Thiouracil in tobacco mosaic virus

2-Thiouracil is a potent inhibitor of tobacco mosaic virus multiplication[1] and of plant growth[2]. JEENER AND ROSEELS[3], reported that under conditions where yield of virus was reduced by about 50%, thiouracil was incorporated into the virus ribonucleic acid (RNA). Using ^{35}S labelled thiouracil they found activity in the virus RNA but not the virus protein. The amount of thiouracil present in the RNA, calculated from radioactivity data, was equivalent to about 20% of the virus uracil. When acid hydrolysed virus RNA was subjected to chromatography in the *iso*propanol-HCl solvent[4] they found that the activity had the mobility expected for thiouridylic acid. However both thiouracil and inorganic sulphate have mobilities in this solvent system close to that expected for thiouridylic acid.

We were unable to detect any incorporation of unlabelled thiouracil into TMV RNA, although with the analytical methods used 5% incorporation should have been detected[5,6]. In these experiments intact plants were used and thiouracil was applied by leaf sprays. Using JEENER's method of treating leaves with ^{35}S labelled thiouracil we have now been able to confirm the appearance of activity in the virus and to obtain further evidence that the thiouracil is incorporated into the virus RNA.

Virus was isolated from the leaves both by the method used by JEENER AND ROSEELS and by the usual procedure using ammonium sulphate precipitations. RNA was prepared by heating virus at pH 7 in the presence of salt. All the activity present in the intact virus was found in the virus RNA. The mixture of nucleotides obtained from the RNA after hydrolysis in *N* KOH at room temperature for 24 h was subjected to chromatography in the *iso*propanol ammonia solvent[7]. All the activity moved with the R_F expected for pyrimidine mononucleotides. This R_F is substantially less than either thiouracil or inorganic sulphate. The material from the nucleotide band was eluted and subjected to electrophoresis on filter paper at pH 9 in borate buffer. All the activity had about the mobility to be expected for thiouridylic acid (uracil: 0 cm/1 h/20 V/cm, thiouracil: 12 cm, uridylic acid: 12 cm, active material: 23 cm). On the other hand, at pH 3.5 where both uridylic and thiouridylic acids should have only one negative charge from the primary phosphate dissociation all the activity moved at the same rate as uridylic acid (about 6 cm/h/20 V/cm). RNA was hydrolysed in *N* HCl and subjected to chromatography in the *iso*propanol-HCl solvent, when all the activity moved in the same position as uridylic acid.

A further sample of RNA was digested to completion with pancreatic ribonuclease and the digest subjected to chromatography in *iso*propanol ammonia. Uracil and thiouracil have the same mobility in this solvent system and the presumed thiouridylic acid had the same mobility as uridylic acid. Thus if the thiouracil was in fact incorporated into the RNA, we would expect to find activity following chromatography of a ribonuclease digest in *iso*propanol ammonia, in all positions in which uridylic acid was present. Activity was found in the bands of di-, tri-, and poly-nucleotides containing uridylic acid, as well as in the free uridylic acid band.

From these results we conclude that the thiouracil was incorporated into polynucleotides of the virus RNA. From measurements of activity and optical density on both acid and alkaline hydrolysed RNA the amount of incorporation corresponded to about 3.5% of the uracil. This is substantially less than found by JEENER AND ROSEELS[3] or JEENER[8] but is about the same proportion of the normal base as that found for 8-azaguanine in T.M.V.[5]. RNA isolated from uninoculated tobacco leaves treated with ^{35}S thiouracil was found to contain low activity in the nucleotides after chromatography in *iso*propanol ammonia. The activity indicated incorporation of thiouracil equivalent to about 0.3% of the uracil.

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