Intracellular Inclusions Associated with Maize Mosaic Virus Infection

Mosaic disease of maize was first recorded in India by CHONA and SETH[1]. They studied the properties and vectors and determined some alternative hosts of the causal virus. The maize mosaic virus (MMV) was considered distinct from any other recorded so far on maize, as also from sugar-cane mosaic virus as it did not infect sugar-cane. In further studies with this virus, PALIWAL and RAYCHAUDHURI[2] determined the shape and size of MMV particles. In this communication, the intracellular inclusions observed in epidermal cells of mosaic-affected maize plants are described.

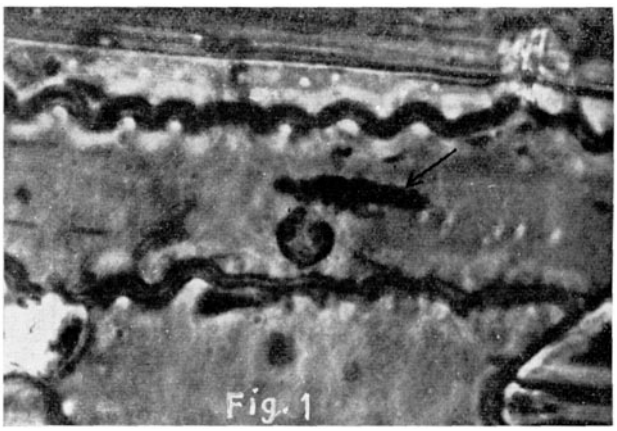

Fig. 1. Elongate shaped intracellular inclusion (arrow) in the epidermal cell of mosaic affected leaf of maize plant. (Magnification: approx. × 2560.)

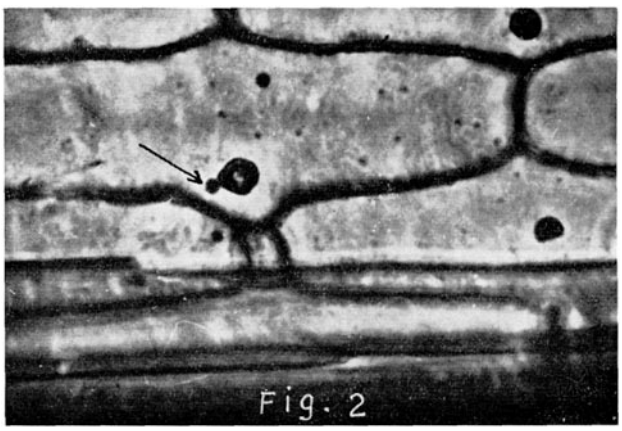

Fig. 2. Spherical shaped intracellular inclusion (arrow) in the epidermal cell of mosaic affected leaf of maize plant. (Magnification: approx. × 2560.)

Preparations were made of the epidermal strips, which were peeled from leaves of the diseased (15 to 20-day-old infection) and healthy maize plants of the same age and cut into suitably small pieces. These pieces were fixed and stained with the Giemsa stain method of BALD[3], as modified by RAWLINS[4], with the further slight modification that the epidermal strips were stained for 45 min in Giemsa solution instead of 15 min as employed by RAWLINS[4]. Strips from diseased and healthy leaves were run through the staining schedule simultaneously, giving exactly similar treatments to both lots. The nuclei and 'inclusion bodies' were stained pinkish purple, while the cell walls, etc., were stained green.

The 'intracellular inclusions' or 'inclusion bodies' were found quite frequently in the epidermal cells of the leaves of mosaic-affected maize plants. These inclusion bodies were either elongated (Figure 1) or spherical to oval (Figure 2) in shape and were found mostly associated with or attached to the cell nucleus. In some cases these structures appeared to be actually connected with the nucleus through a tube-like structure. The elongate-shaped inclusion bodies appeared granular in consistency, while the spherical-shaped inclusion bodies retained the stain more uniformly. The elongate type measured 14–26 μ in length and 3.0–4.6 μ in width, while the spherical and oval types measured 2.0–7.7 μ in diameter.

Spherical to amoeboid inclusion bodies attached to or lying near the cell nucleus were also observed by KUNKEL[5] and BREMER[6] in the cells of maize plants infected with sugar-cane mosaic virus. No information is, however, available regarding the size of these inclusion bodies[7].

Zusammenfassung. Die Epidermiszellen von mit Mais-Mosaik-Virus infizierten Maisblättern enthalten runde oder längliche Einschlusskörper, welche meist dem Kern anhaften oder nahe bei diesem liegen. Sie erscheinen nach Giemsa-Färbung rosa bis purpurn gefärbt. Die Abmessungen der länglichen Einschlüsse betragen 3,0–4,6 · 14–26 μ, der Durchmesser der rundlichen 2,0–7,7 μ.

Y. C. PALIWAL and S. P. RAYCHAUDHURI

Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi (India), May 10, 1965.

[1] B. L. CHONA and M. L. SETH, Indian J. agr. Sci. *30*, 25 (1960).
[2] Y. C. PALIWAL and S. P. RAYCHAUDHURI, in press.
[3] J. G. BALD, Phytopathology *39*, 395 (1949).
[4] T. E. RAWLINS, Phytopathology *47*, 307 (1957).
[5] L. O. KUNKEL, Bull. exp. Sta. Hawaii Sugar Planter's Assoc. Bot., Ser. 3, 44 (1921).
[6] G. BREMER, Meded. Proefstat. Java Sinkerind *11*, 337 (1926).
[7] Our thanks are due to Dr. G. SWARUP, Plant Pathologist, for helping in photomicrography.

Synthetic Peptides Related to Eledoisin[1]

In previous papers[2,3] the chemical data and the biological actions of a large group of synthetic peptides related to eledoisin were presented. We wish now to report briefly on a new group of peptides which throws further light on the problem of structure-activity relationship.

From the Table the following conclusions can be drawn:

(1) The methioninamide residue can be replaced with a variety of alkylhomocysteinamide residues: in all compounds tested the biological activity was found to be enhanced when the alkyl group was larger than the methyl. Maximum activity was observed in compounds No. 2, 3

	Chemical formula[a]	Relative biological activity[g] Hypotensive effect in dog	Contr. of rabbit large intestine	Contraction of guinea-pig ileum	M.p. (dec.)	$[\alpha]_D^{20}$ in 95% AcOH (c = 1%)	Electrophoretic mobility[f]	Molecular formula	Elementary analysis	C	H	N
1	H-Ala-Phe-Ile-Gly-Leu-(S-methyl) Hcys-NH_2[b, 3]	15	30	15								
2	H-Ala-Phe-Ile-Gly-Leu-(S-ethyl) Hcys-NH_2[3]	65–100	220	85								
3	H-Ala-Phe-Ile-Gly-Leu-(S-propyl) Hcys-NH_2	80–90	300	55	300–303°	– 22°	0.50	$C_{33}H_{55}O_6N_7S \cdot HCl \cdot H_2O$	Calcd.	54.1	8.0	
									Found	54.0	7.9	
4	H-Ala-Phe-Ile-Gly-Leu-(S-isopropyl) Hcys-NH_2	50	115	65–70	292–294°	– 20°	0.48	$C_{33}H_{55}O_6N_7S \cdot HCl \cdot H_2O$	Calcd.	54.1	8.0	
									Found	53.9	7.9	
5	H-Ala-Phe-Ile-Gly-Leu-(S-allyl) Hcys-NH_2	60–65	125	90–100	291–292°	– 24°	0.40	$C_{33}H_{53}O_6N_7S \cdot HCl \cdot H_2O$	Calcd.	54.3	7.7	
									Found	54.1	7.8	
6	H-Ala-Phe-Ile-Gly-Leu-(S-β-chloroallyl) Hcys-NH_2	35–50	150	35	288–290°	– 20°	0.42	$C_{33}H_{53}O_6N_7SCl \cdot HCl \cdot H_2O$	Calcd.	51.8	7.2	
									Found	51.8	7.3	
7	H-Ala-Phe-Ile-Gly-Leu-(S-benzyl) Hcys-NH_2	20–22	100–110	80	290–292°	– 24°	0.40	$C_{37}H_{55}O_6N_7S \cdot HCl \cdot H_2O$	Calcd.	56.9	7.5	
									Found	57.0	7.5	
8	H-Ala-Phe-Ile-Gly-Leu-(S-methyl) Cys-NH_2	< 0.1	0.2	< 0.1	223–226°	– 43°	0.43	$C_{30}H_{49}O_6N_7S \cdot HCl \cdot H_2O$	Calcd.	52.2	7.6	
									Found	52.0	7.5	
9	H-Ala-Phe-Ile-Gly-Leu-(S-ethyl) Cys-NH_2	1	5	2	224–227°	– 38°	0.43	$C_{31}H_{51}O_6N_7S \cdot HCl \cdot 1{,}5\ H_2O$	Calcd.	52.2	7.8	
									Found	52.3	7.8	
10	H-Ala-Phe-Ile-Gly-Leu-(S-benzyl) Cys-NH_2	1	5	1	223–225°	– 48°	0.43	$C_{36}H_{53}O_6N_7S \cdot HCl \cdot {}^1/_2\ H_2O$	Calcd.	57.1	7.3	
									Found	57.0	7.4	
11	$(CTB)_2$Lys-Phe-Ile-Gly-Leu-Met-NH_2[c]	20	110	50	244–245°	– 28° (c = 1 in DMF)		$C_{44}H_{74}O_{10}N_8S$	Calcd.	58.2	8.2	
									Found	58.1	8.4	
12	$(CTB)_2$Lys-Phe-Ile-Gly-Leu-Eti-NH_2[d]	25	180–200	180–200	244–246°	– 25° (c = 0,5 in DMF)		$C_{45}H_{76}O_{10}N_8S$	Calcd.	58.8	8.3	
									Found	58.7	8.4	
13	H-Ala-Tyr-Ile-Gly-Leu-Met-NH_2[3]	1	3	0.8								
14	H-Ala-Phe-Ile-Gly-Leu-Met-CN[e]	12–16			180°		0.49	$C_{31}H_{49}O_5N_7S \cdot HCl \cdot 2\ H_2O$	Calcd.	52.8	7.7	
									Found	52.4	7.6	
15	CTB-Ala-Phe-Ile-Gly-Leu-Met-CN[e]	9	8		242°	– 38.5° (c = 1 in DMF)		$C_{36}H_{57}O_7N_7S$	Calcd.	58.7	7.7	13.3
									Found	59.1	7.8	13.4
16	H-(D)Lys-Phe-Ile-Gly-Leu-Met-NH_2	25	90	20–25	225–227°	– 32.5°	0.84	$C_{54}H_{58}O_6N_8S \cdot 2\ HCl$	Calcd.	52.3	7.8	14.4
									Found	52.3	7.8	13.9
17	H-(D)Lys-(D)Phe-(D)Phe-Gly-(D)Leu-(D)Met-NH_2 H-(N^ε-R)Lys-Phe-Ile-Gly-Leu-Met-NH_2	< 0.05	0.1	0.1	170°	+ 11,4°	0.84	$C_{37}H_{54}O_6N_8S \cdot 2\,HCl \cdot 0.5\,H_2O$	Calcd.	54.1	7.0	13.6
									Found	54.1	7.2	13.4
18	R = H[3]	60–70	175	110								
19	R = nicotinyl	220		160	219–220°	– 16°	0.79	$C_{40}H_{61}O_7N_9S \cdot 1.5\ H_2O$	Calcd.	57.2	7.7	15.0
									Found	57.2	7.6	14.9
20	R = butyryl	60–65		55	240°	– 18.4°	0.39	$C_{38}H_{64}O_8N_8S$	Calcd.	57.5	8.1	14.1
									Found	57.9	8.3	14.0
21	R = valeryl	50–60	140–150	75	250°	– 17°	0.43	$C_{39}H_{66}O_7N_8S \cdot HCl$	Calcd.	56.6	8.2	13.5
									Found	56.1	8.2	13.0
22	R = caproyl	35–40		55–60	221–224°	– 16,5°	0.36	$C_{40}H_{68}O_7N_8S \cdot 0.5\ H_2O$	Calcd.	59.0	8.6	13.8
									Found	59.0	8.6	13.6
23	R = cyclopentylpropionyl	30–35	135–140	50–55	248–250°	– 15.5°	0.42	$C_{42}H_{70}O_7N_8S \cdot HCl$	Calcd.	58.1	8.2	12.9
									Found	57.9	8.2	12.5
24	R = benzoyl	50–60	150–160	60–65	250°	– 17.5°	0.43	$C_{41}H_{62}O_7N_8S \cdot HCl$	Calcd.	57.6	7.5	12.7
									Found	58.1	7.5	13.2
25	R = cinnamyl	20–25		37	222°	– 14.5°	0.40	$C_{43}H_{64}O_7N_8S \cdot 0.5\ H_2O$	Calcd.	61.0	7.8	13.2
									Found	60.8	7.8	13.0

[a] Unless otherwise stated, all amino acids have L configuration. [b] Hcys = homocysteine. [c] CTB = carbo-*ter*-butyloxy. [d] Eti = ethionine. [e] Met-CN = methionine nitrile. [f] In $HCOOH/CH_3COOH/H_2O$ (15:10:75) with leucine as standard. [g] The activity of eledoisin is taken as 100. The activity of the other peptides is compared on a weight basis and expressed in %.

and 12, where the alkyl group is either ethyl or propyl. However, when the methionine residue is substituted with an alkylcysteinamide residue the biological activity is drastically reduced. It is apparent, in accord also with previous observations[2,4], that whereas methioninamide cannot be replaced by other naturally occurring amino acids, it may be substituted, even with advantage, by synthetic non-natural sulphur-containing amino acids.

(2) The terminal amide group is not essential for biological activity: methionine nitrile (compounds No. 14 and 15) can replace the methioninamide residue with limited loss of activity.

(3) The all D-enantiomer of a highly active hexapeptide[5] is devoid of activity and does not antagonize either the L-enantiomer or eledoisin[6]. On the contrary, the presence of a single D amino acid can have influence on the biological activity[3] (cf. No. 16) provided the C-terminal pentapeptide fragment is the same as in eledoisin.

(4) N-ε-acylation of the highly active hexapeptide No. 18 does not appreciably alter the overall activity: in one case (No. 19) a 3-fold increase was observed.

Riassunto. Vengono descritte le proprietà di una serie di peptidi sintetici affini per struttura ed attività all'eledoisina.

L. BERNARDI*, G. BOSISIO*, F. CHILLEMI*, G. DE CARO**, R. DE CASTIGLIONE*, V. ERSPAMER**, A. GLAESSER*, and O. GOFFREDO*

Laboratori Ricerche Farmitalia, Milano and Istituto di Farmacologia dell'Università di Parma** (Italy), July 29, 1965.*

1 Part IV, for part III see [3].
2 B. CAMERINO, G. DE CARO, R. A. BOISSONNAS, ED. SANDRIN, and E. STÜRMER, Exper. *19*, 339 (1963).
3 L. BERNARDI, G. BOSISIO, F. CHILLEMI, G. DE CARO, R. DE CASTIGLIONE, V. ERSPAMER, A. GLAESSER, and O. GOFFREDO, Exper. *20*, 306 (1964).
4 E. SCHRÖDER and K. LÜBKE, Exper. *20*, 19 (1964).
5 Compound 29 of [3].
6 After this work was completed we learnt of a similar study by Prof. SCHRÖDER et al.[7]: our results point to the same conclusions.
7 E. SCHRÖDER, K. LÜBKE, and R. HEMPEL, Exper. *21*, 70 (1965).
8 F. CHILLEMI and G. BOSISIO, Gazz. Chim. Ital. *95*, 393 (1965).

Inhibition of the Infective Activity of Bacteriophage f_2 by Spermine

It was shown recently that spermine antagonizes the inhibition of reproduction of phage f_2 caused by streptomycin (SCHINDLER[1]), in spite of that it exerts a high inhibitory activity itself. REITER observed that spermine at a concentration of 1 mg/ml (approximately 3 mM) inhibits adsorption and penetration of phages T 1, T 3, T 7, and PLT 22. T even phages were not inhibited (REITER[2]). FERROLUZZI-AMES and AMES[3] report that the reproduction of T 4r$^+$ phage is about 95% inhibited by spermine at 20 mM concentration. Phage P 22, even though it adsorbs normally to *Salmonella typhimurium*, does not develop in the presence of spermine (AMES and DUBIN[4]). Spermine (1 mg/ml) stimulates injection of streptococcus-P9 phage DNA into the host cell (BROCK and WOOLEY[5]).

Spermine, as well as other polyamines, interact with nucleic acids. Spermine interacts with DNA, changing its thermal denaturation profile (MAHLER and MEHROTRA[6], MANDEL[7], TABOR[8]). It protects DNA against hydrodynamic shear, strengthening its molecule longitudinally (KAISER, TABOR, and TABOR[9]). Interactions with RNA of low molecular weight were described. Spermine becomes bound to polyuridylic acid (HUANG and FELSENFELD[10]) and s-RNA (CANTONI[11]). It is capable of blocking the messenger activity of polyuridylic acid in cell-free protein synthesizing systems (OCHOA and WEINSTEIN[12]). MITRA and KAESBERG[13] have shown that spermine brings about a compact tertiary structure in turnip yellow mosaic virus RNA.

In view of these facts, a brief study was undertaken into the effect of spermine on phage f_2 reproduction. Throughout these experiments, experimental procedures were used as described in detail elsewhere (SCHINDLER[1]). Spermine hydrochloride dissolved in distilled water was used.

First, the inhibitory effect of spermine on phage f_2 reproduction in *E. coli* K 13 Hfr was demonstrated: Bacteria growing in exponential phase in broth ($4 \cdot 10^7$ cells/ml) were infected with f_2 (0.3 phage particles per bacterium) with simultaneous addition of spermine. After 5 min, infective centres were titrated in a control flask without spermine. After 90 min, chloroform was added to all flasks and phage was titrated. In another experiment infected cells were centrifuged 5 min after infection and sedimented cells were resuspended in warm broth without spermine. Average yield of phage particles was calculated by dividing phage titre by infective centre titre. Table 1 shows that spermine inhibits phage development in concentrations ranging from 50–200 μg/ml (approximately $1.5 \cdot 10^{-4} M$ to $6 \cdot 10^{-4} M$). Although spermine inactivates about 30% of phage T 2 (MORA and YOUNG[14]), the inhibition of f_2 is not due to the interaction of spermine with

1 J. SCHINDLER, Folia microbiol. *9*, 269 (1964).
2 H. REITER, Virology *21*, 636 (1963).
3 G. FERROLUZZI-AMES and B. N. AMES, Biochem. biophys. Res. Comm. *18*, 639 (1965).
4 B. N. AMES and D. T. DUBIN, J. biol. Chem. *235*, 769 (1960).
5 T. D. BROCK and S. O. WOOLEY, Science *141*, 1065 (1963).
6 H. R. MAHLER and B. D. MEHROTRA, Biochim. biophys. Acta *68*, 211 (1963).
7 M. MANDEL, J. mol. Biol. *6*, 141 (1962).
8 H. TABOR, Biochemistry *1*, 496 (1962).
9 D. KAISER, H. TABOR, and C. W. TABOR, J. mol. Biol. *6*, 141 (1963).
10 S. L. HUANG and G. FELSENFELD, Nature *188*, 302 (1960).
11 G. L. CANTONI, Nature *188*, 300 (1960).
12 M. OCHOA and I. B. WEINSTEIN, Biochim. biophys. Acta *95*, 176 (1965).
13 S. MITRA and P. KAESBERG, Biochem. biophys. Res. Comm. *11*, 146 (1963).
14 P. T. MORA and B. G. YOUNG, J. biol. Chem. *237*, 1880 (1962).