Asian J. of Bio Sci. (April, 2008) Vol. 3 No. 1 : (115-119)

Estimation of percent conjugation of cypermethric acid hapten to BSA and tetanus toxoid using 2,4,6 trinitrobenzenesulfonic acid method

A.G. DESHMUKH
Biotechnology Centre, Dr Panjabrao Deshmukh Krishi Vidyapeeth, AKOLA (M.S.) INDIA

(Accepted : February, 2008)

The use of immunoassays for analysis of small molecules has been extensive in endocrinology, clinical chemistry and other fields. However, small molecules such as pesticides are not able to induce immunogenic response by themselves but can generate immune response when conjugated to higher molecular weight compounds such as proteins. When the haptens are coupled to proteins the estimation of per cent conjugation is important as higher the concentration of hapten to protein more can be the specificity of developed antibodies. Here 2,4,6, trinitrobenzene sulphonic acid method was used to determine the per cent conjugation. Cypermethric acid, a metabolite of cypermethrin was used as hapten and was coupled to BSA and tetanus toxoid and the per cent conjugation was found to be 40% and 27 %, respectively.

Key words : Cypermethric acid, Hapten, Tetanus toxoid, Per cent conjugation.

INTRODUCTION

The use of immunoassays for analysis of small molecules has been extensive in endocrinology, clinical chemistry and other fields (Christopoulos and Diamondis, 1996). Immunoassays fro 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. The antibodies are produced by immunizing mammals with analyte carrier. For development of a highly sensitive immunoassay, antigen recognition by the antibody is very essential. Many large molecules of higher molecular weight are able to generate immune response and produce specific antibodies. However, small molecules such as pesticides are not able to induce immunogenic response by themselves but can generate immune response when conjugated to higher molecular weight compounds such as proteins (Mellenburg *et al.*, 1995). Thus generation of antibodies to such small molecules becomes easy for the development of their immunoassay which ultimately helps in their analysis at very low concentration. The perfect haptens contains as much as of the structure of target molecule as possible plus a handle to facilitate recognition of target structure by antibodies. This is usually 3-6 carbon atoms long and contains a functional group such as $–NH_2$, -COOH, -SH, and –OH (Harrison *et al.*, 1988).

For this work Cypermethrin, a synthetic parathroid 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 6 aminohexanoic acid as spacer arm.

Protein contains various functional groups (Table 1) at which small molecules can be attached through various methods.

The proteins can be conjugated to haptens by various methods like mixed anhydride, carbodiimide (Sheehan and Hess, 1995), glutaraldehyde (Holzapple *et al.*, 1994), n-hydroxysuccinimide ester (Anderson *et al.*, 1964) method etc. Various methods that can be used to estimate of

Table 1 : Functional groups of carrier proteins for hapten conjugation (Butler, 1977).

S. No.	Functional Groups	Amino acid (s)
1	Amino	Lysine ($-NH_2$ terminal amino acid)
2	Carboxyl	Glutamic acid, Aspartic acid (COOH-terminal amino acid)
3	Phenolic	Tyrosine
4	Imidazo	Histidine
5	Sulphhydril	Cysteine
6	Indolyl	Tryptophan
7	Guanidino	Arginine