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DNA INSECTICIDES VERSUS DNA STIMULATORS: EVERY DRUG IS A POISON, EVERY POISON IS A DRUG

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The basic idea of our work is that exogenous DNA fragments, which coincide with sequence of DNA of the testing cell, should influence its biochemical reactions because they bear some of the information about management of the cell. We used two short single strand fragments of Lymantria dispar multiple nucleocapsid polyhedrosis virus iap3 gene as testing DNA fragments and Drosophila melanogaster larvae as a model object for the experiments. We have found that the same DNA fragments may act as DNA insecticides ($\chi 2 = 4,87$; d. f. = 1; $P < 0,05$) in 100 pmol/mcl concentration and as DNA stimulators ($\chi 2 = 7,99$; d. f. = 1; $P < 0,01$) in 20 pmol/mcl concentration, decreasing mortality of the insect,. In our opinion, the DNA fragments showed insecticidal activity due to mechanisms similar to DNA and RNA interference. These mechanisms are based on blocking of target genes that coincide by sequence with the used DNA and RNA fragments. In our case, mortality increased probably due to activation of cell apoptosis. Using the virus DNA fragments as specific primers for PCR, we have found that there are many target areas in the Drosophila melanogaster's genome that are similar to them. The nature of acting DNA fragments as DNA stimulators is unknown and requires further investigation. The data obtained may serve as a base for creation of DNA insecticides against agricultural and forest pests, and DNA drugs
Keywords: DNA stimulators, DNA insecticides, gene inhibitor of apoptosis, *Drosophila melanogaster*.

INTRODUCTION

The idea of using DNA fragments as DNA insecticides or DNA medicines is quite new and perspective since DNA was recognized as the most important molecule for control of cell life. Such phenomena as the change or destruction of a cell DNA, penetration of foreign DNA into a cell always affect the further development of the organism. Examples of this are epigenetic phenomena, apoptosis, cancer caused by oncogenic viruses *etc*. One of the possible ways, in which DNA fragments may influence a cell, is the DNA interference.

Researches, studying the DNA and RNA interference [1, 2], have persuasively shown that the application of exogenous double strand DNA fragments and single or double strand RNA fragments (20-30 nucleotides long) of an organism cause a specific inhibition of those genes which coincide with the sequence of the used fragments. Several dozen of double strand RNA molecules can lead to the degradation of several thousand molecules of the target RNA. Fine details of this process are not studied enough. It is believed that RNA interference is a protective mechanism that protects the cell from RNA viruses and mobile genetic elements. There are few research works on DNA interference at the moment. It should be added that there is no available data in scientific literature about

researches which test the influence of single strand DNA fragments that are expected to act according to the DNA interference mechanism.

Baculoviruses have two classes of antiapoptosis genes, iap and p35 genes that can block apoptosis from in a phylogenetically wide range of organisms [3]. These genes help baculovirus to deal with premature death of the infected cells, shifting cell biochemical reactions toward antiapoptosis. It is known, that antiapoptosis genes are very conservative and homologous in baculoviruses, worms, insects, humans [4]. We presupposed that short single strand fragments of viral antiapoptosis genes may affect target organisms as DNA insecticides or DNA stimulators since they play a very important role in the virus-host relationship. Thus, viral iap gene fragments are supposed to cause changes (interfere) in the biochemical reactions of the insect cells (for example, reactions similar to RNA and DNA interference). The consequence of the external application of viral iap gene fragments on *Drosophila melanogaster* larvae is the goal of our paper.

MATERIALS AND METHODS

We used two short single strand fragments of *Lymantria dispar* multiple nucleocapsid polyhedrosis virus iap3 gene as a testing DNA fragments and *Drosophila melanogaster* larvae as a model object for the experiments.

DNA fragments were designed according to the viral genome sequence found in ICTVdb and then synthesized by metabion international AG (Germany) with HPLC clearance. The sequences of these 2 single chain DNA fragments were the following: a) 5′- GCC GGC GGA ACT GGC CCA -3′ (134843-134860; antisense chain); b) 5′-CGA CGT GGT GGC ACG GCG -3′ (135159-135142; sense chain). The used DNA fragments are the part of iap3 gene which belongs to genes that inhibit apoptosis of host cells [5].

Single strand DNA molecules have both hydrophilic (sugar-phosphate backbone) and hydrophobic (bases) parts that allow them to penetrate through the polar and non-polar obstacles of insect tissues, and this, for instance has been shown for organophosphorous insecticides [6].

Influence of aqueous solutions of DNA fragments was tested on 1-2 day old larvae of *D. melanogaster* (*ebony* and *white* phenotypes). Larvae of *ebony* phenotype were divided into three groups (25-45 specimens per group). The first group was treated with iap3 DNA fragments (100 pmol/mcl; water solution), the second was treated with random DNA fragment (TGCGCAGCCC (olig 35); SibEnzyme, Russia; 4.3 OU/ml), and the third was treated with distilled water. The *white phenotype* larvae were divided into four groups. Three of them were the same as in *ebony* phenotype experiments, and the forth group was treated with iap3 DNA fragments in a 20 pmol/mcl concentration. Each larva, after 20-minutes exposure in a solution, was placed in a corresponding vial and reared at laboratory conditions till death or imago emerged. In each of the vials we counted number of individuals who had reached the stage of imago. Pearson's χ^2-criteria were calculated to compare viabilities of the specimens among the variants.

PCR method with specific primers was used to find whether the tested DNA fragments are homologous to the antiapoptosis genes found in the *Drosophila*

melanogaster's genome. Extraction of total DNA for PCR was performed according to standard methods using [7] the kit "DNA- Sorb A" (AmpliSens, Moscow). DNA amplification was performed on thermocycler "Tercyc" (DNA Technology, Russia) using reagents for polymerase chain reaction "AmpliSens-200-1" (AmpliSens, Moscow). We used the tested fragments of viral iap3 gene as specific primers for PCR.

RESULTS AND DISCUSSION

Experiments with *ebony* phenotype of *Drosophila melanogaster*

Results of the experiments with the *ebony* phenotype of *D. melanogaster* are presented in Fig.1. A statistically significant difference in viability has been found when comparing only groups treated with water and iap3 gene DNA solution (χ^2 = 4,87; d.f.=1; P <0,05). Application of random DNA fragment olig 35 did not result in significant difference of mortality range with both "water" and "iap (100)" groups. Thus, viral iap3 gene fragments served as DNA insecticide for *ebony* phenotype individuals of *D. melanogaster*.

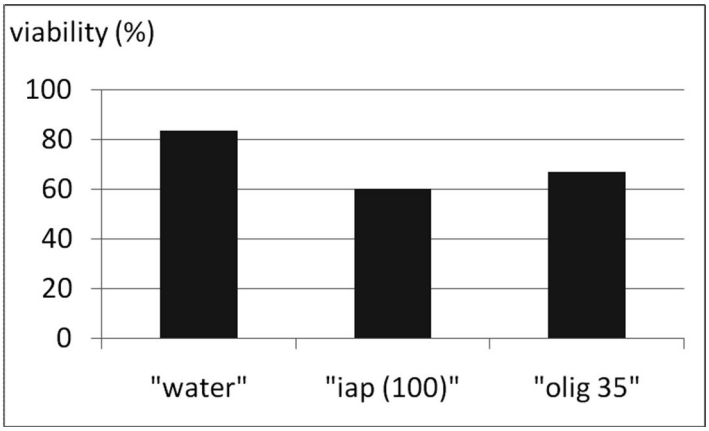

Fig. 1. Viability of the *ebony* phenotype of *D. melanogaster* (from two day old larvae to imago) in three variants of treatment; "iap (100)" – iap gene DNA in 100 pmol/mcl concentration.

Application of PCR procedure with iap3 gene DNA fragments as primers allowed to find fragments of the *D. melanogaster* genome that are similar to the viral iap3 gene.

From 6 to 10 DNA fragments were found in each of the individual spectra of the flies. Each of the individual amplicon sets from the genomes of the *ebony* phenotype flies contains DNA fragments homologous to primers originating from viral iap3 gene (fig. 2). These results can explain the influence of viral DNA fragments on fly cells according to the mechanism of DNA interference or another similar process.

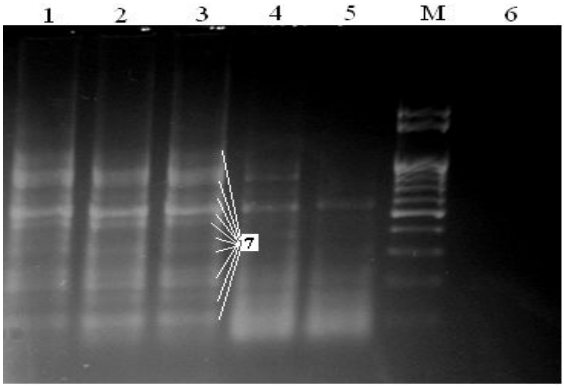

Fig. 2. Electrophoregram of DNA amplification products (with iap3 gene DNA fragments as primers) of the *ebony*-phenotype flies: 1-5 – electrophoretic DNA spectra of individuals; M – marker of DNA length from 100 to 1000 bp with a step of 100 bp and from 1000 to 3000 bp with a step of 1000 bp (from bottom to top); 6 – control; 7 – individual set of amplicons.

D. melanogaster larvae, which died in the experimental group, were morphologically different from those that died in the control group (fig. 3). Dead insect larvae from experimental groups were black and dead larvae from the control group were of normal white color.

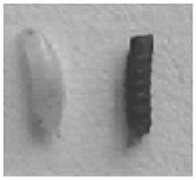

Fig. 3. Perished larvae of *D. melanogaster* from the control (a) and experimental (b) groups.

Blackening of the body began with the rear end of the larvae (fig.4).

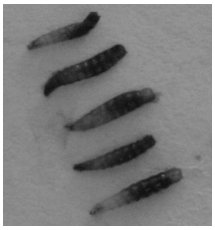

Fig. 4. Early changes in body color of individuals from the experimental group.

After extraction of total DNA from tissues of individuals, died in experimental group, and electrophoresis we have found fragmented DNA (fig. 4; 1) that might be a pattern of cell apoptosis. At the same time, DNA from control flies did not show any fragmentation (fig. 5; 2).

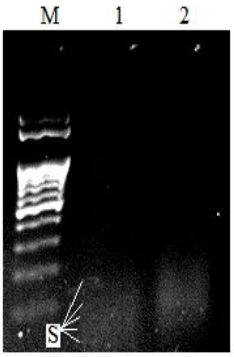

Fig. 5. DNA isolated from perished individuals of experimental (1) and control (2) groups; M – molecular weight marker of DNA length from 100 to 1000 bp long with a step of 100 bp long and from 1000 to 3000 bp long with a step of 1000 bp long (from bottom to top); S – fragmented DNA.

Fragmented DNA from those treated with iap3 gene DNA flies consists of 5 DNA fragments approximately from 50 to 180 bp in their lengths. It is known that the DNA fragments, which characterize apoptosis, are usually of 180 bp or greater lengths, but multiple of 180 bp. In our opinion, lack of DNA fragments of greater lengths may be explained by the fact that apoptosis was caught at later stage when DNA fragments already were deeply degraded by nucleases [8]. The results suggest that apoptosis was probably the cause of the cell death of individuals in the experimental group. We also can not exclude the role of necrosis in this phenomenon.

It is known that inside a cell there are proteins that increase and proteins that decrease the speed of apoptosis. Very often the result of their influence depends on their relative concentration [9]. In our opinion this process can be presented in the form of apoptosis-antiapoptosis «scales». Different factors influence this binary process, and as a result apoptosis-antiapoptosis «scales» bend down to the one or the another side. It lasts to a certain critical mark after which the signal to apoptosis or antiapotosis becomes unchangeable (for antiapoptosis only temporary). The virus DNA fragments displace the biochemical reactions in the cell towards apoptosis and herein, apparently, the main role plays the phenomenon of the DNA interference or a process similar to it. The DNA interference blocks the synthesis of the antiapoptosis proteins, which results in the apoptosis of separate cells, and in a number of cases – death of an organism.

The found effect of the viral DNA fragments on its host can be used for the creation of selective fast-acting insecticides to protect plants from pest insects [10; 11].

Experiments with *white* phenotype of *Drosophila melanogaster*

Results of the experiments with the *white* phenotype of *D. melanogaster* are presented in Fig. 6.

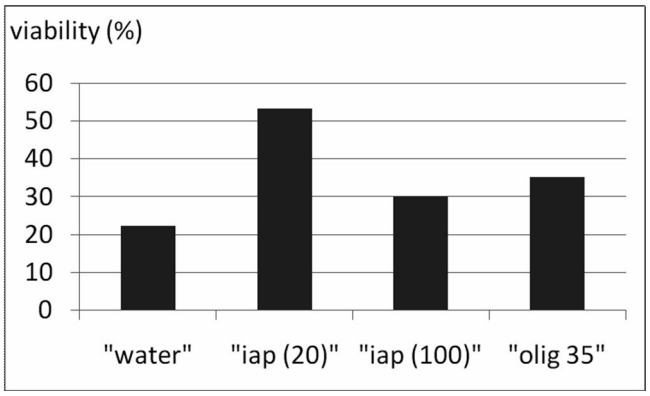

Fig. 6. Viability of the *white* phenotype of *D. melanogaster* (from two day old larvae to imago) in four variants of treatment; "iap (20)" and "iap (100)" – iap3 gene DNA in 20 and 100 pmol/mcl concentrations correspondingly.

Flies from the experimental group, which were treated with iap3 gene DNA fragments in concentration 20 pmol/mcl, significantly differed in viability from control individuals, treated with distilled water ($\chi^2 = 7{,}99$; d. f. = 1; $P < 0{,}01$), while there were no differences among other variants of treatments and the control. Thus, *D. melanogaster* individuals of *white* phenotype were quite weak by nature and the viral iap3 gene fragments served as DNA stimulator for them.

Application of PCR procedure with iap3 gene DNA fragments as primers has shown that genome of the *white* phenotype flies contains only two DNA fragments homologous to the viral iap3 gene DNA fragments (fig. 7).

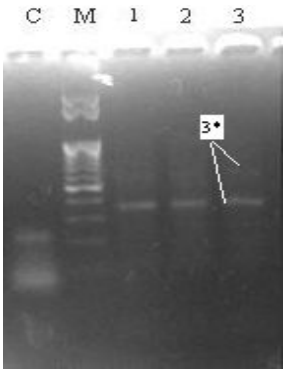

Fig. 7. Electrophoregram of DNA amplification products (with iap3 gene DNA fragments as primers) from the *white* phenotype of *D. melanogaster* flies: 1-3 – electrophoretic DNA spectra of individuals; M – marker of DNA length from 100 to 1000 bp with a step of 100 bp and from 1000 to 3000 bp with a step of 1000 bp (from bottom to top); C – control; 3* – individual set of amplicons.

The results of our experiments show that the response in *D. melanogaster* to external treatment with exogenous DNA depends both on the insect phenotype and on the DNA fragments concentration. Fragments of the viral iap3 gene, being applied on *D. melanogaster* larvae, promote mortality of the *ebony* phenotype flies in 100 pmol/mcl concentration, and stimulate viability of *white* phenotype flies in 20 pmol/mcl concentration. The gene fragments found many homologous sites in the genomes of *ebony* phenotype flies, and increase in their mortality probably due to DNA interference, blocking anti-apoptosis genes of the insect and causing apoptosis. The same viral iap3 fragments allowed to find only two homologous sites in the genomes of *white* phenotype flies. Only low concentration of the gene fragments stimulate viability of the *white* phenotype flies, and the mechanism of the found effect is not clear yet.

The investigated effect of the viral DNA fragments on *Drosophila melanogaster* can be used for the creation of selective fast-acting DNA insecticides, DNA stimulators and DNA medicines. Each living organism has its unique genome and thus has its unique genetic based diseases. DNA drugs may solve this problem by blocking the genes which do not work properly. DNA insecticides may become an effective means of pest control.

CONCLUSIONS

1. Processing of *Drosophila melanogaster* larvae (*ebony* phenotype) with solution containing two short single strand DNA fragments in 100 pmol/mcl concentration causes increased mortality of insects ($P < 0,05$). Thus, fragments of the *Lymantria dispar* multiple nucleocapsid polyhedrosis virus iap3 gene act as DNA insecticides by leading insect cells to death and the main role in this is probably played by apoptosis.
2. Processing of *Drosophila melanogaster* larvae (*white* phenotype) with solution containing two short single strand DNA fragments in 20 pmol/mcl concentration causes increased viability of insects ($P < 0,01$). Thus, fragments of the *Lymantria dispar* multiple nucleocapsid polyhedrosis virus iap3 gene can act as DNA stimulators. The nature of acting DNA fragments as DNA stimulators is unknown and needs further investigation.

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Оберемок В.В. ДНК-инсектициды против ДНК-стимуляторов: каждое лекарство есть яд, каждый яд есть лекарство / В.В. Оберемок, А.С. Зайцев, А.П. Симчук // Ученые записки Таврического национального университета им. В.И. Вернадского. Серия «Биология, химия». – 2011. – Т. 24 (63), № 1. – С.136-143

Основной идеей нашей работы является то, что экзогенные ДНК-фрагменты, которые совпадают с последовательностью ДНК тестируемой клетки, должны влиять на её биохимические реакции, потому что они несут некоторую информацию об управлении клеткой. Мы использовали два коротких одноцепочечных ДНК-фрагмента iap3 гена вируса ядерного полиэдроза непарного шелкопряда и личинок *Drosophila melanogaster* в качестве модельного объекта для экспериментов. Мы обнаружили, что ДНК-фрагменты могут выступать в роли ДНК-инсектицидов ($\chi 2=4,87$; d.f.=1; $P<0,05$) в концентрации 100 пмоль/мкл, а также в роли ДНК-стимуляторов ($\chi 2=7,99$; d.f.=1; $P<0,01$) в концентрации 20 пмоль/мкл, снижая смертность насекомых. По нашему мнению, ДНК-фрагменты проявили инсектицидную активность в связи с механизмами, схожими с ДНК и РНК-интерференцией. Эти механизмы основаны на блокировке генов-мишеней, которые совпадают по последовательности с использованными фрагментами ДНК и РНК. В нашем случае смертность увеличилась, вероятно, за счет активации клеточного апоптоза. При использовании ДНК-фрагментов вируса в роли специфических праймеров для ПЦР мы обнаружили много спектров в геноме *Drosophila melanogaster*, схожих с ними. Причина действия ДНК-фрагментов в качестве ДНК-стимуляторов неизвестна и требует дальнейшего изучения. Полученные данные могут служить основой для создания ДНК-инсектицидов против сельскохозяйственных и лесных вредителей, а также ДНК-лекарств.
Ключевые слова: ДНК-стимуляторы, ДНК-инсектициды, ген-ингибитор апоптоза, *Drosophila melanogaster*.

Оберемок В.В. ДНК-інсектициди проти ДНК-стимуляторів: кожні ліки є отрута, кожна отрута є ліки / В.В. Оберемок, О.С. Зайцев, А.П. Сімчук // Вчені записки Таврійського національного університету ім.В.І. Вернадського. Серія „Біологія, хімія". – 2011. – Т. 24 (63), № 1. – С. 136-143.

Основною думкою нашої роботи є те, що екзогенні ДНК-фрагменти, які збігаються з послідовністю ДНК тестованої клітини, повинні впливати на її біохімічні реакції, тому що вони несуть певну інформацію про управління клітиною. Ми використали два коротких одноланцюгових ДНК-фрагменти iap3 гена вірусу ядерного поліедрозу непарного шовкопряда і личинок *Drosophila melanogaster* в якості модельного об'єкту для експеременті. Ми виявили, що ДНК-фрагменти можуть виступати в якості ДНК-інсектицидів ($\chi 2=4,87$; d.f.=1; $P<0,05$) в концентрації 100 пмоль/мкл, а також як ДНК-стимулятори ($\chi 2=7,99$; d.f.=1; $P<0,01$) у концентрації 20 пмоль/мкл, знижуючи смертність комах. На нашу думку, ДНК-фрагменти виявили інсектицидну активність у зв'язку з механізмами, схожими на ДНК та РНК-інтерференцію. Вони грунтуються на блокуванні генів-мішеней, які збігаються з послідовністю використаних фрагментів ДНК та РНК. У нашому випадку смертність збільшилась, ймовірно, за рахунок активації клітинного апоптозу. Використання ДНК-фрагментів вірусу в якості специфічних праймерів для ПЛР допомогло знайти багато спектрів в геномі *Drosophila melanogaster*, подібних до них. Характер дії фрагментів ДНК у якості ДНК-стимуляторів невідомий і вимагає подальшого дослідження. Отримані дані можуть бути основою для створення ДНК-інсектицидів проти сільськогосподарських та лісових шкідників та ДНК-ліків.
Ключові слова: ДНК-стимулятори, ДНК-інсектициди, ген-інгібітор апоптозу, *Drosophila melanogaster*.

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