HISTONE-LIKE BASIC PROTEINS ON PURIFIED TOBACCO MOSAIC VIRUS

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SUMMARY

The U1 strain of tobacco mosaic virus (TMV) was shown to be intimately associated with basic proteins. The virus was purified by two cycles of differential centrifugation and the histone-like basic proteins were extracted from the purified virus with 0.25 *N* HCl and Reinecke salt. After urea polyacrylamide gel electrophoresis, basic proteins separated as 6–7 bands which corresponded approximately to the position of calf thymus histones. The presence of histone-like basic proteins on the purified tobacco mosaic virus were also demonstrated on cellulose acetate membrane.

Such basic protein may play an important role in virus replication but the location of the histones, their origin and their role in virus-host interaction remain to be determined.

INTRODUCTION

It is a well known phenomenon that in the eukaryotes the nucleic acids are in close association with basic proteins (histones). The chromatin of higher plants and animals contains characteristic basic proteins. These proteins are extremely similar in their number, in biochemical characteristics and in some instances, even in their primary structure (Fambrough and Bonner, 1966, De Lange *et al.*, 1969). On the presence of histones in the lower organisms (viruses, bacteria, algae, fungi) there are conflicting opinions in the literature, especially in the case of viruses. Many workers are using various primitive eukaryotes as model organism for the study of the control of gene expression and development (Cantino, 1961, Daniel, 1966, Nanney, 1968, Sussman and Sussman, 1969).

Basic proteins similar to those in higher eukaryotes have been reported for green algae (Iwai, 1964), slime mold and cellular slime mold (Horgen and O'Day, 1973) and the protozoan (Iwai *et al.*, 1965). However, the chromatin of the *Dinoflagellatae* alga appears to have only a single acid-extractable, electrophoretic band suggesting a nuclear organization quite different from higher eukaryotes (Rizzo and Nooden, 1972). True fungi (*Chytridiomycetes, Zygomycetes, Ascomycetes* and *Deuteromycetes*) have been shown to lack the histones characteristic of the chromosomes of the other eukaryotes (Stumm and Van Went, 1968 and Leighton *et al.*, 1971). The uniquely different *Oomycetes*, however possess basic proteins associated with their nuclei (Horgen and O'Day,1973). The DNA of viruses, including the bacteriophages and certain animal agents is associated with basic proteins and polyamines (Hershey, 1955, Russel *et al.*, 1968, Fine *et al.*, 1968, Gibson and Roizman, 1971,

Fleissner, 1971, Freason and Crawford, 1972). These basic proteins may play an important role in neutralizing the charges of DNA. It has been demonstrated that basic proteins can modify the configurational state bacteriophage DNA as manifest in altered thermal denaturation, viscosity and activity as a template in transcription by a DNA dependent RNA polymerase (Bachrach and Friedman, 1967 and Chaproniere-Rickenberg *et al.*, 1964).

Such observations imply that basic proteins within viruses may have a role in the regulation of gene expression. The DNA of vaccinia is synthesized early in infection within the cytoplasm, specifically within viroplasmic matrices. The isolated basic protein from these materials is arginine-rich polypeptide of molecular weight 11–12000. These proteins occur both in the virus cores and the cytoplasmic "factories" (Pogo and Dales, 1969 and Pogo *et al.*, 1975). Moreover, Freason and Crawford (1972) reported that the polyoma virus particles contain three small basic proteins, but they emphasized that these polypeptides are not virus coded.

So far, the occurrence of histone-like basic proteins on plant virus has not been investigated. Considering the presence of such basic proteins on tobacco mosaic virus (TMV) we connected experiments with TMV U_1 strain.

MATERIALS AND METHODS

The virus was maintained in Samsun tobacco (*Nicotiana tabacum* L.) in the greenhouse at 22–26°C. Infected tobacco leaves served as the source for purification of the virus. Systemically infected leaves were harvested about 3–4 weeks after inoculation and frozen at -20°C. The virus was purified by a modification of the methods of Fraenkel-Conrat (1966) and Gooding and Hebert (1967).

The purified virus was precipitated with diluted HCl by adding 3 ml 0.5 *N* HCl to 3 ml of virus for 15 min and the preparation was then centrifuged at 5,500 *g* for 20 min. The supernatant was used for the isolation of the histone-like basic proteins. To each ml of supernatant 1 ml of a 2 per cent aqueous solution of Reinecke salt ($NH_4/Cr/NH_3/_2SCN/_4$) was added and the preparation left overnight. The precipitated basic protein-reineckates were centrifuged at 5,500 *g* for 20 min. The supernatant was discarded and the pellet twice washed with 5 ml acetone. Proteins were suspended in 1 ml running buffer containing 15 per cent sucrose. All operations were performed at 0–4°C.

For electrophoretic analysis the sample was dissolved in a solution of 15 per cent sucrose and 0,9 *N* acetic acid and applied to preelectrophoresed 15% polyacrylamide gels (in 2.5 *M* urea 0.9 *N* acetic acid pH 2.7). Gel dimensions were 0.6 × 8.5 cm. Electrophoresis was performed at 130 V for 2 h. The electrophoresis buffer contained 0.9 *N* acetic acid and 2.5 *M* urea (pH 2.7). Samples of 100–200 μl, containing 10–20 μg protein, were layered onto the gels. Above each sample was layered 8 *M* urea (50–100 μl). The preparations were run at 4 °C (Panyim and Chalkley, 1969).

Gels were immersed for 10 minutes in a solution of 20% ethanol, 7% amido black 10 B (Merck) for protein staining. The gels were destained twice in a solution of 20% methanol – 7% acetic acid, and finally destained in a solution of 1 *M* H_2SO_4 containing 3 *M* urea (Wray and Stubblefield, 1970).

For investigation of basic proteins we used cellulose acetate membrane, too (Cellogel RS membrane strips, Chemetron, Milano). The pure virus sample (30 μl) and control was put on the cellulose acetate membrane strips. The electrophoresis was carried out in a *Chemetron* tank at 240 V for 60 minutes. The low ionic strength (0 · 03) electrophoresis buffer contained 0.4 *M* tris-glycine (pH 9.5). For staining the membrane strips were immersed in Lissamine green (Eriogreen BDH) solution (0.5% in 45 ml methanol, 45 ml water and 10 ml acetic acid). For destaining we used 5% acetic acid. For intensification of the separated fractions we fixed the strips in 40% formalin for 1 minute and substituted by 7% glycerol, 3 minutes later we put the strips under an infrared lamp at 80–90 °C.

The method of Lowry *et al.*, (1951) was used to determine the protein content. A sample from healthy Samsun tobacco plants, processed as described above, served as the control. Calf thymus histone was purchased from Sigma Co.

RESULTS AND DISCUSSION

Polyacrylamide gel electrophoresis revealed 6 protein bands in the extract prepared from purified virus (Plate I (1)). Such protein bands were not observed in control preparations. Virus coat protein was also detected in the same system but at a different position in the gel to that of the basic proteins. When sulphuric acid was applied to intensify the protein fractions basic protein bands were visible, but if this destaining solution was not used the low molecular weight protein bands could not be detected with certainty.

In the other system at pH 9.5 we electrophoresed the purified virus solution on cellulose acetate membrane strips. On the *Chemetron* strips we could detect 4–5 basic proteins running towards the cathode while the virus (coat protein and viral-ribonucleic acid) moved to anode (Plate I (2)).

In the above mentioned two electrophoresis systems we were able to demonstrate the presence of histone-like basic proteins on tobacco mosaic virus. In the urea polyacrylamide gels 5–7 electrophoretic bands could be detected approximately co-migrating with calf thymus histones. The TMV purified by two cycles differential centrifugation contains 4–5 basic proteins as we demonstrated it on cellulose acetate membrane strips, too.

Regarding the presence of histones in lower living organisms opinions are divided. We wish to emphasize that the proteins isolated in this study may be virus coded, but they could also originate from host plant. However, if they originate from host cells they are strongly bound to the virus. This was supported by the fact that there was a variable number of basic protein bands, between 5–7. It is possible that these

proteins might play an important role in virus replication. Using vaccinia virus, Pogo *et al.* (1975) detected one arginine-rich protein, and in polyoma virus three basic proteins have been demonstrated (Freason and Crawford, 1972); data which strengthen our results and opinions.

Note added in proof: Using the above-mentioned methods we were able to demonstrate also from TMV U 2 strain the basic proteins, which run in polyacrylamide gels similar to the arginine-rich calf thymus histones. Analising the histone changes of host plants after infection we found that the arginine-rich histone fractions bound to both strains of tobacco mosaic virus.

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