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Chapter 22

Applications of Immunochemistry in Crop Protection and Biotechnology

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It is very appropriate that a symposium on immunochemical applications be included in a text on Biotechnology in Crop Protection for a number of reasons. Immunoassays are physical assays yet clearly represent a biotechnology themselves which will play an increasing role in many aspects of crop protection. In addition, immunochemical methods are central to the discovery and analysis of other biotechnology products (1). Rather than repeat other texts in describing how immunochemical applications can be carried out, this symposium was designed to provide a sampling of the great range of immunochemical applications in crop protection today and in the foreseeable future. These applications range from the very practical work of plant diagnostics which is well accepted in many countries, to analysis of classical and genetically engineered pest control agents which has aroused wide interest in industrial and regulatory circles, to the use of immunochemistry in the fundamental research efforts which will lead to new generations of crop protection agents. In many ways it is a pity that immunochemical assays were not extensively utilized in the crop protection field over a decade before. New assay formats have made assays a little easier to use, but the same techniques available in 1970 still provide excellent assays today. However, the final article of this section introduces the concept that there will be a revolution in immunochemical technology as proteins such as antibodies are coupled with solid state electronic and optical devices.

In 1980 an article appeared on the Potential of Immunochemical Technology for Pesticide Residue Analysis (2). As evidenced by the articles in this section, that potential is beginning to be realized. However, of greater importance, we can envision far more applications in the future.

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Misconceptions, Jargon, and Definitions

A common misconception is that one must be an immunologist to utilize immunochemical technology. This is no more true than the assumption that one must be a physicist to use NMR. Like any other biotechnology, however, immunochemical methods usually are surrounded by layer upon layer of jargon. This verbal insulation, in part, is a natural evolution in any field. However, there also is a commercial reason for the confusing jargon in the immunoassay field. A single antibody pool can be used in a number of different assay formats, each with its own relative advantages with regard to sensitivity, speed, ease of use and other parameters. Usually, development of an antibody pool occurs with the use of well established technology so that one does not have patent protection on the antibody. However, it is possible to patent some assay formats. Thus there is a reward in the commercial sector to make one's assay format (whether patented or not) appear as unique as possible.

Certain assay formats offer clear advantages for a particular application, however all immunoassays are based on the reversible but very specific interaction of a protein antibody with an antigen. This interaction can be described by the law of mass action. To deal with the chapters in this section, one only needs the definition of three specialized terms. First, antibodies are a group of serum proteins that react specifically with an antigen. An antigen is a large molecule (usually a protein) which reacts with an antibody and also is capable of inducing antibody production when injected. Finally, haptens are molecules which can react with antibodies, but which are too small to elicit antibody production unless coupled to a protein carrier.

To use immunoassays one normally employs a reporter substance or label. This label can be one of many materials including a radiochemical, a heavy metal, or commonly, an enzyme. A widely used format is the enzyme linked immunosorbent assay or ELISA. This technology is discussed in the chapters by Cheung, Harrison and Van Vuurde. There are numerous variations on a theme as this assay is adjusted to fit a particular analytical need. However, as an example of the complexity of terminology, one can examine the application of the ELISA system in slightly different formats. For instance when the ELISA is carried out on the surface of a nitrocellulose membrane, it has the clever name of dot blot since the end results appear as tiny dots on a piece of paper. If antigens are separated by electrophoresis before the dot blot is run, one has a Western blot. If the antigen is stained *in situ* one might term it enzyme amplified immunohistochemistry even though the biochemical steps are the same in each case. In the commercial sector this same technology has dozens of names. Thus, when one understands the principle of one immunoassay most others are seen as a simple variation on a theme.

Use of Immunoassay in Plant Diagnostics

Immunoassays have been used in research in the plant diagnostic field for over 25 years (3). Kits for the detection of a variety of plant diseases now are appearing on the market, but regulatory

agencies in this country have not placed an emphasis on the use of immunoassays for plant diagnostics or quarantine. As indicated in the article by Van Vuurde and co-workers, immunochemical diagnostics are a major component of the plant protection effort in the Netherlands and in many other European countries. Considering the labor intensive alternatives often in wide use in this country, we certainly could benefit from the experience of our European colleagues with immunodiagnostics. The major impact of immunodiagnostics for plant pathogens in Europe also indicates that the technology is of sufficient maturity to apply to diagnosis of pesticides and to other problems in the crop protection area. A variety of immunochemical diagnostics are used in Europe, but ELISA based assays are of increasing importance. Considering that over 10^6 ELISA's were used in the Netherlands for regulatory detection of plant viruses alone in 1986, this format seems sufficiently mature for it to be used in other crop protection areas. Van Vuurde *et. al.* also bring up a 'low technology' approach to bacterial identification where antibodies are used to enrich a bacterial population before plating. This innovative approach of immunoisolation illustrates another power of antibodies in enriching or purifying a molecule or even an organism by affinity chromatography (4).

Libraries of antibodies are excellent tools for the identification of any organism whether it is a resistant insect, a crop pest, or even the meal of a predator used in biological control. Immunodiagnostic systems are perfect for integration into expert systems for diagnostic applications. Immunodiagnostics and expert systems will greatly improve the quality of routine diagnostic work and will prove a valuable time saver even to the expert. However, immunodiagnostic systems need to be checked by experts familiar with both the pathogen and host. Poorly defined diagnostic kits, especially if not used by trained individuals, could prove misleading as discussed below.

Use of Immunoassay in Pesticide Residue Analysis

The potential of immunochemical technology in pesticide residue analysis has been clear for many years (2), but its actual application is just being realized. The number of laboratories reporting on the technology at the recent IUPAC Congress of Pesticide Chemistry attests to the fact that widespread use of the technology soon will be a reality (5,6,7,8). Of greater importance are the large number of agricultural chemical companies that have major in house efforts. As with HPLC technology only after registrations are granted based on immunoassay will acceptance of the technology become widespread.

Of equal importance are the in house efforts in regulatory agencies in both the classical chemical and biotechnology field. As regulatory agencies develop the in house expertise to evaluate the technology, industry will feel more comfortable in advancing it.

The manuscript by Harrison *et. al.* provides some examples of immunochemical applications to classical residue analysis. It summarizes some of the advantages and limitations of the technology as it applies to the field and provides an outline for development of the technology in house.

Use of Immunoassay in Detection of Genetically Engineered Materials

Most attempts to develop genetically engineered products have concentrated on the production of single gene products which by necessity are peptides and proteins. If these products are to be used in crop protection one needs analytical methods to answer regulatory questions as well as for quality control. There is some question whether one should monitor the gene, the message or the translated product. The answer is simple, at least in early stages of the technology, in that one should have techniques available for all three. Fortunately, the probes for the analytical methods needed probably were already developed during the research leading to the product. A very common way to isolate a message is to use an antibody to the desired protein product to screen an expression library. This screening procedure is another adaptation of the basic ELISA format. For instance, in our laboratory the isolation of the message for insect juvenile hormone esterase by Hanzlik and co-workers was accomplished by immunochemical screening of an expression library (9). The same antibody used to isolate the message can then be used to monitor the protein produced in an expression system as it is developed for pest control. This principle is amply illustrated in numerous chapters of this volume.

A potential problem is that, in an industrial setting, the probes for the analytical method normally would be developed in a molecular biology laboratory. The developers of the technology probably will lack the analytical skills, time and interest needed to reduce immunochemical and hybridization techniques to reliable analytical methods to be used with a variety of matrices. Unless there is a level of appreciation among analytical chemists on how to use these technologies, they are likely to be lost or the analytical duties thrown on molecular biologists rather than placed in an analytical group where they belong. The manuscript by Cheung *et. al.* addresses the problem of immunochemical detection of biologicals using the *Bacillus thuringiensis* species as an example. The exciting developments reported in this text regarding the production of genetically engineered plants as well as microbial insecticides indicate that there will be an immediate need for immunochemical detection of genetically engineered crop protection agents.

Use of Immunoassay in Fundamental Research and Product Discovery

As has been discussed by many workers, we have reached a point of diminishing returns with regard to random discovery of crop protection agents by classical screening methods (10-13). Immunoassays probably will be introduced into the industrial setting for short term goals in biotechnology and analytical chemistry. However, it is likely that the major impact of the technology will be in fundamental research on crop protection.

A number of simplistic applications could result from assays to a group of candidate pesticides early in development. Such assays would be very cost effective in monitoring penetration and translocation. If a molecule is large, there is hope that immunochemical techniques could even be used to localize the compound in the target species and possibly to purify the target.

Longer term work will involve the use of immunochemical probes to explore the comparative biochemistry of a variety of target and nontarget species. Excellent examples of this work are seen in the insect molecular biology section of this book. A clear example has been the use of antibodies raised against mammalian neurohormones as a lead in the isolation of invertebrate hormones. These materials are potential targets to use as insect control agents when produced in inappropriate levels in insects following infection with an appropriate expression vector. The peptide structures also may provide leads for the development of inhibitors of processing enzymes. Industrial research on insect neurohormones is totally justified even if one never develops an insect control agent based on them. Just as probes derived from the structures of mammalian neurohormones have proven useful in work on insect neuroendocrinology (14), invertebrate neurohormones are likely to provide immunochemical and oligonucleic acid probes for use in investigating peptide neurotransmitters in the human central nervous system leading to new generations of pharmaceuticals.

Antibodies to candidate herbicides can aid in translocation studies, and for large molecules can even assist in localizing binding to receptors. Ayers and coworkers in this section provide an excellent illustration of the application of both classical and innovative immunochemical technology in approaching problems with plant disease resistance. Although this work is fundamental, there is a clear path towards exploitation of such research in crop protection. This study clearly illustrates how important immunochemical technology is to the modern biochemist, and analogous examples appear in each section of this text.

Potential of New Assay Formats

Several of the manuscripts in this section demonstrate that even mature immunoassay formats in common use over a decade ago still provide very valuable analytical data. However, it is clear that competing analytical technologies, especially in the mass spectral area, have advanced dramatically. Thus, some of the great advantages over chromatographic assays offered by immunoassay are not as dramatic when compared to modern physical methods as they were previously. In the next decade we will see antibodies and other biological molecules combined with solid state electronic and optical devices in many ways to yield hybrid biological-physical sensors. These so called biosensors are likely to have a dramatic impact on analytical technology.

There can be very simple ways to combine immunochemistry with classical residue methodology. Possibly the simplest example would be to analyze fractions from an HPLC run by immunoassay. However, direct combinations of immunochemical and microelectronic systems offer the possible advantages of real time analysis, greater linearity, and increased sensitivity due to smaller sample sizes. The article by Stanbro *et. al.* explains the operation of one such evolving biosensor technology. This article is exciting in its own right and even more exciting in prophesizing things to come.

Perils of a Successful Technology

Clinical diagnostics have matured to the point where people ask how well an assay performs rather than worrying about the chemical basis of the assay. By contrast in the environmental field there is a concentration on the technique used rather than the results it produces. The environmental field must mature to the point where there are clear criteria for acceptable assay performance regardless of the technology upon which that assay is based.

Immunochemical technology was virtually ignored for many years, but now is being widely advanced as a panacea. Possibly this notoriety is due to pressures on analysts to accept biotechnology approaches or possibly it is our perpetual desire for an easy solution. Recent attempts to legislate the type of assays used by analytical chemists indicate that our field may face a severe problem.

There is no question that immunochemistry will make a major contribution to many aspects of environmental analysis, but there is a severe danger that it will be viewed as a panacea. Many authors have listed the numerous advantages of immunodiagnostics. It is important to realize that some of these advantages may be mutually exclusive. Even though the same antibody pool is used, one format may sacrifice speed for accuracy and another sacrifice some sensitivity for cost. If the concept is advanced that every assay will have all benefits, people will be greatly disappointed with the technology.

As mentioned earlier, immunochemistry is applicable to a great many structures, however, in this case as well, the promise of immunoassay can be oversold. The reversible binding of antibodies to a molecule is based on the summation of a number of weak molecular interactions. Hydrogen bonds are very important since they provide a great deal of binding energy as well as directional and distance specificity. Many of the other bonds formed are even more dependent upon close fit. These binding energies however do indicate that there is a lower limit to the size of the molecule to which one can expect to raise good antibodies. Although it is possible to obtain antibodies to molecules which have only three or four carbons, the likelihood of generating high affinity and very specific antibodies is not great. Since immunoassays must be run in a predominantly aqueous system, lipophicity of the target compound also is important. Solubility of the target compound in an aqueous system seldom is a problem since the compound can be presented in a water soluble cosolvent or as a micelle. However, separating the target compound from a lipophilic matrix can be a nightmare. Such procedures can be particularly intimidating to an immunochemist unfamiliar with handling of lipophilic materials. If one must perform many clean up steps prior to the assay to partially purify a lipophilic compound, then the advantages of immunoassay over chromatographic methods are lost. Thus, highly lipophilic molecules may not be optimum targets for immunoassays. Similarly one should avoid highly symmetrical, water unstable or volatile compounds.

It is not impossible to develop a successful immunoassay to molecules that do not seem to lend themselves to immunoassay. However, as the number of contraindications increase, development

of the assay will become increasingly difficult. One would not want to try assay development for a small, lipophilic, volatile, symmetrical molecule as an introduction to the technology. The field faces a problem in that there is regulatory pressure to apply simplistic immunochemistry to molecules that are better analyzed by other technologies. In many cases we will be able to develop assays for these materials. However, the great relative advantages of immunochemical approaches will be lost.

The development of the technology is at a critical stage in that it should be advanced rapidly, but if it is oversold the technology could be seen as failing in key applications. Possibly the best strategy is to treat immunochemistry as simply another analytical method which must be carefully validated before use.

Commercial Exploitation

If immunochemical technology in crop protection is to reach its full potential, it must be advanced in the commercial as well as other sectors (15). The greatest commercial benefit will be in money saved by large chemical and biotechnology companies, money made by products which reach the market faster thanks to immunochemical support, and new products generated in part with immunochemical methods incorporated into research programs.

Considering only immunodiagnostics, it will be important for companies to enter the field where profits will be tied to sales of immunochemical technologies. The overhead involved with large companies probably will be too great for them to make a realistic profit from agricultural immunodiagnostics in the short term and small companies or subsidiaries will pioneer the field. For such biotechnology companies there are several clear markets. Probably the best market is in providing a technological service to major companies who want in house methods capable of monitoring product quality, worker exposure, waste disposal, and other housekeeping chores. There is an existing small market in end user assays for many compounds, but only those where many users will be involved offer an immediate profit. Examples of this would be in cases where a farmer would need to know herbicide levels before plant back. When assays are approved for routine use in plant certification and residue analysis, large markets for many assays suddenly will appear. Since assay validation is being pursued on several fronts, it is likely that numerous markets will develop in the near future. Especially at an early stage in the acceptance of the technology, it is critical that well characterized assays be developed for clearly defined goals. Immunochemical technology is certain to represent a major market in the plant protection area. However, the speed with which the market develops depends to a great extent on the quality of the assays used to pioneer the technology.

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