

Fractionation of phosphorus compounds in tumor and callus tissues of *Nicotiana tabacum*

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Abstract

Phosphorus compounds in 2-, 4-, 6- and 8-week old cultures of tumour and callus tissues of *Nicotiana tabacum* were fractionated by the method of Schmidt and Thannhauser (1945) modified by Niemierko (1953). Much higher contents of P fractions were found in tumours as compared with callus tissues. DNA content in tumour tissue increased during 8 weeks. The contents of the acid-soluble P fraction and RNA + phosphoprotein fraction increase until 6 weeks, then it decreases. Callus tissue however shows a decreased nucleic acids P fraction after 4 weeks. The remaining fractions of this tissue increase slightly in this period but they decrease after 6 and 8 weeks.

INTRODUCTION

In connection with our preliminary attempt at DNP isolation from callus and tumour tissues of *Nicotiana tabacum* (Kłyszewko-Stefanowicz et al. 1971) it seemed interesting and suitable to determine exactly the contents of nucleic acids in this material, considering particular difficulties in obtaining non degraded DNP preparations freed from RNP.

In order to determine phosphorus compounds in both kinds of tissue (healthy and tumorous) obtained from 2-, 4-, 6- and 8-week old cultures of *Nicotiana tabacum* we applied the method of fractionation of phosphorus compounds after Schmidt and Thannhauser (1945) modified by Niemierko (1953).

MATERIAL AND METHODS

Crown-gall tumour tissue and normal (callus) tissue of *Nicotiana tabacum* cv. White Burley isolated in 1961 and cultured in vitro (Ren-

nert 1965) were analysed. Callus tissue was cultured on a Murashige and Skoog medium (1962) supplemented with thiamin (Linsmaier, Skoog 1965). Tumour tissue grew on the same medium but without IAA and kinetin. pH was adjusted to 5.5–5.6. Media were sterilized at 120°C for 15–20 minutes. The cultures were placed in an incubator at continuous fluorescent light ("day-light" type produced by Unitra-Polan) and temperature $25 \pm 1^\circ\text{C}$.

The determination of phosphorus was performed by the modified method of Kuttner and Lichtenstein (1932, 1960) after previous mineralization of material in concentrated H_2SO_4 . Perchloric acid was used as an oxidant.

Fractionation of phosphorus compounds

The scheme of fractionation of phosphorus compounds is shown in Fig. 1.

In order to obtain the acid-soluble fraction, in which total phosphorus and inorganic phosphorus of the acid-soluble fraction were determined, a sample of fresh tissue (0.2–2.0 g) was extracted with 5% HClO_4 . Phospholipids were extracted after Niemierko (1953). A tissue residue remaining after removing the acid-soluble and lipid fractions was subjected to alkaline hydrolysis. The cooled alkaline hydrolysate was acidified with 60% HClO_4 until precipitation was completed. This precipitate was washed 3 times with 5% HClO_4 at temperature 0°C — to obtain RNA and phosphoprotein fractions and then with 5% HClO_4 at 90°C — to extract the DNA fraction. After previous mineralization phosphorus was determined in the respective fractions. Total phosphorus in the remaining precipitate confirmed that phosphorus compounds were completely extracted.

RESULTS AND DISCUSSION

Fractionation of phosphorus compounds performed according to the scheme (Fig. 1) was made for tumour and callus tissues of *Nicotiana tabacum* depending on the age of cultures (6 analyses of each sample of 2-, 4-, 6- and 8-week old tissue were performed). The results of analyses are presented in figure 2 and 3.

The average phosphorus content in 2-week old tumour tissue is 54.4 mg%. It is represented by: 1) acid-soluble phosphorus (35.0 ± 1.9 mg%); inorganic phosphorus of this fraction makes about 78% (27.1 ± 4.4 mg%), 2) lipid phosphorus (4.6 ± 0.9 mg%), 3) RNA and phos-

phoprotein phosphorus ($3,1 \pm 0,8$ mg⁰/o) and 4) DNA phosphorus ($1,3 \pm 0,2$ mg⁰/o).

4-week old tumour tissue contains on the average 64,0 mg⁰/o of total phosphorus. Phosphorus of the acid-soluble fraction makes $43,0 \pm 5,8$ mg⁰/o; inorganic phosphorus of this fraction makes 87% ($37,3 \pm 7,2$ mg⁰/o). Lipid phosphorus makes $8,1 \pm 0,4$ mg⁰/o, RNA-P and phosphoprotein-P - $6,6 \pm 0,8$ mg⁰/o, DNA-P however $3,2 \pm 0,4$ mg⁰/o.

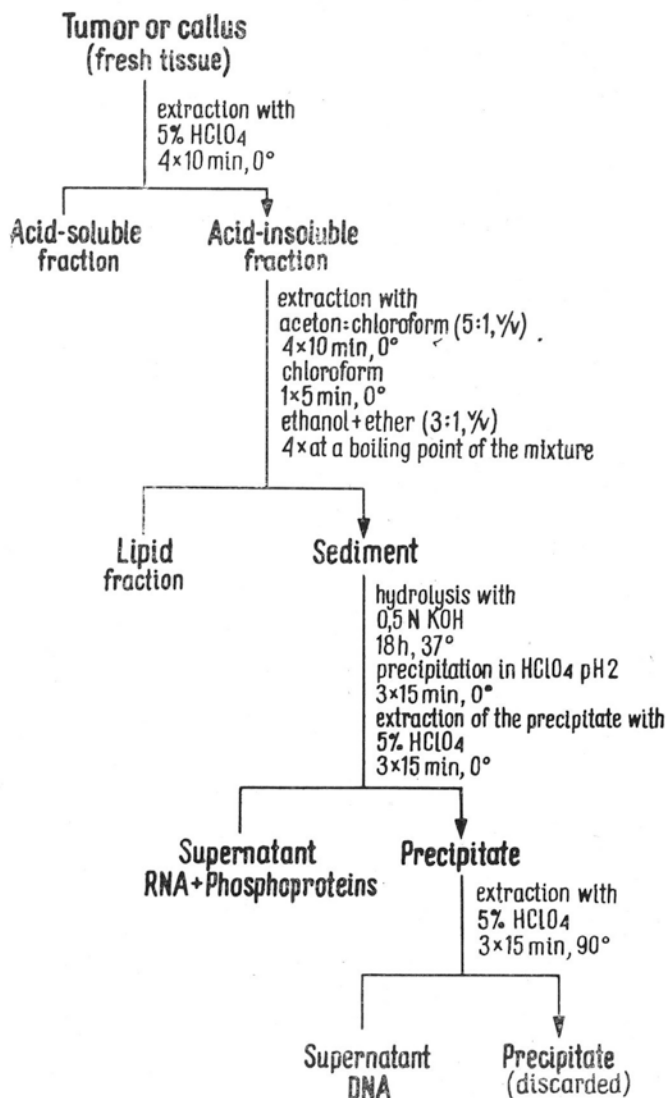


Fig. 1. Scheme of fractionation of phosphorus compounds.

6-week old tumour tissue contains 49,6 mg⁰/o of total phosphorus including acid-soluble-P fraction which makes $46,0 \pm 4,0$ mg⁰/o; 79⁰/o of this fraction makes inorganic P ($38,7 \pm 5,0$ mg⁰/o). Lipid phosphorus makes $4,7 \pm 0,4$ mg⁰/o, RNA-P together with phosphoprotein-P — $6,7 \pm 0,4$ mg⁰/o, DNA-P — $3,1 \pm 0,2$ mg⁰/o.

8-week old tumour tissue contains 41,6 mg⁰/o of total phosphorus. The acid-soluble-P fraction makes $30,5 \pm 0,7$ mg⁰/o of total P; inorganic phosphorus makes 62⁰/o ($19,0 \pm 0,1$ mg⁰/o) of this fraction. Lipid phosphorus makes $5,2 \pm 0,1$ mg⁰/o, RNA-P and phosphoprotein-P — $2,6 \pm 0,2$ mg⁰/o, DNA-P — $3,8 \pm 0,1$ mg⁰/o.

A recovery of total phosphorus as related to the sum of all phosphorus fractions is: for 2-week old tumour tissue — 81⁰/o, for 4-week old — 95⁰/o, for 6-week — 122⁰/o, for 8-week — 101⁰/o.

Total phosphorus in 2-week old callus tissue makes on the average 40,0 mg⁰/o. It includes: the acid-soluble phosphorus ($20,6 \pm 2,8$ mg⁰/o); inorganic phosphorus makes about 90⁰/o of this fraction ($18,5 \pm 5,2$ mg⁰/o), lipid phosphorus ($4,0 \pm 0,4$ mg⁰/o), RNA-P and phosphoprotein-P ($6,0 \pm 0,6$ mg⁰/o) and DNA-P ($2,4 \pm 0,9$ mg⁰/o).

In 4-week old callus tissue total phosphorus makes on the average 48,0 mg⁰/o. The acid-soluble phosphorus makes $27,5 \pm 3,4$ mg⁰/o; inorganic phosphorus is about 74⁰/o ($20,3 \pm 4,2$ mg⁰/o) of this fraction. The lipid

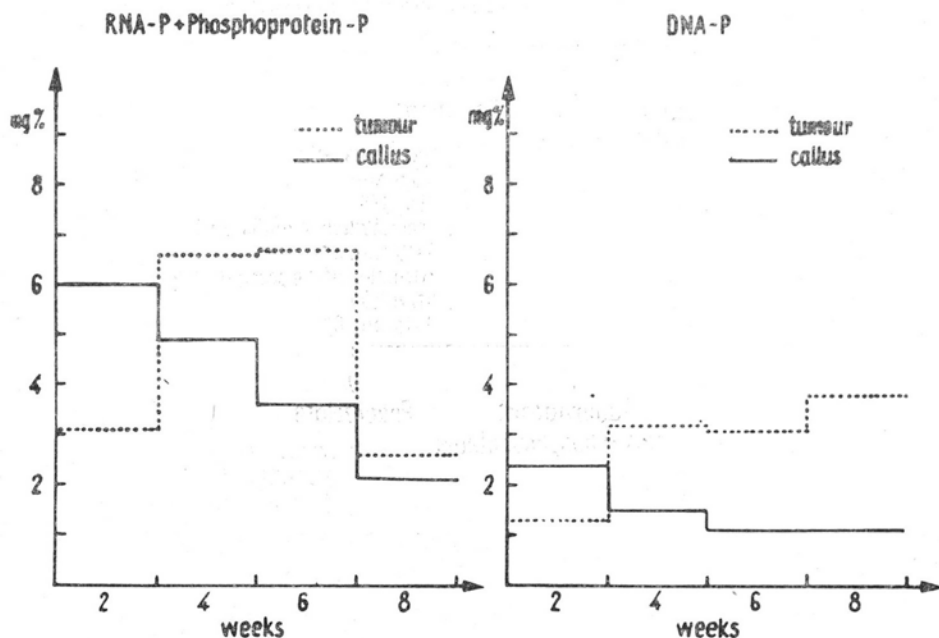


Fig. 2. Changes of nucleic acids phosphorus contents in tumour and callus tissues depending on their age.

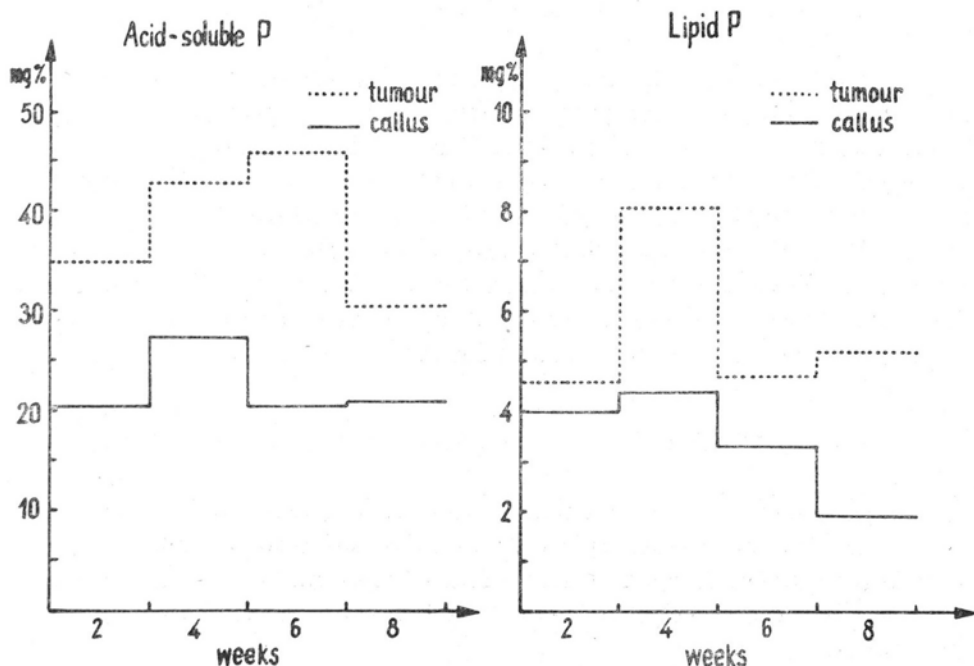


Fig. 3. Changes of acid-soluble and lipid phosphorus contents in tumour and callus tissues depending on their age.

phosphorus makes 4.4 ± 0.8 mg⁰%, RNA-P and phosphoprotein-P — 4.9 ± 0.6 mg⁰%, DNA-P — 1.5 ± 0.2 mg⁰%.

In 6-week old callus tissue the total phosphorus makes 24.0 mg⁰%. The acid-soluble phosphorus makes 20.3 ± 0.5 mg⁰%; inorganic phosphorus makes about 74.0% (15.1 ± 0.8 mg⁰%). An average content of the lipid phosphorus is 3.3 ± 0.2 mg⁰%, RNA-P and phosphoprotein-P — 3.6 ± 0.1 mg⁰%, DNA-P — 1.1 ± 0.1 mg⁰%.

In 8-week old callus tissue the total phosphorus makes about 26.0 mg⁰%. The acid-soluble phosphorus is 21.0 ± 0.3 mg⁰%; inorganic phosphorus makes 62.0% (13.0 ± 0.4 mg⁰%) of this fraction. The lipid phosphorus makes 1.9 ± 0.1 mg⁰%, RNA-P and phosphoprotein-P — 2.1 ± 0.1 mg⁰%, DNA-P — 1.1 ± 0.1 mg⁰% of fresh tissue.

A recovery of total phosphorus as related to the sum of all phosphorus fractions is: for 2-week old callus tissue 84%, for 4-week old — 80%, for 6-week — 115%, for 8-week — 102%.

A comparison of the nucleic acid phosphorus contents in tumour and callus tissue depending on their age is represented in Fig. 2. Fig. 3 is concerned with the phosphorus contents of the acid-soluble and lipid fractions respectively.

DNA-P Fraction

A distinct increase in the phosphorus content of DNA fraction in tumour tissue is noticed: from $1,3 \pm 0,2$ mg⁰/o in 2-week old culture to $3,8 \pm 0,1$ mg⁰/o in 8-week old one. The highest increase was observed in 4-week old cultures. In 6- and 8-week tissue cultures P content of this fraction remained more or less at the constant level.

In callus tissue these values are quite different. The highest P contents of DNA fraction appear in 2-week old tissue. In 4-week old tissue this content decreases to $1,5 \pm 0,2$ mg⁰/o, in 6- and 8-week old ones however it remained at the constant level.

RNA-P and phosphoprotein-P fraction

A rapid increase in the content of this fraction was noticed in 4- and 6-week old tumour tissue. This content increases from $3,1 \pm 0,8$ mg⁰/o in 2-week old culture to $6,6 \pm 0,8$ mg⁰/o and $6,7 \pm 0,4$ mg⁰/o in 4- and 6-week old cultures respectively. In 8-week old culture it decreases to $2,6 \pm 0,2$ mg⁰/o.

In the callus tissue however RNA-P and phosphoprotein-P fraction shows a tendency to decrease in a similar manner, as it was observed in the case of DNA. This value decreases from $6,0 \pm 0,6$ mg⁰/o in 2-week old cultures to $4,9 \pm 0,6$ mg⁰/o in 4-week old cultures and to $2,1 \pm 0,1$ mg⁰/o in 8-week old ones.

Acid-soluble-P fraction

The acid-soluble-P fraction of tumour tissue changes similarly as the acid-insoluble-P fraction. Its level increases with the age of culture: from $35,0 \pm 1,9$ mg⁰/o in 2-week old tissue to $46,0 \pm 4,0$ mg⁰/o in 6-week old one. In 8-week old culture this value decreases and attains $30,5 \pm 0,7$ mg⁰/o. In the case of callus tissue the content of the acid-soluble fraction remained more or less at the same level for 8 weeks. Only a slight increase in this fraction was noticed in 4-week old culture. In 2-, 6- and 8-week old tissues this fraction remains at the same level $21,0 \pm 0,3$ mg⁰/o.

Lipid-P fraction

The lipid-P fraction in 4-week old tumours tissue increases nearly by 100%. In 6- and 8-week old cultures the phosphoprotein content is nearly the same as in 2-week old tissue.

The callus tissue shows a decrease in the lipid phosphorus along with the age of tissue.

On the basis of our experiments it can be concluded that tumour tissue grows intensively during the entire culturing period of 8 weeks. Callus tissue, however, shows a decreased level of RNA-P and phospho-protein-P fraction as well as DNA-P fraction already in the 4-th week of culturing. The remaining fractions increase only slightly during this period as compared with the respective fractions in 2-week old cultures.

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*Fracjonowanie związków fosforowych w tkance tumorowej i kalusowej
Nicotiana tabacum*

Streszczenie

Dla tkanki tumorowej i prawidłowej (kalusowej) *Nicotiana tabacum* przeprowadzono rozdział związków fosforowych według metody Schmidta, Thannhausera (1945) w modyfikacji Niemierki (1953) z 2-, 4-, 6-, i 8-tygodniowych hodowli.

Stwierdzono ogólnie, że ilość fosforu wszystkich frakcji tkanki tumorowej jest znacznie wyższa od ilości fosforu dla tych samych frakcji, ale pochodzących z tkanki kalusowej. Ponadto wykazano, że w tkance tumorowej ilość frakcji DNA wzrasta przez cały 8-tygodniowy okres hodowli. Zawartość fosforu frakcji kwasorozpuszczalnej oraz frakcji RNA+fosfoproteidy wzrasta do 6 tygodnia i dopiero w 8 tygodniu ulega zmniejszeniu. Tkanka kalusowa już w 4 tygodniu hodowli wykazuje zmniejszoną ilość fosforu frakcji kwasów nukleinowych. Pozostałe frakcje tej tkanki wzrastają w tym okresie nieznacznie, ale już w 6 i 8 tygodniu ilość fosforu tych frakcji ulega zmniejszeniu.