The distribution of thiouracil in nucleic acid of tobacco mosaic virus

The incorporation of thiouracil into tobacco mosaic virus, first observed by JEENER AND ROSEELS[1], was confirmed by MATTHEWS[2] by the characterization of thiouridylic acid extracted from the virus nucleic acids. The present study describes the distribution of thiouracil in the polynucleotides.

^{35}S-2-thiouracil was applied to detached tobacco leaves infected with tobacco mosaic virus under the conditions described by JEENER[3]. The leaves were then frozen, ground and the sap expressed and heated to 55°. Coagulated proteins were spun off at 10,000 r.p.m., and the supernatant liquid was centrifuged at 40,000 r.p.m. for 30 min in the "40" rotor of the Spinco preparative centrifuge. The residue was resuspended in water and the centrifuging repeated. The virus was then taken up in 2 *M* NaCl and the solution heated to 100° for 10 minutes. Half a volume of ethanol was added and the coagulated virus protein, which contained a trace of radioactivity, was spun off. The nucleic acid was precipitated from the supernatant liquid by the addition of 3 vol. ethanol.

The nucleic acid was treated with pancreatic ribonuclease at pH 7.0 at 37° for 3 days, and the hydrolysate was concentrated and chromatographed on Whatman No. 3 MM paper in *iso*propanol-NH_3 for 72 hours[4]. A further separation was then obtained by electrophoresis at pH 3.5 at right angles to the chromatographic separation (2 h at 20 V/cm). This treatment separates the constituents of a ribonuclease digest into easily recognizable groups[5]. Because of the close structural similarity between thiouracil and uracil, compounds containing the former substance would be expected to occupy positions near those in which the various uracil compounds appeared. The positions of the various substances were found in three ways. Ultraviolet photography[6] localized absorbing areas due to purine and pyrimidine derivatives, photography of fluorescence under acid conditions showed the position of guanine compounds[7], and radioautography located thiouracil compounds, which were in too small concentration to show absorption. By this means thiouridine, thiouridylic acid, adenylylthiouridylic acid and guanylylthiouridylic acid were recognized. An additional compound characterized by having a low R_F value in *iso*propanol-NH_3 and a high electrophoretic mobility was also noticed. This substance had the properties which one would expect of thiouridine 3′:5′-diphosphate. For confirmation, this compound was electrophoresed mixed with authentic thymidine 3′:5′-diphosphate*, which should be closely similar to the former with respect to charge. At pH's 5.0, 7.4 and 9.2 these two compounds moved with the same electrophoretic mobility, thus confirming the nature of the extra compound. Additional radioactive compounds were present in small amounts evidently corresponding to small poly-

TABLE I

RELATIVE RADIOACTIVITIES IN VARIOUS NUCLEIC ACID HYDROLYSATE FRACTIONS AFTER INCORPORATION OF ^{35}S-THIOURACIL INTO TOBACCO MOSAIC VIRUS

Compound isolated	*% of total* ^{35}S
1. thiouridine	4
2. thiouridylic acid	37
3. thiouridine 3′:5′-diphosphate	25
4. adenylylthiouridylic acid	12
5. guanylylthiouridylic acid	8
6. unhydrolyzed "core"	14

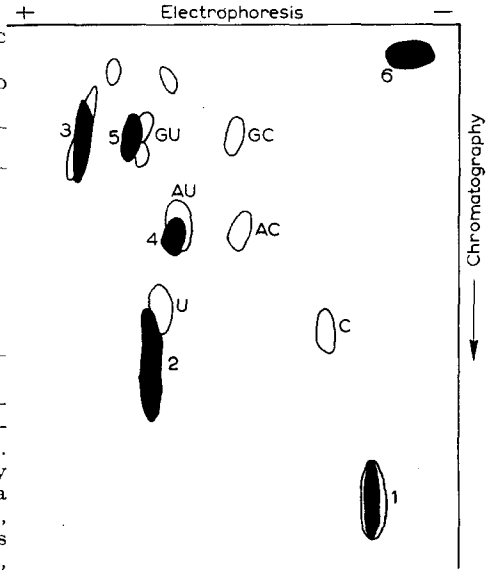

Fig. 1. Separation pattern of compounds derived from ribonuclease treatment of thiouracil-containing tobacco mosaic virus nucleic acid. Mixture was applied at top right, followed by chromatography in *iso*propanol-ammonia in a vertical direction and electrophoresis at pH 3.5, negatively charged compounds moving towards the left. Black spots due to thiouracil metabolites, with numbers corresponding to those in Table I. Other spots due to normal fragments from digest, as indicated, the letters referring to the initial letters of the nucleotides, thus AU is adenylyl-uridylic acid, C is cytidylic acid, etc.

* The thymidine 3′:5′-diphosphate was a gift from Dr. A. M. MICHELSON.

nucleotides. The results of the two-dimensional separation scheme are illustrated in Fig. 1, and the relative distribution of radioactivity is shown in Table I.

Thiouracil thus seems to occupy several of the positions in the nucleic acid of this virus which are normally occupied by uracil. The relatively large proportion oft hiouridine and thiouridine diphosphate liberated by ribonuclease from thiouracil-containing virus nucleic acid is striking. Because of the known specificity of this enzyme, it follows that thiouracil has a tendency to be concentrated at ends of polynucleotide chains, and particularly more at one end than the other.

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On the substrate specificity of glucose oxidase*

Glucose oxidase (notatin) is an enzyme generally considered since the extensive work of KEILIN AND HARTREE[1,2] as highly specific for glucose. The activity relative to glucose with more than fifty related compounds tested was found to be of the order of 1% or less. This high specificity prompted its application to the estimation of glucose in the presence of other sugars[3]. The dependence on a manometric method has acted as some sort of deterrent, which has prevented a wide utilization of glucose oxidase as an analytical tool. The recent adaptation of glucose oxidase activity to colorimetric methods, including its simplification to a paper test[4], will undoubtedly considerably increase the scope of the analytical utilization of glucose oxidase. In attempting to fill in certain gaps of information we have found that 2-deoxyglucose is a fairly good substrate for glucose oxidase. This is important, not only because of the enzymic interest, but also because of the increasing significance of 2-deoxyglucose as an inhibitor of glucose metabolism[5].

For the screening of possible substrates and competitive inhibitors we have utilized the "Tes-Tape" of the Eli Lilly Company, which was kindly made available to us by Dr. J. L. R.-CANDELA. Preliminary tests with low concentrations of glucose indicated that the reciprocal of the time required to obtain a similar degree of color could be used within certain limits as a semiquantitative test of enzyme activity. In some instances the observations were completed manometrically[3], using a preparation of glucose oxidase kindly supplied by the Sigma Chemical Company.

The significant results are summarized in Table I. The relatively high affinity and maximal rate with 2-deoxyglucose obviously prevent the utilization of glucose oxidase for the estimation of glucose in the presence of 2-deoxyglucose. The main reason for other related compounds being poor substrates appears to be a decrease in the affinity of the enzyme for these compounds with respect to glucose. We have observed, both with the Tes-Tape and manometrically, that at 0.05 M concentration the rate with mannose is approximately 1% that with glucose, in agreement with KEILIN AND HARTREE[1]; but the rate of mannose oxidation is essentially proportional to the increase in concentration up to at least 0.5 M. Glucosamine, as could be expected, is not oxidized by the enzyme as the glucosammonium ion, but it is a substrate in the un-ionized form which prevails in alkaline medium. On the other hand, the fact that the apparent activity with maltose can be completely prevented by hexokinase-ATP-Mg clearly indicates that some maltolytic activity is present in the preparation of glucose oxidase. Lack of parallel activity on methyl-α-glucoside makes it easy to understand why the apparent oxidation of maltose was originally interpreted as genuine[1]. The oxidase has no significant affinity for 1,5-sorbitan, although it can

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