

Etapa I : Obținerea conjugatelor imunogene pesticid-proteina și anticorpi antipesticid

Termen: 31.12.2012

Activitate I.1.: Obținerea conjugatelor imunogene 2,4-D-hemocianina, 2,4-D-ovalbumina și 2,4-D-albumina serică de bovină, **CO-IFIN-HH**

Activitate I.2.: Caracterizarea fizico-chimică a conjugatelor imunogene 2,4-D-hemocianina, 2,4-D-ovalbumina și 2,4-D-albumina serică de bovină, **P1-UAIC**

Activitate I.3.: Imunizarea și obținerea anticorpilor anti 2,4-D, separarea imunoglobulinei specifice anti 2,4-D și caracterizarea imunologică și imunochimică a anticorpilor anti acid 2,4-diclorofenoxiacetic, **CO-IFIN-HH**

Rezultate obținute în cadrul primei etape:

1. Stabilirea procedurilor de obținere a conjugatelor imunogene 2,4-D-hemocianina, 2,4-D-ovalbumina și 2,4-D-albumina serică de bovină utilizând ca reactivi de bază pesticidul acid 2,4-diclorofenoxiacetic și proteinele albumina serică de bovină, ovalbumina și hemocianina.

2. Caracterizarea conjugatelor imunogene purificate din punct de vedere al numărului de rezidii pesticidice cuplate la o moleculă de proteină prin metoda diferențelor spectrale. Astfel numărul de rezidii pesticidice cuplate la proteina pentru cele trei conjugate au fost în cazul albuminei serice de bovină de 12,6, 24,5 și 34,0, pentru ovalbumina de 22,4 iar la hemocianina de 67.

3. Imunizarea iepurilor din rasa Noua Zeelanda cu cele trei conjugate imunogene obținute compuse din antigenul pesticid-proteina și adjuvant Freund complet sau incomplet în vederea obținerii anticorpilor anti acid 2,4-diclorofenoxiacetic.

4. Sintetizarea markerului enzimatic 2,4-D-ASB-peroxidaza, marker necesar testării anticorpilor anti 2,4-D.

5. Testarea preliminară după cea de-a 5-a imunizare a anticorpilor pentru a detecta prezența acestora cu ajutorul markerului enzimatic 2,4-D-ASB-peroxidaza, compus component al tehnicii ELISA.

6. Depunerea cererii de brevet de invenție național nr. OSIM A / 00869 / 26.11.2012., „Procedeu de obținere a markerului enzimatic acid 2,4-diclorofenoxiacetic-hexametilendiamin-peroxidaza”, autori: Dorobantu Ioan, Neagu Livia.

Articole:

1. Luiza Buimaga-Iarinca, Adrian Calborean, Electronic structure of the II-cysteine dimers adsorbed on Au(111): a density functional theory study, *PHYSICA SCRIPTA*, 86, 035707 (6 pp), 2012;

2. Alina Asandei, Irina Schiopu, Sorana Iftemi, Loredana Mereuta, Tudor Luchian, Distinct conformational effects induced by Cu²⁺ binding to human and rat amyloid fragments (1-16), probed at uni-molecular level with a protein nanopore, *Analyst*, in revision, 2013;

3. Loredana Mereuta, Mahua Roy, Alina Asandei, Yoonkyung Park, Jong Kook Lee, Ioan Andricioaei, Tudor Luchian, Single-molecule tracing of the pH-controlled, sequential binding of peptides to the inner space of model protein nanopores, *Nature-Physics*, in preparation, 2013.

Cereri de brevet:

1. Cerere de brevet de invenție național nr. OSIM A / 00869 / 26.11.2012, „Procedeu de obținere a markerului enzimatic acid 2,4-diclorofenoxiacetic-hexametilendiamin-peroxidaza”, autori: Dorobantu Ioan, Neagu Livia.

Phase I: Obtainment of immunogenic conjugates pesticide-protein and antipesticide antibodies

Deadline: 31.12.2012

Activity I.1. Obtaining of immunogenic conjugates 2,4-D-hemocyanin, 2,4-D-ovalbumin and 2,4-D-bovine serum albumine, CO-IFIN-HH

Activity I.2. Physico-chemical characterization of immunogenic conjugates 2,4-D-hemocyanin, 2,4-D-ovalbumin and 2,4-D-bovine serum albumine, P1-UAIC

Activity I.3. Immunization and the obtainment of anti 2,4-D antibodies, separation of specific anti 2,4-D immunoglobulin and immunological and immunochemical characterization of anti 2,4-dichlorophenoxyacetic acid antibodies, CO-IFIN-HH

Obtained results

1. Establishing the obtaining procedures of the immunogenic conjugates 2,4-D-hemocyanin, 2,4-D-ovalbumin and 2,4-D-bovine serum albumine using 2,4-dichlorophenoxyacetic acid and the proteins bovine serum albumine, ovalbumin and hemocyanin as the main reagents.

2. The characterization of the purified immunogenic conjugates from the point of view of the number of the pesticide residues coupled to a molecule of protein by spectral differences method. Thus the calculated number of the pesticide residues coupled to protein for the three synthesized conjugates was 12,6, 24,5 and 34,0 for bovine serum albumine, 22,4 for ovalbumine and 67 for hemocyanin.

3. Immunization of the New Zealand rabbits with the three synthesized immunogenic conjugates composed from pesticide-protein antigen and complete or incomplete Freund adjuvant in order to obtain the antibodies anti 2,4-dichlorophenoxyacetic acid.

4. Obtainment of the enzymatic marker 2,4-D-BSA-peroxidase necessary for the testing of the anti 2,4-D antibodies.

5. After the fifth immunization procedure the antipesticides antibodies were tested for the detection of their presence using the enzymatic marker 2,4-D-BSA-peroxidase, constituent reagent of ELISA technique.

6. It was registered one national patent application no. OSIM A / 00869 / 26.11.2012., entitled „Procedure of obtainment of the enzymatic marker 2,4-dichlorophenoxyacetic acid-hexamethylendiamin-peroxidase”, authors: Dorobanțu Ioan, Neagu Livia.

Articles:

1. Luiza Buimaga-Iarinca, Adrian Calborean, Electronic structure of the lI-cysteine dimers adsorbed on Au(111): a density functional theory study, *PHYSICA SCRIPTA*, 86, 035707 (6 pp), 2012;

2. Alina Asandei, Irina Schiopu, Sorana Iftemi, Loredana Mereuta, Tudor Luchian, Distinct conformational effects induced by Cu²⁺ binding to human and rat amyloid fragments (1-16), probed at uni-molecular level with a protein nanopore, *Analyst*, in revision, 2013;

3. Loredana Mereuta, Mahua Roy, AlinaAsandei, Yoonkyung Park, Jong Kook Lee, Ioan Andricioaei, Tudor Luchian, Single-molecule tracing of the pH-controlled, sequential binding of peptides to the inner space of model protein nanopores, *Nature-Physics*, in preparation, 2013.

National patent application

1. National patent application no. OSIM A / 00869 / 26.11.2012, „Procedure of obtainment of the enzymatic marker 2,4-dichlorophenoxyacetic acid-hexamethylendiamin-peroxidase”, authors: Dorobanțu Ioan, Neagu Livia.