Waters
Lab Highlights
An Internal Communication of Applications and Techniques
LAH 0138 12/83
GA/LS/RS/PR/IP
YB 83-0486

LITERATURE CORNER

USE OF PROTEIN-PAK COLUMNS FOR RADIOIMMUNOLOGICAL RESEARCH

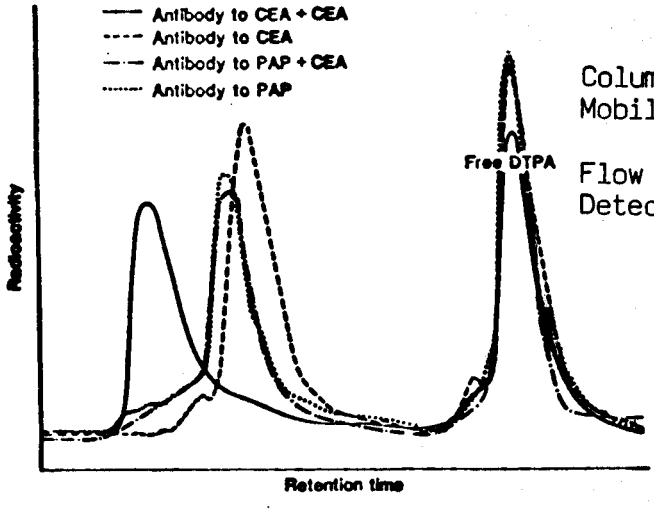
The detection of tumor tissue by radioimmunological methods is receiving considerable attention as a result of hybridoma technology. In this technique, a tumor specific antibody is labelled with ^{131}Iodine and introduced in vivo. The labelled antibody binds to its target tumor forming a typical antigen-antibody complex. By monitoring the ^{131}I emitted gamma radiation, the location and size of the tumor can be identified.

The problems with the use of ^{131}I are poor imaging characteristics, involved labelling and purification procedures, and high degree of in vivo antibody instability. New alternative methods use a strong chelating agent covalently bound to the antibody and various radioactive metal ions.

A group in the Department of Nuclear Medicine at the University of Massachusetts Medical Center has developed a synthetic scheme to covalently bind the strong chelating agent DTPA (diethylenetriamine pentaacetic acid) to antibodies (protein)(1).

They used Waters PROTEIN-PAK I-125 and I-250 columns to evaluate the binding kinetics of DTPA to the antibody as well as the integrity of DTPA antibody-radioactive cation-antigen complex.

The ability of antibody coupled with DTPA to bind to its antigen was investigated with the use of monoclonal antibody to carcinoembryonic antigen (CEA) and as a control a monoclonal antibody to prostatic acid phosphatase (PAP). Figure 1 shows the radioactivity traces obtained by HPLC analysis of ^{111}Indium labelled antibodies to CEA and PAP.



Column: PROTEIN-PAK I-125 and I-250
Mobile Phase: 2.5% Triethylamine and 0.1 M Na_2SO_4, pH 4.5
Flow Rate: 1 ml/min.
Detector: Radioactivity

Waters
Division of MILLIPORE 34 Maple St. / Milford, MA 01757 / 617-478-2000

Jim Krol

All four samples contained free DTPA so that the ^{111}In-labelled free DTPA peak could serve as a marker. The retention time of control antibody to PAP was unchanged by the addition of CEA antigen indicating that no antigen-antibody complex was formed. However, a shift towards shorter retention time, i.e. higher molecular weight, was apparent when CEA antibody was added to CEA antigen indicating the binding of antibody to antigen.

This work demonstrates the stability of the radioactive antigen-antibody complex during HPLC analysis and that the use of size exclusion chromatography is a useful technique to investigate antigen antibody interactions in immunology research.

1. Hnatowich, Layne, Childs, Lanteigne, Davis, Griffin, Doherty, Science, 220, (6 May 83), pp 613-615