Further Studies on the Deoxyribonucleic Acid Exonuclease Induced by Herpes Virus

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Infection of mammalian cells with herpes virus is followed by an increase in alkaline deoxyribonuclease (Keir & Gold, 1963). The virus-induced deoxyribonuclease differs in its properties and is physically separable from the deoxyribonuclease present in non-infected cells (Morrison & Keir, 1968). Analysis of the products of digestion of DNA indicates an exonucleolytic mode of attack, and immunological evidence supports the hypothesis that the enzyme protein is specified by the viral genome (Morrison & Keir, 1967). Experiments have been carried out to characterize further the action of the enzyme, in the hope that this will shed some light on the role of the deoxyribonuclease in the infective process.

DNA was labelled with [^{32}P]TMP residues at the 3'-end in the limited reaction with the DNA polymerase partially purified from *Escherichia coli* or from herpes-infected cells. When this material was digested with herpes exonuclease (DEAE-cellulose fraction), the rapid release of radioactivity into the acid-soluble fraction indicated a stepwise attack from the 3'-terminus of the polydeoxyribonucleotide chain.

This conclusion was confirmed by a study of the attack of herpes exonuclease on (i) 3'-phosphoryl oligonucleotides from DNA digested with micrococcal nuclease; (ii) 5'-phosphoryl oligonucleotides from DNA digested with pancreatic deoxyribonuclease; (iii) chemically synthesized thymidine oligonucleotides of both types. Whereas 3'-phosphoryl oligonucleotides were not attacked, 5'-phosphoryl oligonucleotides of varying chain length, including dinucleotides, were quantitatively hydrolysed to deoxyribonucleoside 5'-monophosphates.

The deoxyribonuclease and DNA polymerase induced by herpes virus co-chromatograph on DEAE-cellulose (J. M. Morrison, R. D. Paton and H. M. Keir, unpublished work) and it was thus of interest to investigate whether these activities were associated. When the four deoxyribonucleoside 5'-triphosphates were added to the deoxyribonuclease assay medium, no change in deoxyribonuclease activity was observed. This observation, along with the fact that the deoxyribonuclease activity is less heat-sensitive than the DNA polymerase, would suggest that, unlike the phage T4-induced DNA polymerase-exonuclease (Goulian, Lucas & Kornberg, 1968), the two enzymes are not directly associated. Attempts are being made to separate the two activities in order that they may be studied independently.

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The Presence of Thiouridylic Acid in Soluble and Ribosomal Ribonucleic Acid and the Sensitivity of Ribosomes to a Thiol Reagent

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Lipsett (1965) showed that thioUMP* is a minor component of sRNA from *Escherichia coli* and described a spectrophotometric assay for the nucleotide in intact RNA molecules. Since this thionucleotide is of potential importance, we have further investigated its distribution in bacteria and also in rat liver. To do this we developed a paper-chromatographic assay for thioUMP in alkaline hydrolysates of RNA, by using as solvent system a mixture of propan-1-ol-aq. ammonia (sp.gr. 0.88)-water (11:2:7, by vol.), in which the thioUMP runs ahead of the common nucleotides. With this system we can detect thioUMP in the hydrolysate of RNA labelled with [^3H]uridine. We also labelled the RNA by growing the bacteria in [^{35}S]sulphate. By using the above methods we have shown that there is an average of 1 thioUMP residue/approx. 1.5 molecules of sRNA in *E. coli*, and that thioUMP is also present in rRNA from this organism.

The presence of thioUMP in sRNA from various sources was detected spectrophotometrically and also chromatographically, and its presence in rRNA from these sources was detected chromatographically. Of the bacteria we studied the RNA of some contained thioUMP, and that of others did not (see below). We never obtained evidence for the presence of thioUMP in sRNA and not rRNA, nor vice versa. We were unable to detect thioUMP in either RNA fraction from rat liver.

We found a correlation between the presence of thioUMP in rRNA and the dissociation of 70S ribosomes into 30S and 50S sub-units with *p*-chloromercuribenzoic acid. The fact that ribosomes of *E. coli* are sensitive to this reagent was shown by Miyazawa and Tamaoki (1967).

Of those bacteria we studied, the rRNA of three (*E. coli*, *Micrococcus lysodeikticus* and *Serratia*

* Abbreviations: thioUMP, 4-thiouridylic acid; sRNA, soluble RNA; rRNA, ribosomal RNA.