

COMPARISON OF ^{14}C 2,4-D AND ^{14}C FLT UPTAKE BY TOBACCO CELL SUSPENSION BY-2

SROVNÁNÍ PŘÍJMŮ ^{14}C 2,4-D A ^{14}C FLT BUNĚČNOU SUSPENZÍ TABÁKU BY-2

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ABSTRACT

The aim of this work was to compare uptake and accumulation of 2,4-dichlorophenoxyacetic acid, a substrate necessary for division and growth of cells of BY-2 suspension with uptake and accumulation of fluoranthene, a toxic substance, which on the contrary negatively influences the cell division. It was observed the kinetics of uptake of both xenobiotics labeled with ^{14}C carbon isotope (1 μM 2,4-D, 1 μM FLT), their metabolism and distribution in cells. The cells of suspension and medium were separated, fractionated ^{14}C activity of cells derivable from both separate marked substances to activity of protoplast and apoplast. For examination of viability of cells after mentioned expositions was carried out standard cultivation of suspension. 2,4-D was accumulated in protoplasts of cells, slight deposition of ^{14}C activity was detected for apoplast. [^{14}C]FLT was incorporated into the protoplast and slightly metabolized in comparison with [^{14}C]2,4-D. The difference in the uptake kinetics, metabolism and accumulation of used xenobiotics in the protoplast can be reached to the toxicity of this substances; it had stood that the fluoranthene was uptaken quicker than 2,4-D.

Key words: fluoranthene, 2,4-dichlorophenoxyacetic acid, ^{14}C activity, protoplast, apoplast, toxicity, uptake

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INTRODUCTION

Uptake of particular substances and their distribution in cell are realized on physico-chemical interactions of particular molecule and biological membrane. Resulting of these interactions are translocation mechanisms, which are important for saturation of biological processes. On protoplasts and cellular suspensions were in two latest centuries elucidated the mechanism of auxin transport throughout cell membranes. (Delbarre et al. 1996, Zažímalová and Petrášek 2000, Hoyerová et al. 2008); this transport determines realization of much physiological processes in plants. Plant cell suspensions are good models for observing substance uptake and kinetic of translocation mechanisms. For a very long time is used tobacco cell suspension BY-2 (Nagata et al. 1992). For cultivation and cell division of BY-2 suspension is necessary to add synthetic auxin 2,4-dichlorophenoxyacetic acid into the medium. Since its uptake mechanism is known, is using ^{14}C -2,4-D appropriate for comparison with ^{14}C -FLT uptake. Fluoranthene is polycyclic aromatic hydrocarbon, toxic for organisms; it's influencing above all bioenergetics processes and cell division. However the dynamics of fluoranthene uptake and its accumulation in plant cells haven't yet been elucidated. The aim of the work was to determine kinetics of uptake of ^{14}C -2,4-D and ^{14}C -FLT, distribution and accumulation of ^{14}C -activity in apoplast and protoplast and bioconcentration factors of substances (BCFs).

MATERIAL AND METHODS

Fractionation of ^{14}C -activity in apoplast and protoplast was performed by Briskin *et al.* (1987). At first, the BY-2 suspension was filtered throughout big sinter. Then was applied the radioactive chemicals. We incubated BY-2 suspension in 2 radioactive chemicals - ^{14}C -2,4-D (specific activity 15,7 mCi/mmol, radioactive concentration 250 kBq/ml) and ^{14}C -FLT (specific activity 50 mCi/mmol, radioactive concentration 100 kBq/ml); there was 8 intervals of suspension output - 1, 2, 3, 5, 7, 9, 12 and 15 minutes. These intervals of time caused different uptake and final content of measured activity. After the incubation the cells and medium were separated by underpressure from -20 to -40 kPa on dorcus (vacuum separator). The medium and filtration papers were than given to ampules. The cells were than given to small conical flasks and 5 ml of infiltration buffer (50 mM MES, 0,3 M NaCl, pH 5,5) was added. Then the suspension was infiltrated by using -80 kPa underpressure in dorcus. The underpressure was reduced slowly. Then we used the dorcus to separate infiltration buffer and cells. The buffer was given to ampules. The cells in separation syringes were given to plastic test glasses and centrifuged by max. 2000 rpm. Gained apoplastic liquid and cells was given into the ampules. To cells was added small amount of methanol (max. 1 ml). Then scintillation liquid (SLD-41) was added into all ampules. After measurement on Packard-TriCarb were determined bioconcentration factors (BCF)- it was used method by Polder *et al.* (1995). In our case the BCF is proportion of ^{14}C -labelled substance concentrations in cell mass and

cultivation medium. Measurements of radioactivity in dpm were three times repeated for statistical purposes.

RESULTS

It was proved that the amount of uptaken ^{14}C -activity into the protoplast and partially also into the apoplast and cell surface has grown up with time (see figures 1,2,3,4). The amount of ^{14}C -2,4-D activity in medium with time decreased (see figures 1 and 3). The biggest amount uptaken into the apoplast was in the seventh minute. This also stand for the cell mass by ^{14}C -2,4-D (see Figures 1,2,4). By ^{14}C -FLT variant the amount of apoplastic ^{14}C -activity decreased from seventh to tenth minute; this activity increased in cell mass (protoplast; see figures 3,4). The percent of apoplastic activity from cellular activity was increasing from second minute to seventh minute by variant ^{14}C -2,4-D and decreasing from seventh minute to twelfth minute (see figure 4). By variant ^{14}C -FLT the percent of apoplastic activity from cellular activity remained low to twelfth minute and from twelfth to fifteenth minute it was increasing (see figure 4). The contained amounts of both radiochemicals in μmol s increased throughout the time in protoplast and decreased in medium. There was ten times more 2,4-D accumulated in protoplast than FLT(see figures 5 and 6). The highest bioconcentration factors were by ^{14}C -FLT variant, the one which is related to the real sample. Bioconcentration factors by ^{14}C -FLT variant related to the theoretical amount in sample (the added amount) were significantly lower. Theoretical and real bioconcentration factors were increasing (see figures 7 and 8).

Figure 1:

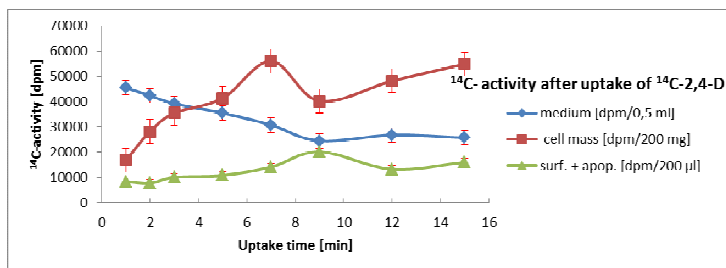


Figure 2:

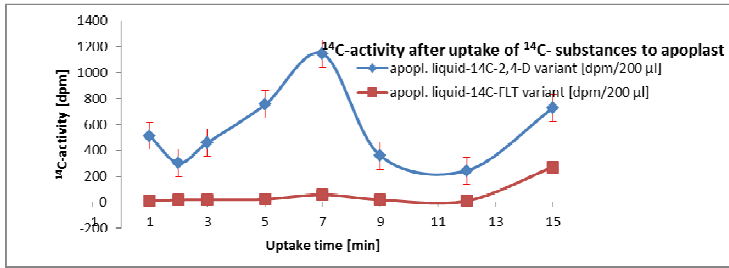


Figure 3:

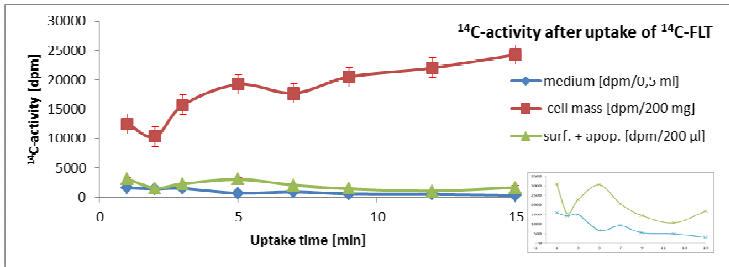


Figure 4:

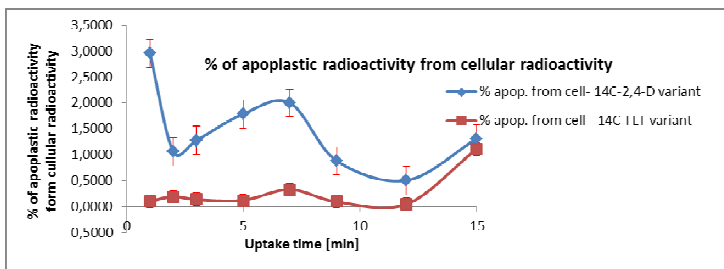


Figure 5:

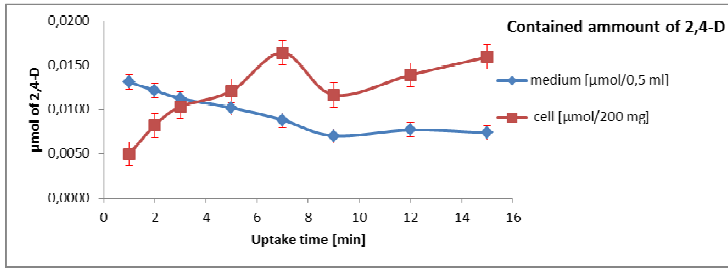


Figure 6:

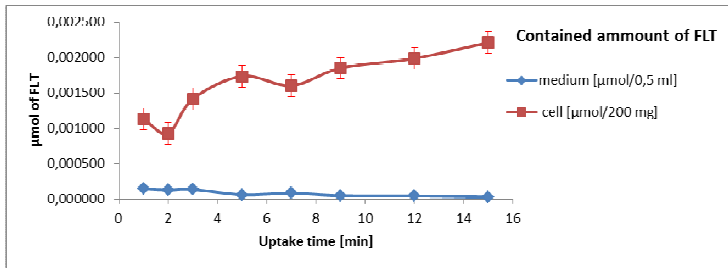


Figure 7:

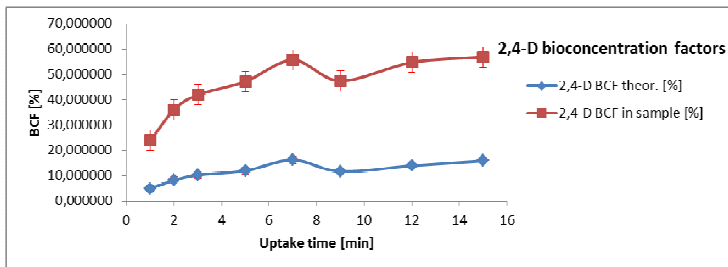
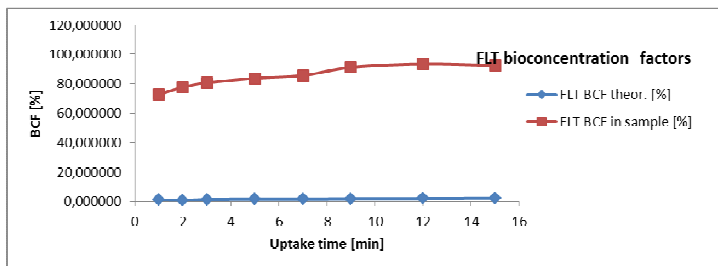


Figure 8:



DISCUSSION

Transport of xenobiotics is a very important area of plant physiology, because it, amongst other factors (eg. concentration of xenobiotic, temperature, insolation, type of xenobiotic...), determines the effects of this xenobiotics on cells. Substance uptake by plant cell suspensions is deciding phenomenon especially in the stage of depletion of nutrients from cultivation medium. The method of observing ^{14}C -activity was used by many other research teams, for example Delbarre *et al.* 1994. In this paper it was used to determine the dynamics of ^{14}C -2,4-D and ^{14}C -FLT uptake. There are still a lot questions that have to be answered. For example, by my experiment by the ^{14}C -FLT variant the amount of apoplastic ^{14}C -activity decreased from seventh to tenth minute and cell mass activity increased, so it's possible that the ^{14}C -activity was translocated from apoplast to protoplast in these periods of time. It was observed that the percent of apoplastic activity from cellular activity was increasing from second minute to seventh minute by variant ^{14}C -2,4-D and decreasing from seventh minute to twelfth minute, so we can say that in the first mentioned time interval the ^{14}C -activity was probably translocated from protoplast to apoplast and in the second mentioned interval it was probably translocated backwards. There is also evidence of extracellular deposition of 2,4-D in explant cultures (Morita *et al.* 1999). Nissen and Minocha (1993) suggested that that the extracellular 2,4-D is transferred by an auxin carrier into the cells in which it acts. Results of ^{14}C -2,4-D and ^{14}C -FLT uptake and deposition of ^{14}C -activity into the apoplast demonstrated that the utilization of both substrates is different. Plant *et al.* (1987) observed quick penetration of hydrophobic molecules throughout plasma membrane on benzo(a)pyrene. From my results ensue that by variant ^{14}C -FLT the percent of apoplastic activity from cellular activity remained low to twelfth minute and from twelfth to fifteenth minute it was increasing, so we can say that in the first mentioned timed it wasn't probably translocating from protoplast to apoplast; it was probably translocating only from the twelfth to fifteenth minute. Kolb and Harms (2000) described metabolism of ^{14}C -FLT in dependence of decrease of cell cycle count, but they haven't observed the mechanism of its uptake. Because there was ten times more 2,4-D accumulated in protoplast than FLT, we can say that the 2,4-D was probably much more taken up into the cells than FLT.

Kinetics of ¹⁴C-2,4-D was described before now by Delbarre *et al.* (1994), Delbarre *et al.* (1996). Authors show on isolated tobacco protoplasts and also on cell suspensions that the uptake of 2,4-D is characteristic by facilitated diffusion, what was confirmed by using transport inhibitors (Imhoff *et al.* 2000), whereas the uptake and utilization of fluoranthene as a hydrophobic molecule proceed by simple diffusion. Results of this work testify to it.

CONCLUSION

Fluoranthene and 2,4-dichlorophenoxyacetic acid have different mechanism of uptake and utilization, 2,4-dichlorophenoxyacetic acid is much more taken up to cell than fluoranthene.

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