

DEVELOPMENT OF POLYCLONAL ANTIBODIES AGAINST CYPERMETHRIC ACID

A.G. DESHMUKH

Correspondence to :
A.G. DESHMUKH
Department of Botany,
Dr. Panjabrao Deshmukh
Krishi Vidyapeeth,
AKOLA (M.S.) INDIA

Accepted : August, 2007

ABSTRACT

The use of immunoassays for analysis of small molecules has been extensive in endocrinology, clinical chemistry and other fields. Pesticides being small molecules are not immunogenic and are known as haptens. However, small molecules such as pesticides when conjugated to higher molecular weight compounds such as proteins are able to generate immune response. The methods generally used for pesticide analysis are HPLC and GC involving extraction of large volumes of water, extensive purification, derivatization, experienced technicians and expensive instruments. Immunoassays have several advantages: high sample throughput, low capital and running costs, excellent specificity and sensitivity, speed, simplicity and applicability. For this work, Cypermethrin, a synthetic pyrethroid was selected. Cypermethric acid, a metabolite of cypermethrin which contains most of the structure of cypermethrin was used as hapten and was coupled to proteins like BSA and tetanus toxoid via 6 aminohexanoic acid as spacer arm to develop immunogenic response.

Key words : Haptens, Cypermethrin, Cypermethric acid, BSA, Tetanus toxoid, Polyclonal antibodies.

Hundreds of different compounds are being used as pesticides. Inherently they show certain degrees of toxicity and especially the less degradable, more persistent compounds pose a problem. Serious problems are encountered with regards to detection methods especially for more polar compounds. The methods generally used for pesticide analysis are HPLC and GC involving extraction of large volumes of water, extensive purification, derivatization, experienced technicians and expensive instruments.

The use of immunoassays for analysis of small molecules has been extensive in endocrinology, clinical chemistry and other fields. Immunoassays for pesticides are the analytical methods, which make the use of antibodies as specific biochemical detector. Pesticides being small molecules are not immunogenic and are known as haptens. However, when conjugated to higher molecular weight compounds such as proteins haptens are able to generate immune response. The perfect hapten contains as much as of the structure of target molecule as possible plus a handle or spacer arm to facilitate recognition of target structure by antibodies. This spacer arm is usually 3-6 carbon atoms long and contains a functional group such as $-NH_2$, $-COOH$, $-SH$, and $-OH$.

Immunoassays have several advantages:

high sample throughput, low capital and running costs, excellent specificity and sensitivity, speed, simplicity and applicability. As a consequence, immunoassay is now recognized as a promising method for screening purposes to reduce sample load for conventional analysis. However the method is subject to specific and nonspecific interferences and requires confirmation by a more definitive method such as GC-MS.

For this work, Cypermethrin, a synthetic pyrethroid was selected. Cypermethrin is used against a wide range of insect pests particularly Lepidoptera in cereals, vegetables and fruits. Cypermethric acid, a metabolite of cypermethrin which contains most of the structure of cypermethrin was used as hapten and was coupled to proteins like BSA and tetanus toxoid via 6 aminohexanoic acid as spacer arm to develop immunogenic response.

MATERIALS AND METHODS

The experiments were carried out at Mumbai University Institute of Chemical Technology, Mumbai. Cypermethrin and cypermethric acid was obtained from Gharada Chemicals, Mumbai as gift sample. Tetanus toxoid was provided by Serum Institute, Poona as gift sample. HRP labeled goat antirabbit antibody, Tetramethylbenzidine/ H_2O_2 and Freund's Complete and incomplete Adjuvant was obtained from Bangalore Geine. TNBS was obtained from Sigma. All other chemicals were obtained from Hi Media and SRL chemicals. The