The Effect of Bacteriophage ϕ X174 Infection on Deoxyribonucleic Acid, Ribonucleic Acid and Protein Synthesis

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Phage ϕ X174 can inhibit bacterial macromolecular synthesis by two distinct mechanisms. At a multiplicity of infection of less than 6 phages/cell, host DNA synthesis is abruptly shut off after a delay of 15 min., but the formation of RNA and protein continues (Lindqvist & Sinsheimer, 1967). This effect is not seen after infection in the presence of 30 μ g. of chloramphenicol/ml. At a higher multiplicity (e.g. 20–40 phages/cell), the formation of DNA, RNA and protein is prevented by a mechanism that is inhibited even by 100 μ g. of chloramphenicol/ml. (A. B. Stone, unpublished work). This may result from phage ϕ X174 causing peripheral damage to the cells. If the damage is slight, the infected cell appears to be able to 'strengthen' its outer layers: infection with 4 phages/cell prevents 20 phages/cell, given 9 min. later, from significantly affecting macromolecular synthesis.

The specific arrest of host DNA synthesis 15 min. after infection with 4 phages/cell has been studied further. Addition of 30 μ g. of chloramphenicol/ml.

at any time up to about the tenth minute prevents the arrest, suggesting that an inhibitory protein is manufactured at about this time. The delay preceding this does not reflect the need to accumulate a pool of viral 'replicative form' DNA, for if a pool of 'replicative form' is made in the presence of 30 μ g. of chloramphenicol/ml. and the chloramphenicol is then removed, a delay of over 15 min. is still observed before bacterial DNA synthesis stops, even though protein synthesis in general is resumed at once. This further suggests that the inhibitory protein is manufactured only after one or several other proteins have been made. The kinetics of inactivation by nitrous acid treatment of the shut-off function of the virus suggest that the synthesis of the inhibitory protein may be dependent on the functioning of a viral gene.

The identity of the responsible gene is not known. Mutations in five of the six known phage ϕ X174 cistrons do not affect the arrest function; the remaining cistron, which specifies one of the proteins of the viral capsid, has not yet been tested. It is inferred that the proteins involved are either capsid components or else are products of cistrons so far unrecognized.

Lindqvist, B. H. & Sinsheimer, R. L. (1967). *J. molec. Biol.* **28**, 87.

DEMONSTRATION

The Detection of Impurities in Tetrazolium Salts by Thin-Layer Chromatography

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It is well known that the concentration of tetrazolium salts can be critical in studies of biological reducing systems. Commercial samples often contain insoluble impurities and residual solvents of crystallization (e.g., see Nineham, 1955), but the presence of other salts of similar chemical constitution is a more important cause of non-uniformity between different batches, since these compounds can also be reduced to formazans of various colours (Jones, 1969). Techniques for the detection of these contaminating tetrazolium salts have been developed.

Monotetrazolium salts have been run on thin-layer chromatograms (DC-Fertigplatten Kieselgel F₂₅₄; E. Merck A.-G., Darmstadt, Germany) with 3-methylbutan-1-ol-formic acid-water (16:1:1, by vol.). Formazans were produced on the dry plates by reduction with an alkaline solution of sodium

ascorbate. Ranges of R_F values, colours of formazan spots, and wavelengths of maximal light absorption by formazans dissolved in pyridine are as follows: 2,5-diphenyl-3-(1-naphthyl)tetrazolium chloride (DNC), 0.56–0.64, violet, 517 nm.; 2,5-diphenyl-3-(4-styrylphenyl)tetrazolium chloride (DSC), 0.70–0.75, violet, 525 nm.; 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT), 0.62–0.70, blood-red, 492 nm.; 2,5-diphenyl-3-(4,5-dimethylthiazol-2-yl)tetrazolium bromide (MTT), 0.46–0.54, blue, 565 nm.; 2,3,5-triphenyltetrazolium chloride (TTC), 0.51–0.58, blood-red, 490 nm. 2,3-Diphenyl-5-methyltetrazolium chloride, (DMC) (orange formazan; $\lambda_{\max}$. 415 nm.) ran more slowly (R_F 0.29–0.35), and tailed badly. Of the samples examined, DMC and MTT each gave rise to only one major formazan spot; all the others each gave rise to at least one very minor secondary spot of identical colour and similar R_F . It is probable that some of these minor spots represent isomers in which the point of attachment of an aryl ring is altered.

Bistetrazolium salts, on the other hand, may be extensively contaminated by monotetrazolium salts, partly because of the difficulty in removing

monoformazan by-products which arise during the synthesis of bisformazan intermediates (e.g., Tsou, Cheng, Nachlas & Seligman, 1956). Such impurities may be readily detected as described above if the proportions of the solvent mixture are altered to 8:1:1. Nine bistretazolum salts have been separated from their contaminants by this means;

information about R_F values, and colours and spectra of the corresponding formazans (Jones, 1969), will be demonstrated.

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Nineham, A. W. (1955). *Chem. Rev.* **55**, 355.

Tsou, K.-C., Cheng, C.-S., Nachlas, M. M. & Seligman, A. M. (1956). *J. Amer. chem. Soc.* **78**, 6139.