Purification and biochemical properties of the 'pathogenesis-related' protein p14 from tomato leaves

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The most prominent alteration found in the protein pattern of tomato leaves after infection with different pathogens and during the subsequent disease development is the accumulation of a 'pathogenesis-related' protein called p14 because of its apparent molecular weight of 14 000.

P14 was purified by a procedure involving acid-extraction of the leaf material, ultrafiltration of the clarified homogenates through Amicon hollow fiber systems, followed by ion exchange chromatography on sulfopropyl (SP-) Sepharose C25 from which it is eluted by a single step with 100 mM NaCl in SA buffer (=100 mM sodium acetate pH 5.5). A final purification step is carried out on DEAE-cellulose equilibrated with SA buffer where p14 does not bind and elutes with the SA-buffer wash. P14 could also be isolated from healthy tissue, where its concentration is about 40 to 50 times lower than in tissue infected with potato spindle tuber viroid. P14 can be stained with Coomassie brilliant blue, silver and ethidium bromide. It is sensitive to digestion with proteases and not altered when treated with RNase and DNase. P14 is a basic protein with an estimated isoelectric point of 10.7 and it seems to differ from any of the described pathogenesis-related proteins appearing in tobacco leaves after the virus-induced hypersensitive reaction. Its amino acid composition and its partially established primary sequence substantiate that p14 is a very basic protein. However, its origin, subcellular location and function (is it a newly synthesized protein or a preexisting membrane protein released during disease development?) are still a matter of conjecture and currently under investigation.

Citrus exocortis viroid (CEV): new data regarding the low-molecular-weight polypeptides associated with viroid infection

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After failure at attempts to find any function of viroids as messengers for specific proteins either in 'in vitro' or 'semi in vivo' synthesizing systems, efforts were devoted to studies of viroid-specified proteins in the native system (viroid-infected plants). Although these investigations were again unsuccessful, enhanced levels of two host-specified low-molecular-weight polypeptides were detected (potato and tomato CEV-P_1: MW 12 000 and $-P_2$: MW 16 300; *Gynura aurantiaca* DC and 'Etrog' citron CEV-P_1: MW 13 700 and $-P_2$: MW 18 000) (1,2). This effect was also detected in naturally senescing *G. aurantiaca* plants, this being the first evidence that neither of the two polypeptides is a translation product of the viroid-RNA or of any mRNA exclusively related to viroid-host interaction (2). More recently, the production of these polypeptides has also been detected in tomato plants *(Lycopersicon esculentum* L. cv. Rutgers)

infected with cucumber mosaic virus (CMV), and tobacco mosaic virus (TMV), and in *Nicotiana tabacum* L. cv. Xanthi-nc infected with TMV (3), as well as in *G. aurantiaca* and tomato (cvs Rutgers, Rentita and Hilda 72) treated with high concentrations of Ag^+ (500-1000 ppm of $AgNO_3$) and ethylene (4800 ppm of ethephon) (4,5).

Using a different extraction procedure and SDS-PAGE system, Camacho Henriquez and Sänger (6,7) have also reported the accumulation of a polypeptide of MW 14 000 (p14) in tomato plants infected with different viroid 'species' (citrus exocortis viroid (CEV), cucumber pale fruit viroid, chrysanthemum stunt viroid and potato spindle tuber viroid), two viruses (CMV and TMV), and a fungus *(Cladosporium fulvum).* This polypeptide, which seems to correspond to the previously described P_1, has been considered by these authors as a pathogenesis-related protein in the sense this term was defined by Antoniw et al. (8).

Regarding the nature of P_1 (p14) and P2 the following should be taken into account:
(1) When the methods described by Gianinazzi et al. (9) for PR (b) proteins (extraction at pH 2.8 followed by non-denaturing PAGE) were applied to leaves from CEV-infected tomato ('Rutgers') plants, we could not find any significant amount of these proteins, as indicated by the lack of detectable bands in the range of Rf values between 0.5 and 1.0. However, after boiling of these extracts with SDS, and SDS-PAGE analysis according to Conejero and Semancik (10) a significant accumulation of P_1 (p14) was observed.
(2) P_1 must occur intracellularly since it was found in protoplasts obtained from CEV-infected *G. aurantiaca* leaves (1).
(3) P_1 and P_2 are not necessarily associated with a hypersensitive reaction since they can be found in systemically infected plants.
(4) Enhancement of P_1 and P_2 does not seem to be related to host defense mechanisms; we found only normal levels in association with the resistance to CEV, induced by Ag^+ and ethylene at low concentrations, in *G. aurantiaca* plants.
(5) A progressive accumulation of P_1 and P_2 was detected in crude extracts of healthy *G. aurantiaca* upon incubation with aliquots of extracts from CEV-infected leaves.

All this suggests that PR (b) proteins and the low-molecular-weight polypeptides P_1 (p14) and P_2 have a different biological significance. PR (b) proteins are 'de novo'-synthesized components of an active (hypersensitive) response which can be elicited by different agents, whereas P_1 and P_2 are proteolytic breakdown products of native proteins associated with the accelerated ageing processes induced by these agents.

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Pathogenesis-related proteins N, O, P, Q, R etc.: some properties and separations

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Extracts from the leaves of tobacco plants *(Nicotiana tabacum* cv. Xanthi-nc) which are reacting hypersensitively to infection with tobacco mosaic virus (TMV) contain the four well-known pathogenesis-related proteins PR-1a, −1b, −1c and − 2, and also five other major protease-resistant proteins which are absent, or present in small amounts in uninfected leaves. These five PRs have not been characterized well enough to be assigned names, according to the proposed system of PR-nomenclature, but, following Van Loon (1), they have been called N, O, P, Q and R in order of their decreasing mobility in electrophoresis. On examination by chromatofocusing, both P and R are further resolved into two components, and the two proteins present as minor constituents are referred to as P′ and R′. None of these proteins N-R′ appears to contain a protein subunit of similar size to those present in PR-1a, b and c and which can be characterized by electrophoresis in the presence of SDS. After electrophoresis, protein peaks (P+P′) and Q stain with the Schiff-periodate reagent as if they contained carbohydrate, and the same is probably true of peak (R+R′). P, Q and R′ are absorbed onto columns of chitin or colloidal chitin: they could not be identified with the chitinases that are present in these extacts and so they may well be lectins which specifically bind to N-acetylglucosamine residues.

When leaves of TMV-infected plants are exposed to $^{14}CO_2$ as lesions develop, radioactivity accumulates in PR-1a, −1b, −1c, −2, and also probably in (R+R′), as judged by the distribution of radioactivity in electrophoretic gels. Incorporation of ^{14}C into PR-1a was confirmed by extraction of the protein from the gels and re-electrophoresis in denaturing conditions. The incorporation into PR-1a was not substantially different when $^{14}CO_2$ was applied to the leaves before, at the same time as, or after inoculation with TMV. The difficulty of establishing conditions of 'pulse-labelling' with these leaves however, makes it difficult to judge if the proteins are stable metabolic end-products, or are actively 'turning-over'.

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