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Benzo(a)pyrene induces nuclear-DNA adducts in plant cell suspension culture

Detection by [^{32}P] postlabelling

B. Rether, A. Pfohl-Leszkowicz°, P. Guillemaut and G. Keith°

Laboratoire de Biologie Végétale Appliquée, IUT, Université Louis Pasteur, 3, rue de l'Argonne, 67000 Strasbourg and °*Institut de Biologie Moléculaire et Cellulaire du CNRS, Université Louis Pasteur, 15, rue René Descartes, 67084 Strasbourg, France*

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Using a [^{32}P] postlabelling technique, we found that plant cells (*Echinacea purpurea*) cultured in the presence of benzo(a)pyrene form nuclear DNA adducts.

[^{32}P] postlabeling; Plant cell; Benzo(a)pyrene; DNA adduct

1. INTRODUCTION

The specific degradation of benzo(a)pyrene into proximal and ultimate carcinogens, by animal cells and particularly by the microsomal system which transforms the benzo(a)pyrene into a diol epoxide product as the ultimate carcinogen, is already known [1,2]. This ultimate carcinogen binds covalently with DNA leading to the formation of adducts. In plants, several authors have also suggested the possible activation of a plant cytochrome P450 microsomal system for the transformation of benzo(a)pyrene [3,4]. Furthermore, in the presence of benzo(a)pyrene, a plant cell suspension culture of *Echinacea purpurea* suffers a loss of vitality (B.R. and P.G., unpublished results). But up to now, there is no information available regarding the possible formation of adducts in plant DNA by benzo(a)pyrene degradation products as already found in human and animal DNAs. We therefore focused our research on a plant system. The present paper deals with the detection of nuclear DNA adducts in plant cells cultured in the presence of benzo(a)pyrene, by the means of [^{32}P] postlabelling of enzymatically hydrolysed DNA.

2. MATERIALS AND METHODS

2.1. *Plant cell suspension culture*

The culture conditions were derived from standard methods from Gautheret [5]. Stem cuttings of *Echinacea purpurea* plants were maintained on a Murashige and Skoog medium [6] supplemented with 2 mg/l of 1-naphthyl-acetic acid(α) and 1 mg/l of kinetin. The cells were subcultured every two weeks and maintained on a rotary shaker at 110 rpm in the absence or presence of benzo(a)pyrene at a concentration of 10^{-5} M.

2.2. *Extraction of nuclear DNA from plant cell suspension*

The cells (10 g in weight) were harvested at several stages of the subcultures by filtration and were incubated in 100 ml Murashige and Skoog medium containing 0.5 M mannitol, 17.5 units of pectolyase (Sigma), 100 units of pectinase (Sigma) and 1 g of cellulase (Onozuka) for 3 h at room temperature to obtain protoplasts. The protoplasts were extensively washed and resuspended in the medium described by Green et al. for plant nucleus purification [7] and homogenized in a Kontes tissue grinder. DNA was finally prepared from purified nuclei according to Green et al. [7].

2.3. *[^{32}P] postlabelling of DNA*

The method used was that of Randerath et al. [8,9]. The DNAs were digested with micrococcal nuclease and spleen phosphodiesterase, and adducts were privileged in the digestion mixture, vs normal nucleotides, by further nuclease P1 digestion.

The remaining nucleotides were [^{32}P] labelled and the labelling mixture was directly applied to PEI-cellulose thin layers. Dimension 1 was on a one-dimension plate; the solvent was Na phosphate 2.3 M, pH 5.7.

After detection of the hydrophobic nucleotides at the lower part of the migration track, the corresponding region (spot) was cut out and transferred onto the origin point of a new plate [8]. Further migrations of the labelled nucleotides were performed using the following solvents: dimension 2: urea 6.25 M, lithium formate 3.25 M, pH 3.5; dimension 3: urea 7.5 M, lithium formate 4.25 M, pH 3.5; dimension 4: NaH_2PO_4, 1.7 M.

3. RESULTS AND DISCUSSION

The cells were cultured, the nuclear DNAs were extracted and the DNA adducts were determined by the [^{32}P] postlabelling method described in section 2. As shown in Fig. 1B,C, benzo(a)pyrene leads to the for-

Correspondence address: B. Rether, Laboratoire de Biologie Végétale Appliquée, IUT, Université Louis Pasteur, 3, rue de l'Argonne, 67000 Strasbourg, France

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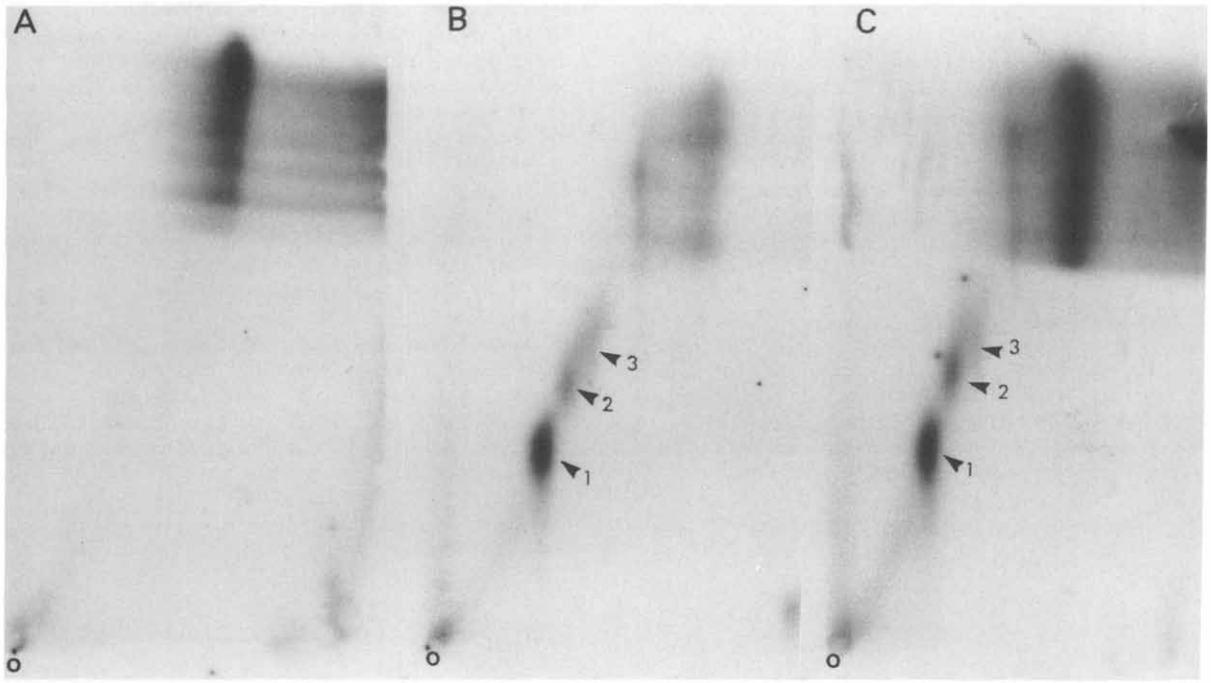

Fig. 1. Autoradiograms of [^{32}P]-labelled digests of DNA isolated from *Echinacea purpurea* cells cultured in the presence of benzo(a)pyrene. (A) Control; (B) 4th subculture; (C) 5th subculture. Notice that adducts appeared already in the 1st subculture (not shown). The first dimension was performed in one dimension on a PEI cellulose thin layer (not shown) as described in section 2. The other migrations were performed on a second PEI thin layer plate after transfer of the labelled material at the origin and in the lower migration region of the first dimension [8,9]. Dimension 2 was from bottom to top; dimension 3 was from left to right; dimension 4 was from left to right (it helped cleaning the background of the autoradiogram).

mation of at least two adducts, whereas none could be found in the control DNA (Fig. 1A). Indeed two adducts (spots 1 and 2) appear on the autoradiogram of the PEI plates: a major one and a fainter one. In addition a third adduct (spot 3) may exist just above spot 2.

Our results show that benzo(a)pyrene induces nuclear DNA adducts in cultured plant cells. However their exact nature remains unknown. These DNA adducts could be formed by the involvement of the already-mentioned microsomal cytochrome P450 system whose role in the metabolism of xenobiotics in higher plants becomes actually well documented. Indeed in plants, there are several pieces of evidence for the existence [3,10], like in mammals [11], of multiple cytochrome P450 forms, which are inducible by benzo(a)pyrene [12] and several other compounds. Furthermore, the plant cytochrome P450 enzymes appear to be very close to the mammalian hepatic enzymes [12]. It is therefore possible, that like in liver, the cytochrome P450 systems generate highly reactive electrophile compounds that bind to the plant nuclear DNA leading to the production of adducts. But we do not know yet, whether these adducts: (i) are directly related to activated benzo(a)pyrene; or (ii) come from other aromatic compounds present in the culture medium which could have been activated by benzo(a)pyrene-induced oxidase. Are these reactive electrophiles identical in mammals and in plants? The ultimate carcinogens of benzo(a)pyrene are already well known in mammals, but nothing is known about the possible ultimate 'mutagens' in the plant systems. The comparison of the DNA adducts obtained in liver DNA of benzo(a)pyrene-treated rats to the plant DNA adducts would lead to the partial answer to the above question. Further experiments to demonstrate this hypothesis are necessary. In addition, it would be interesting to check the possible formation of DNA adducts in chloroplasts and mitochondria, whose DNAs are much less protected in vivo than the nuclear one, meaning that they could be much more modified by electrophile compounds. We are investigating this possibility.

In conclusion, we show that in plants, benzo(a)pyrene induces DNA adducts which can be detected by [^{32}P] postlabelling of hydrolysed DNA. This has never been shown before, for that or for any other mutagen in plants. According to our results, one could imagine that DNA adduct formation may also occur in plants which are exposed to air and/or soil pollutants. It would therefore be very interesting to check whether such plants have DNA adducts or not. We are investigating in this direction, because we have the feeling that the [^{32}P] postlabelling technique, applied to plant DNA adduct detection, could soon become a very powerful tool in the DNA study of plants that have been exposed to pollutants.

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