Published in final edited form as:

Biochem Eng J. 2012 April 15; 63: 50–56. doi:10.1016/j.bej.2012.01.006.

Paclitaxel uptake and transport in *Taxus* cell suspension cultures

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Abstract

The transport of paclitaxel in *Taxus canadensis* suspension cultures was studied with a fluorescence analogue of paclitaxel (Flutax-2®) in combination with flow cytometry detection. Experiments were carried out using both isolated protoplasts and aggregated suspension cell cultures. Flutax-2® was shown to be greater than 90% stable in *Taxus* suspension cultures over the required incubation time (24 hours). Unlabeled paclitaxel was shown to inhibit the cellular uptake of Flutax-2®, although structurally similar taxanes such as cephalomannine, baccatin III, and 10-deacetyl baccatin III did not inhibit Flutax-2® uptake. Saturation kinetics of Flutax-2® uptake was demonstrated. These results indicate the presence of a specific transport system for paclitaxel. Suspension cells elicited with methyl jasmonate accumulated 60% more Flutax-2® than unelicited cells, possibly due to an increased cellular storage capacity following methyl jasmonate elicitation. The presence of a specific mechanism for paclitaxel transport is an important first result that will provide the basis of more detailed studies as well as the development of targeted strategies for increased paclitaxel secretion to the extracellular medium.

Keywords

Paclitaxel; plant cell culture; methyl jasmonate; metabolite transport; diffusion; product inhibition

1. Introduction

Paclitaxel (Taxol® – Bristol-Myers Squibb) is a diterpenoid secondary metabolite produced by *Taxus* species that has been approved by the FDA for the treatment of several types of cancer. The natural supply of paclitaxel is limited, but the use of plant cell culture technology has emerged as a viable, renewable alternative for commercial supply [1]. Although yields in cell culture have been improved to the point that several companies worldwide utilize this technology for industrial production [2], more widespread application of plant cell culture technology to produce paclitaxel and other natural products is limited by several factors, including a high degree of variability and low yields in secondary metabolite production [3–5]. Although most work on secondary metabolism has focused on the understanding and manipulation of biosynthetic pathways, low yields in plant cell culture may also be related to other factors, such as storage capacity or product degradation [6].

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Although cellular storage and secretion can be an important factor in the commercialization of plant cell culture processes, the specific transport processes for secondary metabolites, both within a cell and amongst cells and tissues, can be extremely complex. In addition to specific mechanisms unique to different metabolites, there is a high degree of variability exhibited within the same general system. For paclitaxel secretion, reports have ranged from 10% of paclitaxel in the extracellular medium of *T. x media* suspension cultures [7], to intermediate levels between 50–70% extracellular paclitaxel in *T. chinensis* and *T. cuspidata* cultures [8,9], to very high levels where almost 90% of paclitaxel was found in the extracellular medium of *T. baccata* cultures [10]. As a result, the development of specific processing strategies aimed at improving product secretion has largely been empirical, including the utilization of two-phase culture systems [11,12] with a variety of surfactants and resins [13–16].

Significant progress has been made to understand the mechanisms involved in the transport of several classes of secondary metabolites, including flavonoids [17] and alkaloids [18]. Traditionally, specific transport has been demonstrated in terms of saturation kinetics, specificity, and analogue competition [6,19,20], and more recently, molecular approaches allow for the characterization of the particular transporters involved. Specific carriers, such as ATP-binding cassette proteins, have been identified for some plant secondary products, including alkaloids such as berberine [21,22] and nicotine [23], as well as acylated anthocyanins [24], and coumaryl glycosides [25]. In some cases, a targeted engineering approach aimed at a specific secondary metabolite transporter has proven to be successful [26], but this has been relatively rare due to the lack of knowledge regarding most transport systems as well as difficulty in engineering some plant systems, particularly medicinal plant species such as *Taxus*. There have been several studies aimed at paclitaxel transport in *Taxus* systems, including uptake studies utilizing [¹⁴C]-paclitaxel as a tracer, which suggested there was an active, energy-dependent paclitaxel uptake mechanism [27], as well as studies utilizing a spin probe which indicated a decreased membrane permeability in *Taxus x media* upon elicitation with jasmonic acid [28]. The identification of a specific transport mechanism for paclitaxel has yet to be demonstrated.

Presented here are studies of paclitaxel (Taxol®– Bristol-Myers Squibb) transport in *Taxus* cell suspension cultures. The fluorescent analogue of paclitaxel known as Flutax-2® (Molecular Probes, Eugene, OR) was used to study paclitaxel uptake and storage in *Taxus* cell suspension cultures. The fluorescent moiety Oregon green ($\lambda_{\text{abs}}=496$ nm, $\lambda_{\text{emis}}=524$ nm) was attached by derivitization of the 7- β -hydroxy group of native paclitaxel, a strategy that does not interfere with the microtubule-binding activity of the probe [29–31]. Suspension cells were utilized as a model of aggregated plant cell cultures. Since paclitaxel and related taxanes can bind to the cell wall to some degree [32–34], isolated protoplasts were also used to study transport across the cell membrane [20]. The kinetics of Flutax-2® uptake was investigated in both systems via flow cytometry to determine the time necessary for cell-associated concentrations to reach equilibrium. Cell-associated compounds include both those stored intracellularly as well as those that are associated with the cell wall. The quantitative ability of flow cytometry makes it an excellent technique for both the study of cellular transport and ligand-receptor complexes [35,36]. Further experiments were undertaken to determine the stability of Flutax-2®, specificity of paclitaxel transport, and the stimulation of cellular transport upon elicitation with methyl jasmonate (MJ). This is the first study using Flutax-2® to investigate paclitaxel transport and accumulation in *Taxus* cell cultures.

2. Materials and Methods

2.1 Materials

Flutax-2[®] was obtained from Molecular Probes (Eugene, OR) and the enzyme pectolyase Y-23 was purchased from MP Biomedicals (Aurora, OH). Taxane standards were obtained from either Hauser Chemical Research, Inc. (Boulder, CO) or Sigma Chemical Co. (St. Louis, MO). All other chemicals were obtained from Sigma Chemical Co. unless otherwise noted.

2.2 Cell line maintenance and methyl jasmonate elicitation

The *Taxus canadensis* cell line CO93D and *Taxus cuspidata* cell lines P93AF and P930X were provided by US Plant Soil and Nutrition Laboratory (Ithaca, NY) and maintained in our laboratory as described previously [37]. Cell cultures were elicited with 200 μ M methyl jasmonate on day 7 post-transfer as described previously [37].

2.3 Taxane identification and quantification via HPLC

Cell cultures were sampled for the quantification of extracellular paclitaxel and total paclitaxel levels via HPLC. The samples were then extracted as described previously for the quantification of extracellular [37] and total paclitaxel [38]. All taxanes were identified and quantified using a Waters (Milford, MA) Alliance 2690 separation module equipped with a 996 photodiode array detector as described previously [38]. Peak identification was based upon comparison of retention time and UV absorption spectra with known standards. For quantification of paclitaxel and other taxanes, peak detection at 228 nm was used. Although Flutax-2[®] has an absorbance peak at 228 nm, the absorbance at 494 nm is much stronger due to the presence of the fluorescent label. For Flutax-2[®] quantification, absorbance at 494 nm was used.

2.4 Degradation of Flutax-2[®]

Conditioned medium was isolated via filtration of mid-exponential (days 7–10) cell suspension cultures through Miracloth[®] (EMD Biosciences, San Diego, CA). Flutax-2[®] was added to conditioned medium at 16 mg/L in a 96-well plate or cell suspension cultures at 1.6 mg/L in a 24-well plate and incubated for 24 hours at *Taxus* culture conditions (23–24 °C, 125 rpm, dark). To prepare samples for HPLC analysis, the contents of each well were transferred to 0.65 ml tubes and stored at –50 °C. Samples of conditioned medium only were then dried on a Savant SpeedVac Plus[®] and resuspended in 100 μ l acidified methanol. Following 20 minutes of sonication in a sonicating bath (VWR Scientific), the samples were filtered through a 0.2 μ m PVDF filter and analyzed via HPLC. Suspension culture samples were extracted for total taxanes as described previously [39] and analyzed via HPLC.

2.5 Experiments with aggregated cultures and isolated protoplasts

Flutax-2[®] was added to suspension cells at the desired concentration and incubated at standard culture conditions (23–24 °C, 125 rpm, in the dark) for 24 hours unless otherwise specified. After incubation, protoplasts were prepared from the suspension cell cultures for flow cytometric analysis. Protoplasts were isolated from *Taxus* suspension cultures by digestion with 1.0 % (w/v) cellulase and 0.1 % (w/v) pectolyase Y-23 in an osmoticum of 0.5 M mannitol and 0.3 % dextran sulfate, as previously described [34, 37]. Protoplasts are generally spherical and uniform amongst all *Taxus* cell lines, with an average size of approximately 40 μ m with 15% standard deviation. The protoplast isolation ratio was consistent amongst experiments and protoplasts were representative of cells in aggregated culture. Following filtration through an 80 μ m nylon membrane, the isolated protoplasts were resuspended in osmoticum at a density of 1×10^6 /ml. Alternatively, protoplasts were

isolated prior to addition of Flutax-2[®]. After addition of Flutax-2, the protoplasts were incubated at standard *Taxus* culture conditions (23–24 °C, 125 rpm, in the dark). The protoplast concentration did not change upon incubation. After two hours (unless otherwise specified), samples were pelleted by centrifugation (5 minutes, 1000 × g) and resuspended in 300 µl of osmoticum. Prior to flow cytometry analysis, 200 µl of 10 mM phosphate buffered saline (PBS) was added. Protoplast viability was greater than 90% as determined through staining with fluorescein diacetate.

2.6 Flow cytometry

For analysis, a Becton-Dickinson (San Jose, CA) FACSCalibur model flow cytometer equipped with an Argon laser tuned to 488 nm was used. Samples were gated according to forward (FSC) and side scatter (SSC) to eliminate debris. Fluorescence was measured using both peak height and area. Data were analyzed with CellQuest v3.1 (Becton-Dickinson) and reported as the population median.

3. Results and discussion

3.1 Cell-associated paclitaxel

To determine which cell line was most suitable for paclitaxel uptake experiments, three *Taxus* cell lines, CO93D (*T. canadensis*), PO93X (*T. cuspidata*), and P93AF (*T. cuspidata*), were tested for their ability to incorporate exogenously added paclitaxel. On day 6 post-transfer, with biomass concentrations of ~ 4 g dry weight/L, paclitaxel was added to all three cell lines at a concentration of 10 mg/L. Four days later, the cultures were sampled and analyzed via HPLC (Figure 1). The percent cell-associated paclitaxel was determined by comparing the amount of paclitaxel in the extracellular medium to that measured in the total culture (cells and medium). Although all cell lines did accumulate exogenous added paclitaxel, the *T. canadensis* cell line CO93D was shown to have the highest cell-associated paclitaxel (30%). The differences in paclitaxel uptake amongst cell lines are expected, as different species and cell lines have shown to exhibit variability in the percent of cell-associated paclitaxel [7–10] as well as other properties. For this reason, CO93D cultures were utilized in all further experiments. For each cell line, over 90% of the amount of paclitaxel added on day 6 was recovered in the total culture samples on day 10. In addition, independent experiments showed that the cell lines were not accumulating significant (i.e., detectable via HPLC) levels of paclitaxel under the experimental conditions described above.

3.2 Stability of Flutax-2[®] in suspension culture

To use Flutax-2[®] for the study of paclitaxel storage and transport, it must be stable in cell culture both with regards to the paclitaxel molecule and the fluorescent moiety. Flutax-2[®] in the extracellular medium should not be converted into other taxanes, nor should the fluorescent label be degraded. To investigate its stability, Flutax-2[®] was incubated in cell-free conditioned medium, and cell-free conditioned medium without Flutax-2[®] was used as a control. The only peak that was statistically different between these sample sets corresponded to the Flutax-2[®] molecule. Additional peaks were identified as nontaxanes through both retention time and PDA spectral comparison with a mixture of taxane standards (Hauser, Inc., Boulder, CO). All peaks were identical in both the conditioned medium and Flutax-2[®] spiked samples, indicating that the paclitaxel moiety is not significantly degraded. In addition to absorbance at 228 nm, the HPLC chromatograms were also examined at 494 nm, where Flutax-2[®] has an absorption maximum. None of the taxanes exhibit any significant absorbance at this wavelength. Only one peak was observed at 494 nm, which was determined by comparison to standards to be Flutax-2[®]. This indicates that Flutax-2[®] was not degraded by a 24 hour incubation in cell-free conditioned

medium. Quantification of the amount of Flutax-2[®] remaining in the conditioned medium sample after 24 hours (13.5 ± 2.6 mg/L) was statistically similar to the amount initially added (16 mg/L). These experiments indicate that Flutax-2[®] is stable, in that it is neither degraded nor converted to other taxanes, in cell-free conditioned medium for up to 24 hours.

Previous work has shown that plant cell cultures can degrade paclitaxel to other structurally similar taxanes. For example, *Eucalyptus perriniana* cultures converted ~ 50% of paclitaxel to baccatin III, 10-deacetylbaaccatin III, and 2-debenzoyletaxol over a three-day period [39]. However, there are no direct reports on paclitaxel stability in *Taxus* cultures. Flutax-2[®] was added to suspension cultures at a concentration of 1.6 mg/L to determine its stability in the presence of *Taxus* cells. Culture samples were analyzed via HPLC after 24 hours. Two separate peaks were identified at 494 nm, indicating that there is some conversion of Flutax-2[®] by *Taxus* cells. The significantly more abundant of these compounds was identified as Flutax-2[®] by retention time and spectral comparison and accounted for $92.6\% \pm 4.4\%$ of the two observed peaks by area. The combined concentration of these two compounds (1.6 ± 0.2 mg/L) was statistically identical to the amount of Flutax-2[®] initially added (1.6 mg/L). The minor peak eluted earlier and had a similar spectrum to Flutax-2[®] as well as a characteristic taxane spectrum from 200–300 nm and is therefore likely a taxane. Although a degradation product was observed in the presence of *Taxus* cells, greater than 90% of Flutax-2[®] was stable up to 24 hours. This demonstrates that the fluorescence measured can be primarily attributed to intact Flutax-2[®], and that flow cytometric measurements of cellular fluorescence can be used to assess the amount of Flutax-2[®] present.

3.3 Uptake of Flutax-2[®] in *Taxus* cultures

Flutax-2[®] was added to both *Taxus* suspension cultures and isolated protoplasts to determine the kinetics of uptake in each of these systems. Paclitaxel, a hydrophobic metabolite, has been shown to bind to cell walls to some degree [32–34,40], so we used both cultured suspension cells and isolated protoplasts. Since protoplasts do not contain a cell wall, they can be used to study paclitaxel transport across the plasma membrane [20]. To determine how long it took for Flutax-2[®] to equilibrate between the extracellular medium and cells, Flutax-2[®] was added to *Taxus* suspension cultures at 1.5 μ M and to isolated protoplasts at 1.2 μ M and 4.8 μ M (Figure 2). As expected, there was a difference between protoplasts and aggregated suspension cells. While protoplasts reached equilibrium by 1–2 hours, cell suspensions did not equilibrate until 4–12 hours. This difference can be attributed to both the aggregation of suspension cells and the presence of the cell wall. For all future experiments, isolated protoplasts were allowed to equilibrate for two hours and aggregated suspension cultures were incubated with Flutax-2[®] for 24 hours.

3.4 Uptake in the presence of paclitaxel and other taxanes

To determine the influence of other taxanes on the uptake of Flutax-2[®], aggregated suspension cell cultures were used. Suspension cell cultures were incubated with Flutax-2[®] in the presence of other taxanes (paclitaxel, baccatin III, cephalomannine, and 10-deacetyltaxol). These taxanes are structurally similar to paclitaxel, with several differences: baccatin III lacks the side-chain derived from phenylalanine; cephalomannine has a linear carbon chain at the third carbon position of the side-chain instead of a phenyl group; 10-deacetyltaxol lacks the acetyl group at position C-10 (Figure 3). Only paclitaxel was found to significantly ($p < 0.05$) inhibit the cellular uptake of Flutax-2[®] (Figure 4A). These results clearly demonstrate the specificity of paclitaxel uptake. Additionally, the inhibition of Flutax-2[®] was shown to depend linearly on the amount of unlabeled paclitaxel present (Figure 4B). At a constant concentration of Flutax-2[®], the addition of paclitaxel is able to competitively inhibit the cellular association of the fluorescently labeled ligand. The

competitive inhibition indicates that the two compounds are being transported by a specific mechanism [41].

The ability of paclitaxel to inhibit Flutax-2[®] transport, and the fact that cells could discriminate between paclitaxel and several structurally related taxanes, are indicative of a specific transport mechanism. The y-intercept can be used to estimate the amount of background fluorescence associated with nonspecific interactions that are not susceptible to saturation or analogue competition. Here, the background fluorescence is high and may be attributed to association with components of the cell wall or cell membrane as well as transport via nonspecific mechanisms such as diffusion or phagocytosis. Additionally, during the incubation period of 24 hours, other cellular processes such as degradation may contribute to the source of the background fluorescence [41].

3.5 Saturation of Flutax-2[®] uptake

In addition to the specificity and analogue competition that have been demonstrated for the transport of paclitaxel and Flutax-2[®], the presence of saturation kinetics was investigated to confirm a specific transport mechanism. Since protoplasts require shorter incubation times to reach equilibrium (Figure 2), experiments can be conducted more rapidly with protoplasts as compared to suspension cells. The use of shorter incubation times can be used to isolate the process of uptake, as less time is available for metabolic and intracellular trafficking processes following cellular uptake [41]. Flutax-2[®] was added to isolated protoplasts at various concentrations (Figure 5). The amount of cellular fluorescence is linearly proportional at low concentrations, as expected. However, at high concentrations, the total amount of cellular fluorescence does not reach saturation. This can be attributed to the added effect of nonspecific binding, which should be linear with respect to concentration of the fluorescent analogue. Nonspecific binding is assumed to be reversible, not saturable, and directly proportional to the ligand concentration [41].

To compensate for nonspecific binding, the amount of bound labeled ligand can be determined in the presence of very high concentrations of unlabeled ligand that will saturate the binding sites [41]. Here, protoplasts were incubated with both Flutax-2[®] (1.5 μ M) and paclitaxel (46 μ M). At this ratio, paclitaxel saturates the specific receptors, and all cellular fluorescence is due to nonspecific interactions with Flutax-2[®]. Assuming that nonspecific interactions are linearly proportional to the concentration of the fluorescent analogue [42], and using the total fluorescence value at 1.5 μ M Flutax-2[®] and 46 μ M paclitaxel as a proportionality constant, nonspecific fluorescence can be subtracted from the total amount of cellular fluorescence to yield specific fluorescence only (Figure 5). Following subtraction, saturation kinetics is demonstrated by considering the trend in the specific fluorescence curve, where smaller increases in cellular fluorescence are observed as Flutax-2[®] concentration increases. Additionally, specific cellular fluorescence does not increase at Flutax-2[®] concentrations greater than 9 μ M (equal to a paclitaxel concentration of 7.7 mg/L). These results agree with the saturation kinetics demonstrated in experiments with [¹⁴C]-paclitaxel as a tracer [27]. In combination with the specificity and analogue competition analysis, this saturation behavior further supports the existence of a specific paclitaxel transport mechanism.

3.6 Effect of methyl jasmonate on Flutax-2[®] uptake

Both suspension cells and isolated protoplasts were incubated with Flutax-2[®] in the presence of methyl jasmonate (MJ) at 200 μ M. MJ has been shown to elicit secondary metabolite synthesis in a wide range of plant systems [43], and has been shown to induce paclitaxel synthesis in *Taxus* suspension cultures [7,44,45]. Suspension cells showed a 60% increase in Flutax-2[®] accumulation following MJ elicitation as compared to the control

(Figure 6). This increase in cellular uptake may be attributed in part to an increased membrane permeability which has been found in response to jasmonic acid in *Taxus* cells [28]. It is also likely due to the upregulation of processes related to storage and transport, as MJ is a general elicitor of secondary metabolic pathways and has been shown to upregulate many processes, including transport, in addition to biosynthesis [46].

By contrast, there was no change in the cellular fluorescence for protoplasts exposed to methyl jasmonate (Figure 6). The differential response between isolated protoplasts and suspension cells may be attributable to either an increase in storage capacity for paclitaxel or to the length of the incubation period. Protoplasts were only exposed to MJ for 2 hours, as a significant decrease in viability was observed at later time points, compared to 24 hours for suspension cells. Many genes differentially regulated by MJ are not affected within two hours. Transcriptional profiling studies in *Arabidopsis* revealed that although gene transcription was evident as early as 30 minutes following wounding, the genes involved in secondary metabolism and cell wall modifications are part of the late term response, occurring no earlier than six hours after wounding [47]. Studies in *Taxus* have also shown that early paclitaxel biosynthetic pathway genes are upregulated by 6 hours following exposure to methyl jasmonate [48]. This time delay in gene expression may contribute to the differential response observed for Flutax-2[®] uptake between protoplasts and suspension cells. The increase in cellular fluorescence following MJ exposure may correspond to an increase in cell storage capacity of paclitaxel/Flutax-2[®]. Since paclitaxel is cell wall-associated [32–34], the induction of cell wall synthesis may correspond to a higher paclitaxel storage capacity. In addition to its effects on secondary metabolism, MJ also plays a role in cell defense and has been shown to regulate genes responsible for cell wall synthesis [49–51].

4. Conclusions

The transport and storage of paclitaxel in *Taxus* suspension cell cultures has been investigated using a fluorescent paclitaxel analogue (Flutax-2[®]) in combination with flow cytometry. Flutax-2[®] was shown to be stable in cell-free conditioned media for up to 24 hours, and exhibited greater than 90% stability in the presence of *Taxus* cells. Uptake kinetics analysis in both aggregated suspension cells and isolated protoplasts of *T. canadensis* cell cultures indicated that the intracellular concentration of paclitaxel in isolated protoplasts reached equilibrium within two hours, while aggregated suspension cells did not equilibrate until 4–12 hours. Inhibition experiments using several taxanes demonstrated the specificity of Flutax-2[®] uptake, where the addition of paclitaxel linearly inhibited the cellular uptake of Flutax-2[®], but none of the other structurally similar taxanes tested (10-deacetyltaxol, baccatin III, and cephalomannine) significantly affected Flutax-2[®] transport. In addition to selectivity, Flutax-2[®] uptake was shown to display saturation kinetics, indicating the presence of a specific transport mechanism. Finally, the effect of methyl jasmonate (MJ) on Flutax-2[®] transport was studied. With isolated protoplasts, there was no effect on the uptake of Flutax-2[®]. However, in suspension cells, MJ increased Flutax-2[®] uptake by 60% in comparison to the control, and this differential responses between protoplasts and suspension cells is likely explained by a combination of the longer time exposure to MJ for aggregated cells and an increased storage capacity in aggregated cells.

While there is likely some contribution of nonspecific transport processes such as diffusion, these results show that paclitaxel is at least in part transported by a specific mechanism such as a channel membrane protein or a cell surface receptor. In order to differentiate between these possible mechanisms, further experimentation is necessary, including use of inhibitors such as phenylarsineoxide or cytochalasin b to determine the role of vesicular trafficking. A functional genomics approach to identify specific transporters that are upregulated in

response to methyl jasmonate or are similar to those identified for other secondary metabolites could help to identify specific proteins involved in these processes. Once these mechanisms are fully characterized, targeted strategies can be developed to enhance paclitaxel secretion to the extracellular medium, decreasing feedback inhibition and further increasing paclitaxel accumulation. Increased secretion to the extracellular medium will also aid in the development of scalable bioprocesses by simplifying extraction and reducing processing costs.

Acknowledgments

This work was supported by the National Science Foundation (BES 9984463). The authors thank Dr. Donna Gibson of the USDA, Agricultural Research Service for the *Taxus* cell cultures and Dr. Barbara Osborne and coworkers for assistance with flow cytometry. MCN acknowledges support from a National Research Service Award T32 GM08515 from the National Institutes of Health and from a University of Massachusetts Faculty Research Grant. MEK acknowledges support from the National Science Foundation-sponsored Institute for Cellular Engineering IGERT program (DGE-0654128).

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Highlights

- Paclitaxel transport studied via a fluorescent analogue Flutax-2[®]
- Flutax-2[®] stable in suspension culture and cell-free media
- Flutax-2[®] uptake inhibited by paclitaxel but not similar taxanes
- Saturation kinetics of Flutax-2[®] uptake demonstrated
- Methyl jasmonate increases uptake by 60%

Figure 1.

The uptake of exogenous paclitaxel by three *Taxus* suspension culture lines: CO93D (*Taxus canadensis*), P93AF (*T. cuspidata*), and PO93X (*T. cuspidata*). Paclitaxel was added on day 6 post-transfer at a concentration of 10 mg/L and cell cultures were harvested four days later for HPLC analysis. Reported values are the average of three replicate flasks and error bars represent the standard error.

Figure 2. Kinetic uptake of Flutax-2® by (A) *T. canadensis* (CO93D) suspension cultures and (B) isolated protoplasts from *T. canadensis* (CO93D) cultures. Values are reported as the median cellular fluorescence obtained from flow cytometry analysis and error bars represent the standard deviation of the population distribution.

Figure 3.

Structures of (A) paclitaxel and related taxanes, including (B) 10-deacetyltaxol, (C) cephalomannine, and (D) baccatin III. 10-deacetyltaxol (DAT) is missing the acetyl group from position 10, while cephalomannine differs from paclitaxel in the side chain functionality. Baccatin III does not contain the side chain moiety.

Figure 4.

Influence of taxanes on the uptake of Flutax-2[®] by aggregated suspension cell cultures. (A) Paclitaxel, 10-deacetyltaxol (10-DAT), baccatin III, and cephalomannine were incubated with aggregated suspension cultures at 2.4 μ M for 24 hours. Flutax-2[®] was also added to all cultures (including control) at 1.5 μ M. (B) Cell cultures were incubated with a constant amount of Flutax-2[®] (1.5 μ M) and different amounts of paclitaxel for 24 hours prior to flow cytometric analysis. The x-axis represents the percentage of Flutax-2[®] as compared to the total amount of both Flutax-2[®] and paclitaxel. Values for (A) and (B) represent the average of three replicate flasks, and error bars represent the standard error. * represents statistical significance ($p < 0.05$).

Figure 5.

Saturation of Flutax-2[®] uptake in *T. canadensis* (CO93D) protoplasts, which were incubated with Flutax-2[®] for two hours. The reported values for total fluorescence represent median cellular fluorescence as determined with flow cytometry. For determination of nonspecific fluorescence, protoplasts were incubated with Flutax-2[®] (1.5 μM) and paclitaxel (46 μM) for two hours. Specific fluorescence is reported as the difference between total and nonspecific fluorescence values.

Figure 6.

Effect of methyl jasmonate on Flutax-2[®] uptake. Suspension cells and isolated protoplasts were incubated with Flutax-2[®] in the absence (control) and presence of 200 μ M methyl jasmonate (MJ). Suspension cells and protoplasts were incubated with Flutax-2[®] only or Flutax-2[®] and MJ for 24 hours and 2 hours, respectively. Reported values correspond to the median amount of cellular fluorescence and error bars represent standard error from three samples. * - represents statistical significance