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Letter to the Editor

Dot immunobinding assay of high-performance liquid chromatographic fractions on poly(vinylidene difluoride) membranes

Sir,

The presence of immunoreactive peptides in tissue extracts can be demonstrated by radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA) or dot immunobinding assay (DIA). The purification of the immunoreactive peptides is facilitated by the use of high-performance liquid chromatographic (HPLC) techniques. We routinely use DIA in HPLC procedures to identify the peptides because of its great simplicity in comparison with RIA or ELISA. However, many HPLC buffers contain high concentrations of organic solvents which damage the nitrocellulose paper, the most widely used solid support for DIA[1]. We therefore tried a new hydrophobic membrane, made of poly(vinylidene difluoride) (PVDF)[2], as an alternative solid support. We have now made use of the caudodorsal cell hormone (CDCH, MW 4500), a neuropeptide which regulates egg-laying in the mollusc *Lymnaea stagnalis*, to demonstrate the usefulness of the PVDF membrane as a solid support for DIA in HPLC.

An acid extract of cerebral commissures (the neurohaemal storage site of CDCH) was size-fractionated on three high-performance gel permeation columns connected in series (one I_{300} and two I_{125} protein columns from Waters Assoc.) using 7.0 m*M* trifluoroacetic acid (TFA)–acetonitrile (70:30) as running buffer. Fractions of 1 ml were collected and 2 μl per fraction were directly dot blotted on to a PVDF membrane and immunostained using a monoclonal anti-CDCH antibody[3] as described by Batteiger *et al.*[4]. The second antibody was rabbit against mouse peroxidase

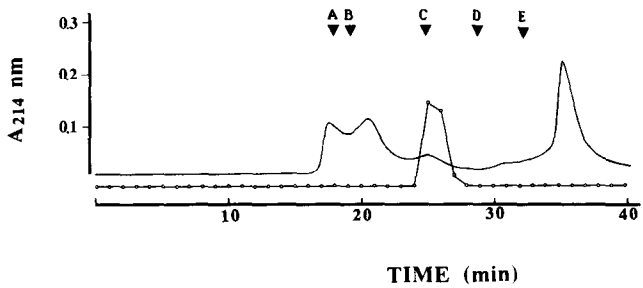

Fig. 1. High-performance gel permeation separation of cerebral commissure extracts. A 2-μl volume of each fraction was dot blotted on to a PVDF membrane, and the absorbance at 214 nm (solid line) and coloured peak areas (open circles) were measured. The molecular weight markers were (A) ovalbumin (43 kDa), (B) myoglobulin (17.8 kDa), (C) synthetic CDCH (4.5 kDa), (D) Glu–Pro–Arg–Leu–Arg–Phe–His–Asp–Val (1.2 kDa) and (E) Arg–Leu–Arg–Ala–Ser (0.6 kDa).

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conjugated antibody from Dako. The assay was developed in diaminobenzidine solution (2 mg per 10 ml of phosphate-buffered saline) containing hydrogen peroxide (2 μl of 30% H_2O_2 per 10 ml of phosphate-buffered saline), which produced coloured spots. After the membrane had been placed in 100% methanol, it was scanned with a densitometer. The coloured spots were then quantified by cutting and weighing. The results are presented in Fig. 1.

We have also used other HPLC buffers for peptide purification, including triethylamine–formate, pyridine–acetate and heptafluorobutyric acid as counter ions in reversed-phase HPLC, and in all instances specific immunostained fractions were demonstrated. We are not aware of any HPLC buffer that is not compatible with the PVDF membrane. This unique property makes the PVDF membrane an ideal solid support for DIA of proteins and peptides separated by HPLC.

Department of Biology, Vrije Universiteit,
De Boelelaan 1087, 1081 HV Amsterdam (The Netherlands)

K. W. LI*
W. P. M. GERAERTS
R. VAN ELK
J. JOOSSE

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