

Small peptide haptens in ELISA: A method for hapten immobilization and improved sensitivity

P. Dagenais and E. Escher

*Department of Pharmacology, Faculty of Medicine, University of Sherbrooke,
Sherbrooke, Quebec, Canada J1H 5N4*

Introduction

Peptide antigens have become increasingly important as diagnostic tools for a wide variety of infections and immune diseases. Enzyme-linked immunosorbent assays (ELISA) of smaller peptides are however often difficult to perform because of poor adherence of small peptides to the commercially available plastic surfaces and therefore, larger peptides (20 AA's and up) are generally preferred as detection antigens. Larger peptides are however composed of several putative epitopes and may hence, be less discriminative as diagnostic tools than single-epitope detection antigens. A possible alternative is the conjugation of small peptides to carrier molecules but it is often difficult to control the specificity of that kind of conjugation [1]. We have developed a new and relatively simple method of stable attachment of small peptides to polystyrene surfaces, material of which most ELISA plates are made of and which permits high sensitivity, stability in storage and even reutilization.

Results and Discussion

We have subjected simple multi-well polystyrene plates to γ -irradiation (^{60}Co 1.1732 MeV) at room temperature and at an average intensity of 38.8 rad/s, up to a cumulative optimal dose of 3.5 Mrad. This treatment was followed by activation with 0.1 M DCC, 0.1 M *N*-hydroxysuccinimide, both dissolved in DMSO and by extensive washings. The peptides were incubated in the activated plates for one night in water. Covalent attachment was evaluated with radioactive (^{125}I) angiotensin II followed by extensive washing with hot 5% SDS, since it has been shown that this peptide had completely detached from ELISA plates during standard ELISA procedures [2]. Immunological tests using this method, CGRP (a 37-peptide) and successively smaller C-terminal fragments of it, showed a very important improvement in sensitivity and especially in stability for the smallest single-epitope fragment (decapeptide). CGRP and its fragments (generous gift of Dr. A. Fournier, Pointe-Claire, Qué.) were plated in conventional manner in carbonate-bicarbonate buffer at pH 9.6 and washed three times with 0.4% Tween 20, 0.1 M phosphate buffer, pH 7.4. Non-specific sites were saturated with reconstituted milk for both plating techniques. With both plating techniques

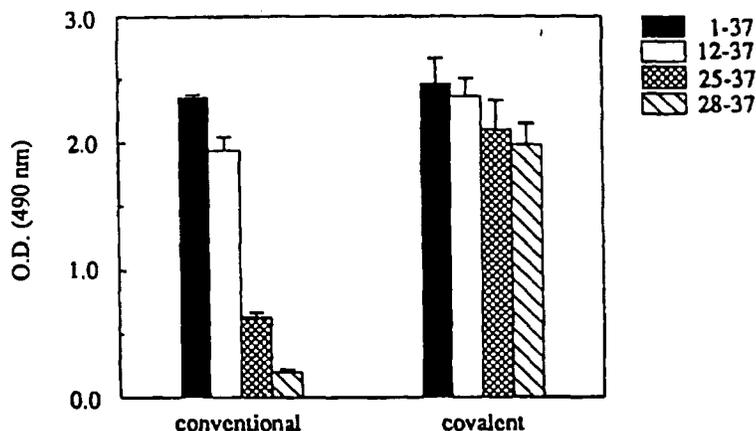


Fig. 1. Influence of peptide size on antigen detection. Polystyrene ELISA plates (Dynatech) were coated by both procedures (conventional and covalent), washed and assayed with anti-CGRP serum (rabbit, polyclonal, Amersham). The plates were then washed with hot SDS and assayed again. OD is the optical density of the ELISA plates after development of the assay coloration. Every point is the mean of at least 4 independent experiments.

peptide 1-37 was equally well recognized. Using the conventional coating technique without activation, the C-terminal decapeptide was only poorly recognized; with our covalent technique, however, the decapeptide was as well recognized as the 37-peptide. Additional washing of the wells with hot 5% SDS did not affect the sensitivity of the covalently attached antigens, regardless of their length; washing of the conventionally coated antigens reduced the sensitivity inversely proportional to the peptide length, the decapeptide being 10-fold less sensitive than the full sequence peptide and the covalently attached decapeptide (Fig. 1). The detection level of covalently coated plates was not reduced considerably even after storage of several month at room temperature.

ELISAs with covalently attached antigen have already been proposed and produced stable and reproducible binding of the antigens to the solid phase, sometimes increasing the sensitivity of the assays [2-5]. The method we developed here is characterized by its simplicity and efficiency; it could become an inexpensive and highly discriminative tool in immunodiagnostics. Such tests may be stored and even re-utilized.

References

1. Briand, J.P., Muller, S. and Van Regenmortel, M.H.V., *J. Immunol. Methods*, 78 (1985) 59.
2. Sondergard-Andersen, J., Lauritzen, E., Lind, K. and Holm, A., *J. Immunol. Methods*, 131 (1990) 99.
3. Rotmans, J.P. and Delwel, H.R., *J. Immunol. Methods*, 57 (1983) 87.
4. Larsson, P.H., Johansson, S.G.O., Hult, A. and Göthe, S., *J. Immunol. Methods*, 98 (1987) 129.
5. Varga, J.M. and Friish, P., *FASEB J.*, 4 (1990) 2671.