vital in proteome analysis because the vast majority of the proteome is expected to be expressed at quite low levels.

There are 19 099 predicted genes expressed by *C. elegans* [4,10,11]. Two proteins, vitellogenin-1 and vitellogenin-5, both of molecular mass 170 000, are identified as yolk proteins [12]. It is tempting to propose that these glycoproteins are the major components in peak 21 of this very early stage embryo.

The data presented in this paper are a preliminary glimpse at the protein expression at the earliest stages of development within this simple multicellular organism. There were two-dozen components resolved in this one-dimensional electropherogram. This resolution is similar to that produced by other one-dimensional separation methods for proteome analysis. A second dimension will be required to resolve the 89 known proteins present in the embryo and to identify other proteins that are not yet known to be involved in embryogenesis [11]. We hope to use a two-dimensional separation, similar to that developed by Bushey and Jorgenson, to characterize further the protein expression of each cell in *C. elegans* throughout its development [13]. The extraordinarily high dynamic range of our electrophoresis system will allow us to monitor trace-level proteins, provided that they are resolved by the more sophisticated two-dimensional separation.

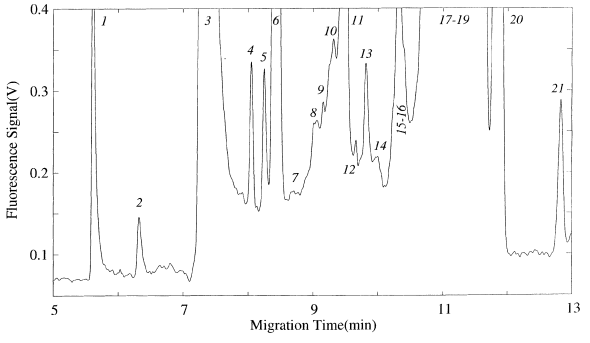

Fig. 3. Expanded view of the data of Fig. 1.

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