

Uptake and Conversion of Three Different 5-Ring Polycyclic Aromatic Hydrocarbons (PAHs) in Cell Suspension Cultures of Various *Chenopodiaceae*-Species

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Three ^{14}C -labelled 5-ring PAHs, benzo(a)pyrene, dibenz(a,h)anthracene and perylene were added to various cell suspension cultures of plants belonging to the *Chenopodiaceae* family. The differently configured 5-ring systems varied in uptake and metabolism. The uptake of benzo(a)pyrene was highest in all cell cultures tested whereas the assimilation of dibenz(a,h)anthracene and perylene was significantly less. The uptake of benzo(a)pyrene and dibenz(a,h)anthracene was highest in those cultures which showed the highest increase in biomass. In contrast the uptake of perylene was highest in cultures whose increase in biomass was less.

Previous investigations (H. Harms, W. Dehnen and W. Mönch, Z. Naturforsch. **32 c**, 321–326 (1977)) showed that benzo(a)pyrene is metabolized to quinones and other oxygenated derivatives by *Chenopodium rubrum* cell cultures. It was demonstrated now that in further reactions these compounds are incorporated into insoluble fractions. With increasing time of incubation the amount of radioactivity in the extracted residue increased. The proportion of oxygenated derivatives formed seems to be correlated with the incorporation of radioactivity into insoluble fractions. Dibenz(a,h)anthracene turned out to be the most stable 5-ring system whereas perylene is metabolized to a certain extent but only small amounts of radioactivity could be detected in the extracted insoluble residue.

The experiments showed that plant cell cultures maintain their specific behaviour over long periods. On the other hand each of the chemical compounds, because of its molecular size and configuration, is subject to its own specific metabolism in plant systems.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are undoubtedly the most widespread carcinogens in the human environment [1]. Among those, benzo(a)pyrene is used as an indicator for the presence of PAHs [2] because this compound is known to be the most carcinogenic one. Benzo(a)pyrene is converted metabolically to oxygenated derivatives by microsomal mixed function oxygenases from animal cells [3]. The metabolic products include quinones, dihydrodiols, phenols and further unidentified conjugated derivatives. As was demonstrated recently benzo(a)pyrene is also metabolized by microorganisms [4], by intact plants [5–7], and by plant cell suspension cultures [5, 6, 8, 9]. Previous investigations showed that the uptake and metabolism of PAHs depend not only on the size of the molecule but also on the structure of the particular compound [6].

This paper reports the uptake and metabolism of three different 5-Ring PAHs, benzo(a)pyrene, dibenz(a,h)anthracene and perylene in cell suspension cultures of various *Chenopodiaceae*-species. *Chenopodium rubrum* cell cultures were found to metabolize benzo(a)pyrene to a large extent [10]. Therefore some further cell cultures of this plant family have been propagated in order to compare the ability of closely related plant species for metabolizing these PAHs.

Materials and Methods

Cell suspension cultures

Cell cultures of *Chenopodium bonus henricus*, *Chenopodium quinoa* and *Atriplex hortensis* were first established as callus cultures from root or stem sections of sterile grown seedlings. After several months of subculture and selection of fast growing cell material these tissues were transferred to liquid mediums and agitated on a shaking machine. The culture techniques [11] and the measurement of the

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growth cycle of *Chenopodium rubrum* cell suspension cultures have been described previously [10]. The suspension cultures of the Chenopodium-species are grown on a Murashige Skoog-medium containing 0.033 mg/l 2,4 D and 0.25 mg/l Kinetin whereas *Atriplex* cells are grown on B5-medium with 2 mg/l of Kinetin.

Chemicals

Labelled [7,10-¹⁴C]benzo(a)pyrene and [7-¹⁴C]dibenz(a,h)anthracene were purchased from Amersham-Buchler, Braunschweig. [6a, 6b, 12a, 12b-¹⁴C]perylene was synthesized by Natec, Hamburg. Non-radioactive compounds were obtained from Fluka Feinchemikalien GmbH, Neu-Ulm.

Experimental

The labelled PAHs were added to the nutrient solution during the last two days of the linear phase of the growth cycle. The concentration of the PAHs was 5×10^{-5} molar. The desired amount of the PAH was added to 0.5 ml 8 mm soybean lecithin (Roth No. 2-9812) in benzene. The benzene was evaporated under a gentle stream of nitrogen and the residue redissolved in 0.4 ml of methanol under sonication. The resulting suspension was added to the cell suspension cultures. The incubation time was 48 h. Thereafter the cells were harvested by vacuum filtration and washed several times with distilled H₂O before lyophilization. Cell materials as well as growth media were analyzed for PAHs and metabolites.

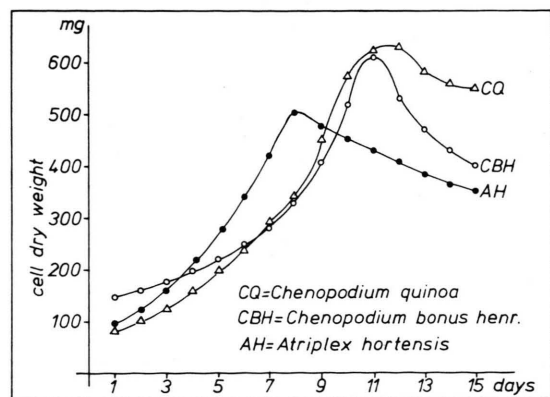


Fig. 1. Growth cycles of *Chenopodium quinoa*, *Chenopodium bonus henricus* and *Atriplex hortensis* cell suspension cultures.

The extraction procedure of the cell material was nearly the same as that of Bligh and Dyer [12] and has been described in detail by Sandermann *et al.* [13]. 500–800 mg dry weight was suspended in 40 ml chloroform/methanol/water, 1:2:0.8 (v/v) and extracted by sonication. The cell debris was finally washed with 10 ml chloroform. After addition of 10 ml water the mixture was left for 15 h at 4 °C to allow phase separation. The organic (chloroform) and aqueous phase were separated, evaporated to 0.5 ml and used for chromatographic analysis. The determination techniques used for chromatographic procedures as well as for radioactivity have been already described [10].

Results

Growth cycles of cell suspension cultures of different Chenopodiaceae species

When cell dry weight of a suspension culture is plotted against growth time, curves of the form shown in Fig. 1 are obtained.

These growth cycles give information not only about the viability of the cell cultures and when they have to be subcultured, but also make it possible to determine the physiological state of the cultures at which PAHs should be added. The new propagated cell cultures of the three plant species show different growth behaviour. *Atriplex hortensis* cultures are very fast growing cells. Within 8 days they reach the stationary phase of the growth cycle. The increase in biomass was 5 fold as compared to the inoculated amount. The *Chenopodium quinoa* and *bonus henricus* cell cultures grow much slower. In the case of *Ch. quinoa*, however, the biomass increases about 7fold.

Uptake of the three 5-ring PAHs

Previous experiments [6] with PAHs and aseptic seedlings and cell suspension cultures showed that the uptake of these substances depends not only on the size of the molecule but also on the structure of the particular compound. Of the three compounds tested here (Fig. 2) benzo(a)pyrene is assimilated most strongly by all four plant cell cultures. The uptake by *Ch. rubrum* and *Ch. quinoa* mounted up to more than 90% of the added amount. These two cultures were also the ones which showed the highest increase (7.5- and 7fold, respectively, see reference

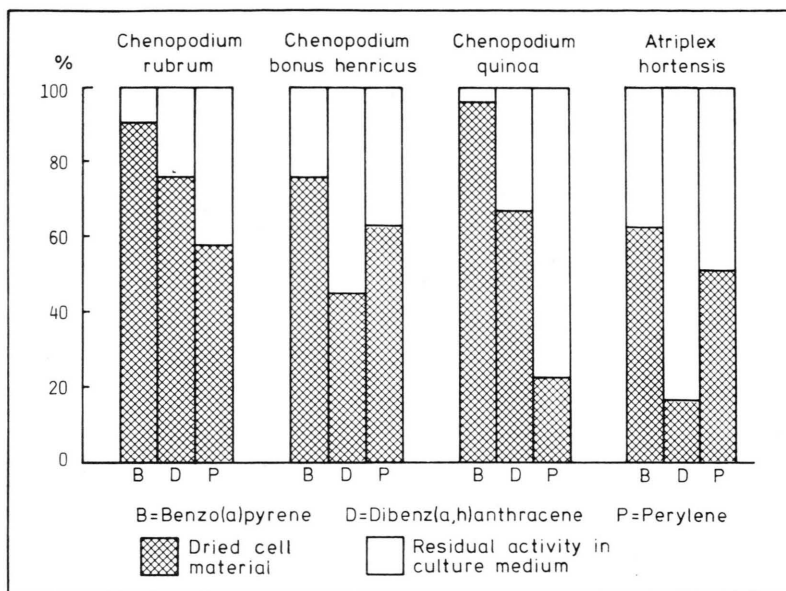


Fig. 2. Uptake and distribution (%) of the radioactivity of benzo(a)pyrene, dibenz(a,h)anthracene and perylene added to various cell suspension cultures of the family Chenopodiaceae.

10 and Fig. 1) in biomass during growth time. For the cell cultures of *Ch. bonus henricus* and *Atriplex hortensis* the comparative increase in biomass was only 4- or 5fold, and the uptake of benzo(a)pyrene varied between 75 and 60%, respectively.

Dibenz(a,h)anthracene is assimilated by the same cell cultures to a smaller extent (more than 30% less), but the tendency of uptake behaviour by the cells was similar to that of benzo(a)pyrene. The species with the higher increase in biomass also showed the higher rate of uptake.

The assimilation of perylene is smallest from all compounds tested. In contrast to the experiments with benzo(a)pyrene and dibenz(a,h)anthracene the uptake of perylene is higher in *Ch. bonus henricus* and *Atriplex hortensis* cultures which show a smaller increase in biomass as compared with the two other cultures.

Metabolism of the three 5-ring PAHs

Previous investigations [6] concerning the metabolism of benzo(a)pyrene in intact plants and cell cultures showed that this compound is metabolized to oxygenated derivatives. As *Ch. rubrum* was found to metabolize benzo(a)pyrene to a great extent, Chenopodiaceae-cell cultures were chosen to determine if the ability to metabolize PAHs is limited to this defined species or if other varieties of the same

family are also able to form oxygenated derivatives. The results are summarized in Fig. 3.

In general the results confirm that benzo(a)pyrene and dibenz(a,h)anthracene as well as perylene are metabolized by plant cell cultures. Benzo(a)pyrene and perylene are mainly metabolized by *Ch. rubrum* cells, while dibenz(a,h)anthracene is degraded by *Atriplex* cells.

It is obvious that in *Ch. rubrum* cells only a very small amount of benzo(a)pyrene is still present in the added form. Most of the radioactivity is detected in the extracted residue. Only small portions of this bound material can be hydrolyzed with 2 N methanolic potassium hydroxide and are present as water-soluble compounds. All the other cell cultures tested show that 80 to 88% of the radioactivity is still detectable as benzo(a)pyrene. Only up to 2% can be isolated as metabolites, whereas the amount of radioactivity in the extracted residue ranges between 10 and 17%, respectively.

Dibenz(a,h)anthracene proves to be the most stable compound among the three 5-ring PAHs. *Atriplex* cell cultures, however, metabolize nearly 10% of this compound, but the main part of radioactivity in all cell cultures remains in the form of the added compound. In contrast to benzo(a)pyrene only small amounts of radioactivity are found in the extracted residue.

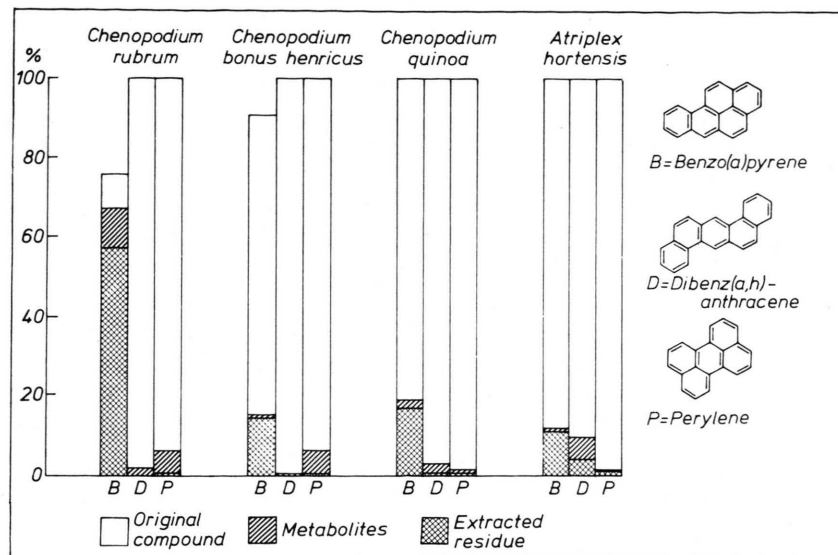


Fig. 3. Distribution (%) of the added radioactivity in different fractions of Chenopodiaceae cell suspension cultures after incubation with ^{14}C -labelled benzo(a)pyrene, dibenz(a,h)anthracene and perylene.

Perylene is measurably metabolized only by *Ch. rubrum* and *Ch. bonus henricus* cells. All cell cultures show very little radioactivity in the extracted residue. From this fact it may be concluded that the metabolites formed in the cells are not covalently linked to nucleic acids, proteins or lignin.

Discussion

Benzo(a)pyrene and dibenz(a,h)anthracene are known to be highly carcinogenic polycyclic hydrocarbons. Therefore discussions have arisen to use the not toxic perylene instead of these compounds as a test substance in trials. Perylene has the same molecular weight and solubility.

The experiments herein, however, establish that the differently configured 5-ring systems benzo(a)pyrene, dibenz(a,h)anthracene and perylene vary greatly in uptake and metabolism by Chenopodiaceae-cell suspension cultures. Each of the three compounds shows its own specific behaviour. The uptake of benzo(a)pyrene is high in all the plant cell cultures tested whereas the assimilation of dibenz(a,h)anthracene and perylene are much less. Similar results have been reported for intact plants grown under sterile conditions on nutrient solutions containing

PAHs in high concentrations [6] as well as for plants from pot and field trials which were cultivated on soils treated with town waste composts with high contents of PAHs [14, 15]. The uptake of benzo(a)pyrene and dibenz(a,h)anthracene is highest in those cell cultures which show the highest increase in biomass during their growth cycle. In contrast perylene assimilation is highest in cultures with less increase in biomass. This demonstrates that plant cell cultures keep the characteristic properties of the original plant variety even though they are subcultured for years. Hence plant cell cultures appear to be a very stable system.

In *Chenopodium rubrum* cell cultures, only a small amount of the applied benzo(a)pyrene can be isolated in the added form while a high part of its radioactivity is incorporated in the extracted residue. In recent studies v.d. Trenck *et al.* [8] demonstrated that a significant amount of the radioactivity of benzo(a)pyrene became associated with metabolic fractions which were insoluble in aqueous buffer or organic solvent or boiling SDS-solution. Such fractions were isolated from parsley and soybean cells as well as from the culture fluid of the soybean cells. In further studies [9] the same authors showed that benzo(a)pyrene is oxygenated

to isomeric 1.6, 3.6 and 6.12-quinones by plant microsomal fractions which in a further pathway are incorporated into lignin [16].

Previous time course studies [10] with benzo(a)pyrene and *Chenopodium rubrum* cell cultures have shown that these isomeric quinones are the first detectable metabolites formed. With increasing time of incubation the amount of quinones decreased, and radioactivity could be detected in the diol and polar compound fraction instead. With extended incubation the amount of radioactivity in the extracted residue also increased. There seems to be a relationship between the amount of oxygenated metabolites formed and their incorporation into insoluble fractions.

Dibenz(a,h)anthracene is the most stable 5-ring system tested. Only *Atriplex hortensis* cells metabolize this compound. As compared to the other cultures tested, the radioactivity in the extracted residue is significantly higher. However, in all cultures

the main part of the radioactivity can still be detected in the added compound.

Conversely, perylene is metabolized by *Ch. rubrum* and *Ch. bonus henricus* cells to a certain extent. However, only small amounts of radioactivity can be detected in the extracted residue of all cultures. This demonstrates that the metabolites formed from perylene must be different from those of benzo(a)pyrene.

The experiments with the three 5-ring systems benzo(a)pyrene, dibenz(a,h)anthracene and perylene and the four Chenopodiaceae cell cultures show that plant cell cultures maintain their specific peculiarities. The chemical compounds also have their own metabolism in plant systems because of their molecular size and configuration.

Acknowledgement

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