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A COMPARATIVE STUDY OF THE SOLUBLE LEAF PROTEINS FROM HEALTHY AND POTATO VIRUS X-INFECTED TOBACCO PLANTS

Ever-growing interest is aroused by the question whether in the case of virus infection new proteins are synthesized in the plant or not. This problem was raised by L. C van Loon and A. van Kammen (1970), who found four new protein fractions in TMV-infected tobacco plants. Additional data supporting the fact that new proteins existed were given both by the same authors (van Loon, 1975a, b) and others (Zaitlin, Hariharasubramanian, 1972; Gianinazzi, Kassanis, 1974; Kassanis et al., 1974; Gianinazzi et al., 1977). On the other hand, some authors take the opposite point of view. So H. Barker (1975) found that three from the four new proteins may be normal plant constituents. A. Ziemiecki and K. R. Wood (1975) did not discover any qualitative changes in the protein constitution of diseased plants as compared with the healthy ones. According to the data of some authors the only protein that arises as a result of infection is the virus coat protein (Singer, 1971). Most investigations are made with TMV. Results are contradictory with the potato X virus. So S. Gianinazzi and B. Kassanis (1974) found three, but L. C. van Loon (1975b) no new proteins in the tobacco plants infected with the potato X virus.

The purpose of this work was to investigate the problem raised above using a form of the local strain of the potato X virus.

Materials and methods

Plant material and virus. Tobacco plants (*Nicotiana tabacum* L.) were grown in a greenhouse. Young plants in the phase of 3—4 leaves were inoculated with the sap from diseased plants infected with potato X_{17} virus (PVX_{17}). Plants inoculated with the sap from healthy plants served as a control.

Strain X_{17} was obtained from the purified preparation of potato X_3 virus by electrophoretical fractionation in sucrose density gradient (Хёдреярв et al., 1977).

Protein extraction. Inoculated leaves were harvested 4 and 11 days after infection. Leaf material (30 g) was rinsed with distilled water, blotted dry on filter paper, and precooled for 15 min. All subsequent procedures were carried out at 0...4 °C.

The leaves were ground in a blendor (type RT 2) with 0.8 vol (v/w) phosphate-citrate buffer, pH 3.0, containing 0.5 M NaCl and 0.1% (v/v) mercaptoethanol (van Loon, 1976). The resulting slurry was squeezed

through several layers of gauze and centrifuged for 30 min at 5400 g. The supernatant was passed through a glass filter N 2 and the resulting filtrate through a Sephadex G 25 column (3.3×44 cm), equilibrated with 0.05 M tris-HCl, pH 7.5. The column was eluted with the same buffer (van Loon, van Kammen, 1968). Absorptivity of the eluate was determined at 280 nm. The protein-containing fractions were pooled and centrifuged for 75 min at 130 000 g. The supernatant was brought to 95 per cent saturation with solid ammonium sulphate. After standing for at least 30 min, protein was collected by centrifugation at 30 000 g for 10 min. The precipitate was dissolved in 1 ml of 0.005 M tris-glycine buffer, pH 8.2 and centrifuged for 30 min at 25 000 g. The resulting supernatant was passed through a Sephadex G 25 column (2.0×18 cm) which was eluted with the same buffer. The partially purified protein solution obtained was subjected to disc electrophoresis.

Electrophoresis. Gels and solutions for separation of proteins in basic conditions were prepared by the modified method of Davis (Сафонов, Сафонова, 1959). 10% polyacrylamide gels were used. Samples contained glycerine (Майзель, 1972) and tracking dye of bromephenol blue. About 50...100 μg of protein were applied into each tube. To achieve such a concentration, the initial protein solution was thickened by adding dry Molselect G 25. The electrophoresis buffer (tris-glycine buffer, pH 8.3) contained 0.5 per cent 2-mercaptoethanol (van Loon, van Kammen, 1968).

Electrophoresis was conducted in an apparatus of «Reanal» for 20 min at 2 mA per tube and subsequently for about 1 hr at 4 mA per tube, until the tracking dye had migrated a few mm beyond the lower end of the gel.

After electrophoresis, gels were fixed overnight in 20% solution of sulphosalicylic acid and stained for 4 hr in 0.25% solution of Coomassie brilliant blue R-250. Excess dye was removed by soaking gels in 7% acetic acid (Майзель, 1972). Protein patterns were recorded by densitometry, using the type UT 7312 Tartu densitometer. The position of the protein bands on the gels was expressed by the R_f value, taking the distance travelled by the bromephenol blue tracking dye as 1.00. The R_f values presented here are the average values from four series of experiments.

Other methods. Peroxidase isozymes were detected by incubating the gels in 0.005 M benzidine and subsequently placing them into a solution of 0.002% H_2O_2 (Ладыгина, 1975). Isozymes of o-diphenoloxidase were revealed by incubating the gels in the solution of 0.01 M D-3-(3,4-dihydroxyphenyl)-alanine (van Loon, van Kammen, 1970). Protein concentrations were determined by the method of O. G. Lowry et al. (1951). The coat protein of the X_{17} virus was prepared from the purified virus (Hödrejärv et al., 1971) by its degradation with 2 M guanidine hydrochloride (Miki, Knight, 1968) or 2 M lithium chloride (Francki, McLean, 1968).

Results and Discussion

Trying to find in diseased plants new, lacking in healthy plants protein fractions, the experimental conditions were chosen. According to the data in literature (van Loon, van Kammen, 1970; Gianinazzi, Kassanis, 1974; Gianinazzi et al., 1977) such proteins were discovered in the fast-moving zone of 10% gel. The grinding of leaves with phosphate-citrate buffer, pH 3.0, makes it possible to liberate all new components

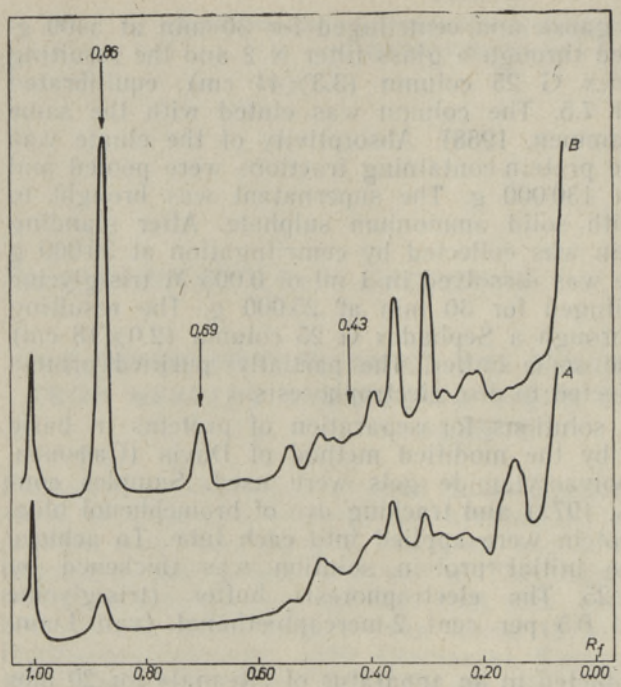

Fig. 1. Densitometer tracings of electrophoretic patterns of soluble protein extracts from tobacco leaves, 4 days after inoculation in 10% polyacrylamide gels. A — healthy plants; B — PVX_{17}-infected plants.

quantitatively, despite the fact that only about 30% of the total protein amount extractable at pH 8.0 were isolated under these conditions (van Loon, 1976).

The time for collecting leaves was chosen according to the virus infection development: the 4th day after the inoculation — primary appearance of disease symptoms, and the 11th day — the maximal virus concentration in the inoculated leaves.

Densitometer tracings of protein patterns are presented in Figs. 1 and 2.

Whereas we did not set the task to study quantitative changes in protein fractions, only a few protein bands were of interest to us. In the gel electrophoresis of protein from the leaves of 4-day-old infection, a fraction at position 0.86 attracted attention. Its quantity in the healthy plants was hardly observed (Fig. 1,A), in the diseased plants, however, it was high enough (Fig. 1,B). Other changes in the composition of the protein bands in that period of infection were also only quantitative. We did not succeed in discovering qualitatively new protein fractions.

The picture was in some respects different when protein solution from the leaves harvested on the 11th day after inoculation, was electrophoresed (Fig. 2). Fraction at position 0.86 increased noticeably in diseased plants (Fig. 2,B). At that phase of infection, this fraction was one of the three most noticeable fractions; at the same time it did not increase, or increased scarcely, in the healthy plants (Fig. 2,A). Apparently, virus infection induces an intensive synthesis of this protein fraction.

Moreover, we succeeded in discovering a new protein fraction at position 0.69 (Fig. 2,B), which was lacking in healthy plants. This fraction had quite a high concentration by the 11th day of the disease, whereas in the early period of infection it was hardly detectable (Fig. 1,B). A weak band at position 0.43, lacking in healthy plants, was found in addition (Fig. 2,B).

Comparing our R_f values with those of other authors, some coincidence occurs. L. C. van Loon and A. van Kammen (1970) found a new band at position 0.68; however, in a relatively recent work (van Loon, 1976) — at position 0.70 — the result was obtained by S. Gianinazzi

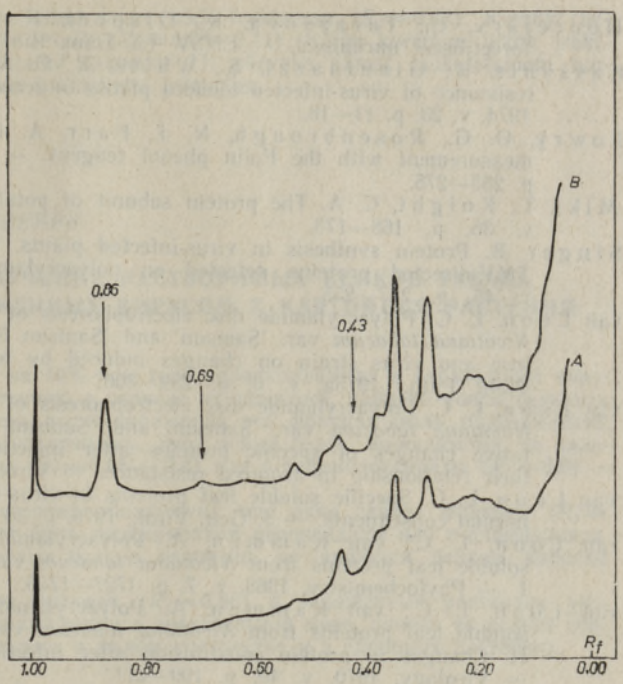

Fig. 2. Densitometer tracings of electrophoretic patterns of soluble protein extracts from tobacco leaves, 11 days after inoculation in 10% polyacrylamide gels. A — healthy plants; B — PVX_{17}-infected plants.

et al. (1977) as well. It is to say that all these authors worked with TMV. S. Gianinazzi and B. Kassanis (1974), using alongside of several viruses also potato X virus, found a band at position 0.67. These bands may be identical with our fraction at position 0.69. All above-named authors inform of finding a new protein band in the region 0.82 . . . 0.87. Our fraction 0.86 was found in the same region. But as was said before, according to our data, such a protein existed not only in the diseased but also in the healthy plants. As regards the fraction at position 0.43 found by us, the authors available to us have not referred to it. On the other hand, we did not succeed in finding a band in the region 0.55 . . . 0.60 mentioned by others.

As regards the nature of the new bands, this question remains unclear as yet. In developing peroxidase isozymes, a number of fractions were found in the region 0.50 . . . 0.43. o-Diphenoloxidase isozymes appeared in our experiments in the region 0.01 . . . 0.45. Whereas the major new protein fraction found by us had R_f value 0.69, it evidently was not an isozyme of peroxidase or o-diphenoloxidase. Minor fraction at position 0.43 may be either the one or the other.

In a separate experiment the relative electrophoretic mobility of the virus coat protein was determined. On the grounds of our data, coat protein obtained in two different ways moved in one band in the region 0.08 . . . 0.11. It follows from the fact that neither of the new components found by us was likely to be related to potato X virus coat protein.

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TERVEST JA KARTULI-X-VIIRUSEGA NAKATATUD TUBAKAST ERALDATUD LAHUSTUVATE VALKUDE VÕRDLEV UURIMINE

Tubaka lahustuvate valkude elektroforeesil 10%-lises polüakrüülamiidgeelis leiti kartuli-X-viirusega ($KX_{17}V$) nakatatud taimedes uus valgufraktsioon (R_f=0,69), mida tervetes taimedes ei esinenud. 4. päeval pärast inokulatsiooni oli see fraktsioon vähemärgatav, kuid suurenes tunduvalt 11. päevaks. Arvatavasti ei ole kõnealune valk peroksüdaasi (R_f=0,05—0,43) ega o-difenooloksüdaasi (R_f=0,01—0,45) isoensüüm, küll võib

seda aga olla haigetes taimedes leitud teine, nõrk fraktsioon (R_f=0,43). Kumbki leituist ei ole tõenäoliselt $KX_{17}V$ kattevalk (R_f=0,08—0,11). Kõige kiiremini liikuv fraktsioon (R_f=0,86) suurenes nakatatud taimedes 11. päevaks pärast inokulatsiooni tunduvalt; teda esines vähesel määral ka tervetes taimedes.

Керсти ОЛСПЕРТ, Улрих ХЕДРЕЯРВ

СРАВНИТЕЛЬНОЕ ИЗУЧЕНИЕ РАСТВОРИМЫХ БЕЛКОВ ТАБАКА ИЗ ЗДОРОВЫХ И ЗАРАЖЕННЫХ ВИРУСОМ Х КАРТОФЕЛЯ РАСТЕНИЙ

При проведении электрофореза на 10%-ном геле полиакриламида в зараженных вирусом X_{17} картофеля ($BX_{17}K$) растениях, в отличие от здоровых, найдена новая белковая фракция (R_f=0,69). На 4-й день после заражения эта фракция была малозаметной, а к 11-му дню она значительно увеличилась. Этот новый белок, по-видимому, не является изоферментом пероксидазы (R_f=0,05 — 0,4) или *о*-дифенолоксидазы (R_f=0,01 — 0,45).

Кроме того, в больных растениях обнаружена еще одна слабая фракция (R_f= 0,43), которая, очевидно, может быть изоферментом пероксидазы или *о*-дифенолоксидазы. Ни один из обоих найденных белков, вероятно, не является белком капсида $BX_{17}K$ (R_f=0,08 — 0,11).

Наиболее быстродвижущаяся фракция (R_f=0,86) в зараженных растениях увеличивалась к 11-му дню после инокуляции в значительной мере, однако ее нельзя считать новой, так как она наблюдалась в малом количестве и в здоровых растениях.