Short Communications and Preliminary Notes

INCORPORATION OF 2-THIOURACIL-^{35}S IN THE RIBOSE NUCLEIC ACID OF TOBACCO MOSAIC VIRUS

by

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The inhibitory action of thiouracil on tobacco mosaic virus development[1] is suppressed by uracil, and appears to result from a competition of both substrates in some enzymic reaction during the synthesis of ribose nucleic acid (RNA). But, as one of us observed, the inhibition of virus synthesis is greater the smaller the amount of virus present in the leaves where thiouracil is added. This fact is not explained by a competition between uracil and thiouracil. We thus were led to believe that thiouracil could be incorporated into the virus nucleic acid and that this incorporation hindered the multiplication of the modified particles. If this interpretation were correct, the proportion of modified particles would influence the speed of virus synthesis and we could expect a greater inhibition when thiouracil is allowed to act earlier.

To check this hypothesis, we added ^{35}S labelled thiouracil* to VICKERY's saline solution on which tobacco leaves, infected two days previously, were cultured. The concentration of thiouracil was such that the speed of growth of the virus was reduced by 50%.

The virus is then isolated by the method of COMMONER[2] and recrystallized three times. The RNA is extracted by heating the virus solution at 100° C[3] and is completely deproteinized by prolonged shaking with chloroform/butanol. The product obtained is checked spectrophotometrically. The protein moiety of the virus is not radioactive, the RNA on the contrary is very active. Calculations show that the quantity of thiouracil incorporated amounts to about 20% of the normal quantity of uracil.

The tagged RNA is hydrolyzed for 30 minutes at 100° C in 1 *N* HCl. The hydrolyzate, which contains the intact pyrimidine nucleotides, is chromatographed on paper according to MARKHAM[4]. The total quantity of ^{35}S chromatographed belongs to a component with an R_F value 6% greater than uridylic acid, and consequently very different from the R_F values of all the other components of nucleic acid. It appears thus very probable that it is thiouridylic acid.

However, after two hours hydrolysis in concentrated formic acid at 175° C, in order to obtain free pyrimidine bases, we only found in the thiouracil 20% of the ^{35}S incorporated in the RNA. This might be due to a partial destruction of the thiouracil during hydrolysis or to an incomplete hydrolysis of thiouridylic acid.

We would further like to mention that HEINRICH, DEWEY, PARKS AND KIDDER[5] have already demonstrated that 8-azaguanine could be incorporated in RNA of *Tetrahymena geleii*.

REFERENCES

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