VIVASCIENCE

Concentration of Soluble and Membrane proteins - Concentration of Soluble and Membrane Proteins with the Vivacell 250 Centrifugal Ultrafiltration Device

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Introduction

Protein Estimation

Protein purification is characterised by a series of important tasks such as extraction, capture and polishing of the protein of interest. One of the most crucial steps at every stage of the protein purification protocol is concentration of the sample. This step enables the gathering of important data including specific activity, yield, recovery and the degree of/or fold purification. Protein samples can be concentrated using centrifugal ultrafiltration devices with membranes that have a specific molecular weight cut-off. For small volumes of protein solutions ranging from 0.1-10 ml there are many devices available on the market produced by a range of manufacturers. Similarly, it is possible to concentrate large volumes ranging from 1-100 litres since the devices are available and these include the Sartocon Slice (Sartorius) which consists of a membrane sandwiched between plastic supports. The extract is pumped across the membrane under pressure and salts and molecules of lower mass than the size of the membrane pores permeate through the membrane whilst higher mass molecules are retained. However, if the extract to be concentrated is less than 1 litre there is a problem since this falls between the range of the low and high volumes devices available.
In this study, we have tested the Vivacell 250, an uftrafiltration device which fills this gap in concentration devices. The Vivacell 250 allows the concentration of 250 ml sample at any one time and uses gas pressure to force the samples against the membrane which is housed in a plastic support structure.

Axonal Growth Inhibition in the CNS of Higher Vertebrates

The axons of neurons in the mammalian CNS, including the human brain and spinal cord, fail to regrow following injury. Recent evidence from a variety of studies point towards axon-growth inibitory factors in the environment of damaged axons as being responsible for the lack of axonal regeneration. In our laboratory we have purified an axonal growth inhibitory protein that induces the collapse of axonal growth cones in culture. At present, we are initiating the molecular cloning of the gene coding for this protein and producing larger quantities of the native protein in order to raise polyclonal antibodies. We have used this protein as an example of a membrane protein to test the performance of the Vivacell 250.

Axon Guidance in the Vertebrate Retina

During development of the visual system the axons of retinal ganglion cells navigate out of the retina eventually connecting to their target cells in the optic tectum. In order to leave the retina the axons are steered towards the optic nerve head possibly by an inhibitory molecule. The axonal outgrowth is restricted to a narrow channel which terminates in the optic nerve head. It is possible that this inhibitory molecule is secreted by the lens epithelial cells and diffuses across the vitreous humour towards the ganglion cell bodies in the retina. We have purified a diffusible protein that induces growth cone collapse of retinal ganglion cell growth cones and inhibits axonal outgrowth in collagen gel culture (Fig.1). Experiments are underway to confirm the in vivo function of this protein. We have used this soluble protein to test the performance of the Vivacell 250.

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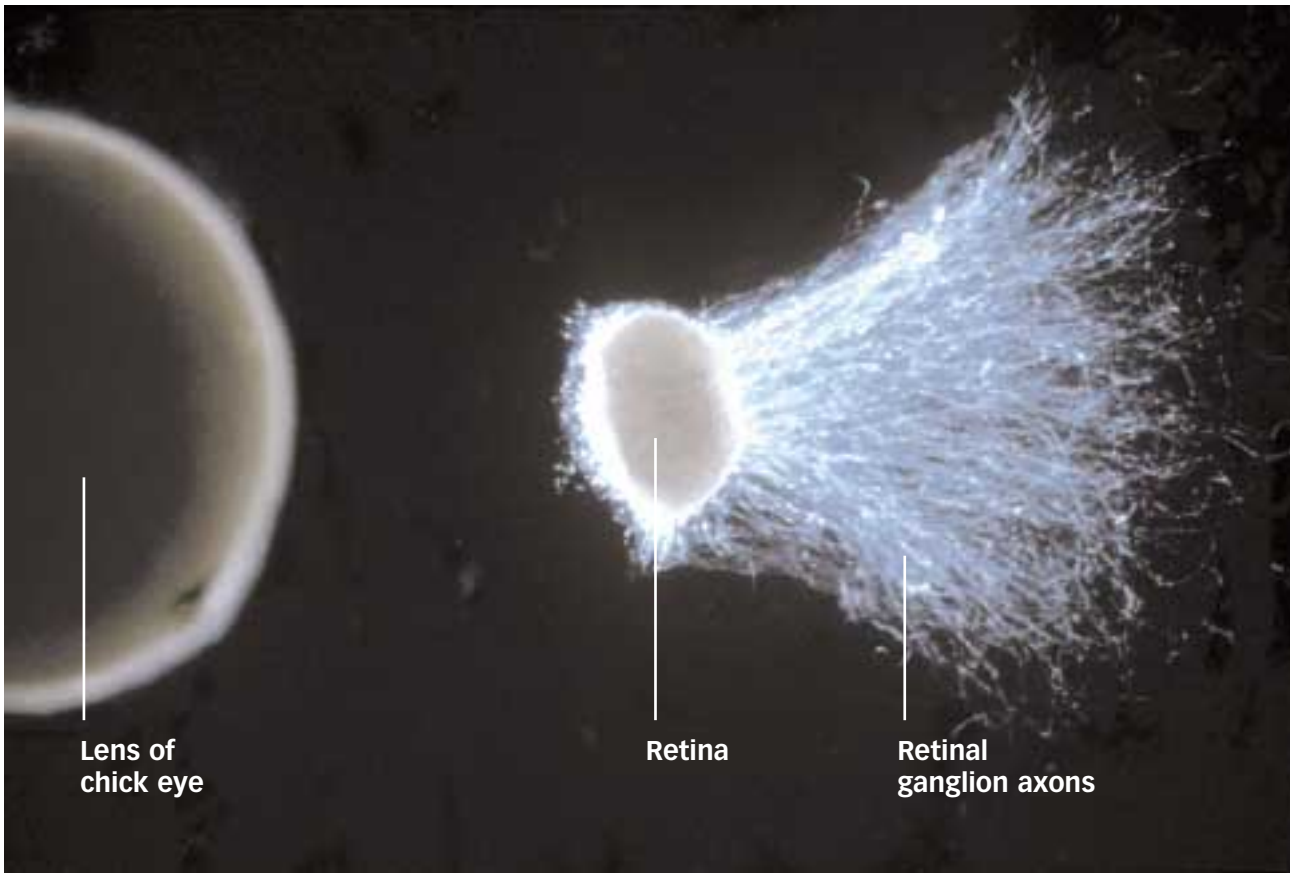

Figure 1: Co-culture of embryonic day 6 chick retinal explant with the lens of the same age.
The lens is secreting a diffusible axon growth inhibitory protein which causes the retinal axons to grow in the oposite direction i.e. the lens protein repels the retinal axons.

Materials and Methods

Collagen Gel Culture

400 µls of Rat tail collagen 1 (Sigma) was mixed with 40 µl of 10x Dulbecco's Minimal Essential Medium (DMEM) and 25 µl of 7.5% sodium bicarbonate and vortexed for 1 minute. A small drop of the collagen was pipetted into the well of a 4-well Nunc tissue culture plate. A small, 600 μm^2, retinal explant and one embryonic day 6 chick lens were placed together on the drop of collagen separated by a distance of 8 mm.

Another drop of collagen mixture was pipetted on to the tissues so that they were completely enclosed within collagen. The collagen was allowed to polymerise by placing the culture plate in a CO^2 incubator for 15 minutes at 37 °C. The culture wells were then filled with growth medium (3% Chick embryo extract in 1x DMEM).

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Growth Cone Collapse Assay:
Growing axons produce hand like structures at their tips known as growth cones. The addition of inhibitory proteins to cultures of dorsal root ganglion or retinal neurons induces a change in the morphology of growth cones from a spread structure to a collapsed stump. Thus, the growth cone collapse assay can be used to detect the presence of these proteins and quantify the amount of inhibition present in a sample.
Membrane proteins need to be purified in the presence of detergents in order to keep them in solution. However, detergents need to be removed before their activity can be tested on growing neurons. This can be achieved either by dialysis or, by ultrafiltration or with the use of Vivapure spin columns. In order to mimic the natural environment of membrane proteins it is useful to incorporate them in to lipid liposomes. Liposomes are obtained by mixing the proteins with a combination of phospholipids (phosphatidylcholine and phospatidylserine) and dialysing them against PBS. The liposome mixture is then added to cultures of growing neurons which are incubated for one hour and then fixed in 4 % paraformaldehyde in PBS, pH 7.4. Growth cone collapse activity is expressed as the percentage of the total number of axons with collapsed growth cones.

Protein Estimation
The protein content of the filtrate and the retentate was measured using the Advanced Protein Assay.

Ultrafiltration
For the concentration of both the adult rat inhibitory membrane and the soluble embryonic lens protein in the Vivacell 250, a membrane with a 100 kDa molecular weight cut-off was used. In separate experiments 250 ml of adult rat membrane protein and soluble embryonic lens protein was poured into the upper chamber of the Vivacell 250. To apply pressure across the membrane, we used nitrogen gas at 4 bars. To keep the active proteins viable, the Vivacell 250 was packed on ice.

Results

Growth Cone Collapse
After concentration of the adult rat brain membrane and soluble embryonic lens proteins, the growth cone collapse activity remains in the retentate of the Vivacell 250 (Table 1).

Table 1.

	Filtrate		Retentate	
Sample Percentage of	Percentage of total number of spread growth cones	Percentage of total number of collapsed growth cones	Percentage of total number of spread growth cones	total number of collapsed growth cones
Adult rat brain inhibitory membrane protein	98	1	5	86
Soluble embryonic lens protein	96	2	6	93
Phosphate buffered saline	99	1	NA	NA
Liposomes only	97	3	NA	NA

NA = Not applicable

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Both the adult rat brain membrane protein and the soluble embryonic lens protein were concentrated 10 fold in the Vivacell 250. The protein concentrations before and after ultrafiltration are shown in Table 2.

Table 2.

Sample	Protein concentration at start of experiment (mg/ml)	Protein concentration mg/ml (Vivacell 250 Retentate)	Protein concentration mg/ml (Vivacell 250 Filtrate)	% Protein recovered
Adult rat brain membrane protein	2.8	21.9	0.6	96
Soluble embryonic lens protein	1.6	5.2	0.7	95

Conclusions

The excellent performance of the Vivacell 250 is reflected in the recovery of active protein. In this respect, most of the active protein is recovered in the retentate with very little loss. Additionally, there are no differences in the performance of the Vivacell 250 when concentrating either soluble or membrane proteins.