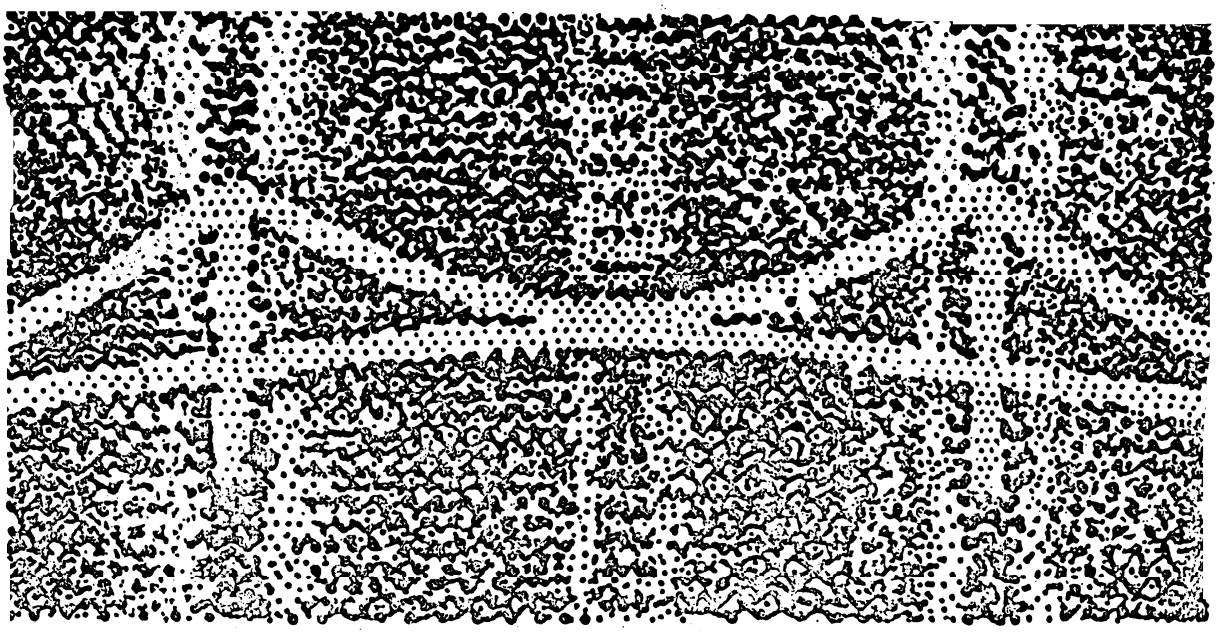
BIOKÉMIA

A MAGYAR BIOKÉMIAI EGYESÜLET TÁJÉKOZTATÓJA
XV. ÉVF. 4. SZÁM 1991. DECEMBER



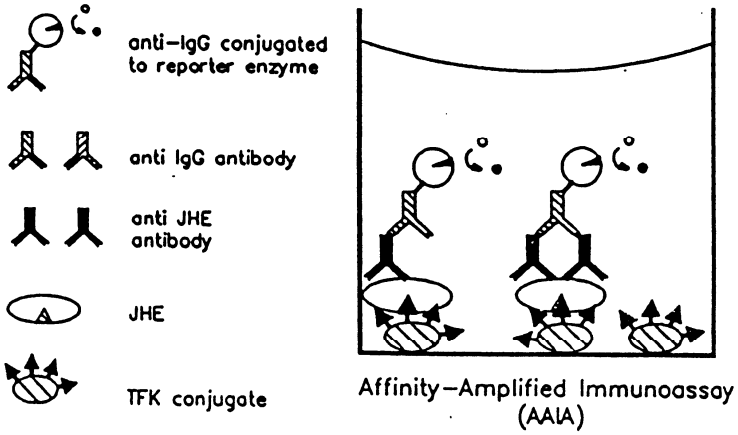
AFFINITY-AMPLIFIED IMMUNOASSAY FOR THE DETECTION OF INSECT JUVENILE HORMONE ESTERASE

András Székács[x+] and Bruce D. Hammock[+]

[x]Departments of Biotechnology and Organic Chemistry, Plant Protection Institute of the Hungarian Academy of Sciences, 1525 Budapest, P.O.Box 102, Hungary.
[+]Departments of Entomology and Environmental Toxicology, University of California, Davis CA 95616, USA

Insect juvenile hormone esterase (JHE) is believed to function as a key element in the metabolism of juvenile hormones (JHs) in several insect species, therefore playing an important role in the regulation of insect metamorphosis [1]. Monitoring its catalytic activity in various tissues of the insect body is equally important for better following the developmental stage of a given insect and for characterizing the enzymatic behavior and possible inhibition. The presently used radiometric partition method [2] to measure JHE activity, based on the hydrolysis of ^{3}H-labeled substrates, is well established and provides reliable information, however, it has several drawbacks for at least two reasons. One, applying radioisotopes, it is relatively expensive and requires caution. Two, it can be used only with tissues containing no other esterases that can cleave the substrate, JH.

Another way to selectively detect JHE is with the use of antibodies. In a collaboration between our laboratories, we targeted to design an immundetection system specific for JHE. In the Davis laboratory, rabbit antibodies have been raised against JHE from several insect species, i.e. *Trichoplusia ni* (cabbage looper), *Manduca sexta* (tobacco hornworm), *Heliothis virescens* (tobacco budworm), *Helicoverpa zea* (corn earworm) and *Bombyx mori* (silkworm) in order to assist the purification and characterization of this enzyme. These antibodies, in enzyme-linked immunosorbent assay (ELISA) or Western blot formats, generally offer selective recognition at peak levels in insect hemolymph, but are not adequate to monitor physiologically interesting low levels of the enzyme present in hemolymph at other developmental stages or in other tissues. In addition, the antibody response does not necessarily measure the enzyme protein in its catalytically active form.



Affinity-Amplified Immunoassay (AAIA)

To ensure the selective binding of only the catalytically active form of the enzyme protein to the solid surface, we decided to develop a new format for ELISA with the use of affinity ligands. Immobilized tight binding inhibitors, i.e. trifluoromethyl ketone sulfides (TFKs), putative transition state mimic inhibitors of JHE [3-8], offer a way to specifically amplify the sensitivity of the antibody

recognition towards the catalytically active enzyme. The assay format, we named **Affinity-Amplified Indirect Immunoassay** (AAIA), is similar in principle to affinity-separation.

For the assay, TFK haptens, *in vitro* inhibitors of JHE, have been bound to various proteins. To synthesize the immobilized (peptide-conjugated) TFKs, four haptenic compounds with thiol and carboxyl functional groups were prepared. Conjugation has been carried out using heterobifunctional coupling reagents (spacers) or the mixed anhydride method. Three proteins, keyhole limpet hemocyanin (KLH) and chicken embryo conalbumin (CONA) were applied as carrier proteins, alkaline phosphatase (AP) and horseradish peroxidase (HRP) enzymes were used as reporter enzymes. The TFK-protein conjugates have been characterized as esterase inhibitors and have been applied in several formats of solid-phase ELISA using specific antibodies raised in rabbits against purified JHE.

The new format of the affinity-amplified immunoassay has been successfully applied to simultaneous separation and monitoring low levels of insect JHE in diluted hemolymph and egg homogenate from several insect species. The new immunoassay system - like an *in situ* affinity chromatography purification - not only increased assay sensitivity for the target esterase but detected it only in its catalytically active form. In the assay, the TFK conjugate forms a tight-bound complex with the enzyme, which is then recognized by the first antibody. A color signal is then created by a second antibody conjugated to an enzyme and its colorimetric substrate added in the last step.

Since the TFK conjugates in the AAIA format retained inhibitory activity towards JHE according to the radiopartition assay, two difficulties associated with using ELISA formats for the detection of JHE in hemolymph can be solved by the use of these conjugates. First, JHE levels in hemolymph are often so low that our current antibodies cannot detect it specifically from other hemolymph proteins present at far greater concentrations. Second, applied alone the JHE antibody may not specifically detect the catalytically active form of JHE. Using the TFK-protein conjugate as coating solves both these problems. Only active JHE binds to the TFK conjugate, other hemolymph components are washed from the plate. This then increases the signal/noise ratio enabling the antibody to specifically detect low levels of active JHE.

This work was supported by Grants ES02170-07, DCB-8518697 and 85-crcr-1-1715 from NIEHS, NSF and USDA, respectively.

References

[1] B.D. Hammock *In:* Comprehensive Insect Physiology, Biochemistry, and Pharmacology, G.A. Kerkut and L. Gilbert, Eds., Pergamon Press, N.Y., pp. 431 (1985).
[2] B.D. Hammock and R.M. Roe *In:* Methods in Enzymology **111**, 487 (1985).
[3] B.D. Hammock, Y.A.I. Abdel-Aal, C.A. Mullin, T.N. Hanzlik and R.M. Roe *Pestic. Biochem. Physiol.* **22**, 209 (1984).
[4] R.J. Linderman, J. Leazer, K. Venkatesh and R.M. Roe *Pestic. Biochem. Physiol.* **29**, 266 (1987).
[5] A. Székács, B.D. Hammock, Y.A.I. Abdel-Aal, P.P. Halarnkar, M.L. Philpott and Gy. Matolcsy *Pestic. Biochem. Physiol.* **33**, 112 (1989).
[6] A. Székács, B. Bordás, Gy. Matolcsy and B.D. Hammock *In:* Probing Bioactive Mechanisms, P. Magee, J. Block, and D. Henry, Eds., American Chemical Society Symp. Ser. **413**, pp. 169 (1989).
[7] A. Székács, P.P. Halarnkar, M.M. Olmstead, K.A. Prag and B.D. Hammock *Chem. Res. Tox.* **3**, 325 (1990).
[8] T.C. Thomas, A. Székács, S. Rojas, B.D. Hammock, B.W. Wilson and M.G. McNamee *Biochem. Pharm.* **40**, 2587 (1990).