NEW TECHNIQUE FOR SYNTHETIC ANTIGEN OF AFLATOXIN

Ch. Junjie
Scientific adviser - R.V. Petrov, professor
Sumy National Agrarian University, Faculty of Veterinary Medicine
40021, Sumy, H.Kondratiieva str., 160
Department of Virology, Pathological Anatomy and Poultry Diseases Professor Panikar I.I.
Tel. +38(066)392-79-28, E-mail: 63409522@qq.com

As we all know, the very small amount of aflatoxin in food will bring a great threat to human health, so in the world trade, a lot of countries have made clear requirements for the minimum amount of aflatoxin in food. Rapid detection of trace aflatoxin has become a research hotspot in food safety field. Among many detection techniques, immunological methods have the advantages of simple operation, short detection time, high accuracy and low detection limit, and the preparation of artificial antigen of aflatoxin and the acquisition of high quality antibodies are the basis of aflatoxin immunological methods.

An innovative aflatoxin artificial antigen preparation technique was investigated during my PhD work. An aflatoxin artificial antigen with a higher molecular weight was synthesized using a combination of chelation (chelating agent with 4 aflatoxin B1 molecules) and coupling (aflatoxin B1 molecule with bovine serum albumin) techniques. In view of the existence of active ketones in molecular structure, artificial antigens were prepared by coupling carrier proteins BSA and OVA with hapten by active ester method and mixed anhydride method. For preparation of universal antigen of aflatoxin protruding mother nucleus, according to the mother nuclear structure of dihydrofuran in the molecules of AFB1, AFB2, AFG1 and AFG2, carboxyl groups of active groups were introduced by oximation method, and carrier proteins BSA (bovine serum albumin), OVA(Ovalbumin) and so on were crosslinked with hapten by EDC[1-ethyl-3-(3-dimethylaminopropyl) carbodiimide] and DCC (N, N-dicyclohexylcarbodiimide) methods. The coupling rate of synthesized antigen was identified by infrared scanning, ultraviolet scanning, protein electrophoresis and mass spectrometry.

Monoclonal antibodies to AFB1, AFB2, AFG1 and AFG2 were prepared, including animal immunity, establishment of hybridoma cell lines, cell fusion, screening, cloning and production of monoclonal antibodies. After that, two kinds of antibodies were mixed to form a mixed universal antibody. Through testing, this artificial antigen has better stability and immunogenicity. Accordingly, the antibody prepared by using the antigen has better sensitivity and specificity. However, the synthetic process of this artificial antigen is complex, and its preparation conditions need to be further optimized.