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

A. CAMACHO-HENRIQUEZ, J. LUCAS and H.L. SÄNGER (Max-Planck-Institut für Biochemie, Abteilung Viroidforschung, D-8033 Planegg-Martinsried bei München, F.R. Germany)

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

V. CONEJERO, P. SEGADO, J.M. BELLES and A. GRANELL (Cátedra de Bioquímica y Química Agrícola, E.T.S.I. Agrónomos, Universidad Polytécnica, Valencia, Spain)

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)