THE STRUCTURE OF THE BACTERIOPHAGE ALPHA DNA MOLECULE

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WILLIAM DAVID SUTTON

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ABSTRACT

Selective precipitation with polyethylene glycol is an efficient method for concentrating and purifying bacteriophage α and other phages.

The phage α DNA molecule has a molecular weight of 33 million. When prepared by phenol extraction of crude phage suspensions, it contains many single-strand breaks. When prepared by phenol extraction of purified phage, it contains approximately one randomly-located single-strand break per molecule. The number of single-strand breaks can be further reduced by changing the conditions of the phenol extraction.

The complementary single strands of α DNA can be separated by MAK chromatography followed by self-annealing and hydroxylapatite chromatography, but this procedure results in extensive breakage of the strands. An alternative procedure has been developed using CsCl gradient centrifuging in the presence of polyguanylic acid (polyG) to give an efficient separation of the intact strands in 100 μg. quantities.

Both the L strand and the H strand of α DNA form complexes with polyG, although to different extents. The PolyG binding sites in the L strand appear to be confined to a small segment having a similar buoyant density to the H strand.

Sequences of consecutive pyrimidine nucleotides of all lengths up to 13 have been detected in diphenylamine-formic acid digests of α DNA. There is a slight general tendency towards clustering of the pyrimidine nucleotides, sequences of lengths 1-4 being present at

below random frequencies, and longer sequences being present at above random frequencies. These same general features are found in diphenylamine digests of the separated H and L strands. The distribution of pyrimidine nucleotide sequence lengths in α DNA does not appear to follow a rhythmic code of the type found in RNA phages.

Preliminary analyses have been made of the longest pyrimidine nucleotide sequences in α DNA, and of the distribution of various sequences between the two strands.

The dialysis of pyrimidine deoxyoligonucleotides was investigated, and found to be strongly influenced by cytosine content. This may reflect an unusual conformation of cytosine-rich oligonucleotides at low ionic strength. Gel filtration was found to provide a satisfactory method for the preliminary fractionation of diphenylamine digests on the basis of chain length.

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ABBREVIATIONS

α	alpha
p.f.u.	plaque-forming units
DNA, RNA	deoxyribonucleic acid, ribonucleic acid
L strand	purine-rich strand of alpha DNA
H strand	pyrimidine-rich strand of alpha DNA
DNase	deoxyribonuclease
A,G,C,T	adenine, guanine, cytosine, thymine
polyG	polyriboguanylic acid
polyIG, polyUG	copolymer of guanylic acid with inosinic acid, uridylic acid
PEG	polyethylene glycol
MAK	methylated albumin adsorbed on kieselguhr
SDDC	sodium diethyldithiocarbamate
EDTA	diaminoethanetetra-acetic acid
tris	tris(hydroxymethyl) aminomethane
SSC	0.15 M NaCl, 0.015 M sodium citrate (pH 7)
SP	saline phosphate buffer pH 6.8, containing 0.05 M phosphate
PB	phosphate buffer pH 7.0
DEAE-	N,N-diethylamino-ethyl-
TCA	trichloroacetic acid
PPO	2,5-diphenyloxazole
POPOP	1,4-bis-(5-phenyloxazolyl-2)-benzene
Py	pyrimidine nucleotide
p	esterified phosphate (5' to the left, 3' to the right)
d	deoxy-
A, n, ε	absorbance, refractive index, extinction
η, ρ	intrinsic viscosity, density
S^0	sedimentation coefficient at infinite dilution
I, M	ionic strength, molarity
P.S.F.	percentage sequence frequency
S.E.	standard error

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