THE SUBUNIT OF DEOXYRIBONUCLEIC ACID*

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The DNA molecules of E. coli B have been shown by Meselson and Stahl (1958) to be composed of two subunits, one of which is parental and the other, newly synthesized. Similar results have been obtained at the chromosomal level by Taylor, Woods and Hughes (1957). It is obviously essential to know the nature of the molecular subunit in considering possible mechanisms of replication at both levels.

Experiments carried out in this laboratory show that the subunit consists of two strands, rather than the single polynucleotide chain necessitated by the Watson-Crick (1953) replication hypothesis. Molecular-weight measurements by the light-scattering method confirm the results obtained by Meselson and Stahl, who used the method of equilibrium sedimentation in a density gradient, that the molecular weight of E. coli DNA is reduced to half by heating in cesium chloride. However, the DNA used by them has been found to be an aggregate held together by protein links; this type of linkage differs from that occurring between the two subunits.

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DNA isolated from E. coli B grown under the identical conditions used by Meselson and Stahl and deproteinized by the Duponol method had a molecular weight of 11 x 10^6 (light scattering) and, when centrifuged in CsCl, formed a band identical with that obtained by Meselson and Stahl. The molecular weight dropped to 5.6 x 10^6 on heating to 100° in CsCl, and the band width increased. Heating in the absence of CsCl did not alter the molecular weight.

When the 11 x 10^6 DNA was either treated with chymotrypsin, or shaken with a chloroform-octanol mixture, its molecular weight dropped to 2.4 ± 0.2 x 10^6 while its length actually increased. Repeated treatments with either agent had no further effect. That both treatments lead to the same molecular weight indicates that chloroform-octanol does not degrade. This 2.4 x 10^6 DNA had typical properties: a hyperchromic shift with acid of 40%; a normal optical density-temperature curve (Cavalieri and Rosenberg (1959)) which is sigmoid with a midpoint at 92°; a large radius of gyration, indicating a stiff chain. The molecular weight of this DNA was reduced to one half by heating in CsCl; from 2.4 x 10^6 to 1.3 x 10^6.

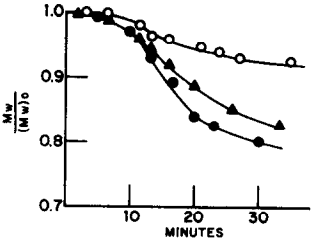

Figure 1. Enzyme kinetics for heated DNA's. The abscissa gives the ratio of the weight-average weight at any time to the initial weight average weight. ● Sample 4; 0 Sample 5; ▲ Denatured calf thymus DNA obtained by heating in NaCl.

Enzyme kinetics (Fig. 1) using DNase II showed that the DNA so split (1.3 x 10^6) was not a single strand, as evidenced by the induction period (Shumaker, Richards, and Schachman (1956); Thomas (1956)). The number

of strands per molecule in the split DNA was found to be 2.3 when calculated from the initial part of the curve, according to a method similar to that developed by Shumaker, Richards and Schachman. A control kinetic study carried out on DNA heated in the absence of CsCl and thus unsplit showed the number of strands to be 4.0. Calf thymus DNA, which does not split when heated in CsCl, showed 2.2 strands.

We deduce that the unaggregated, unit DNA molecule of E. coli is actually a dimer composed of two double helices, laterally bonded together; that each double helix is conserved intact during cell division; and that the bonds holding the dimer together are ruptured by heating in CsCl, as well as by some part of the replication cycle in the cell. The dimer bonds are clearly different from the protein links in the 11×10^6 aggregate, which are unaffected by heating in CsCl.

On these grounds it might be predicted that the DNA molecule would be monomeric just before DNA synthesis occurs, and dimeric thereafter. This hypothesis was tested by preparing DNA from E. coli 15_T- (strain kindly supplied by Dr. Seymour S. Cohen) which had been synchronized by withholding thymine. Barner and Cohen (1956) have shown that when thymine is added DNA synthesis occurs immediately, followed by synchronous cell division. This DNA had a molecular weight of 1.3×10^6, but was found to split to 0.65×10^6 on heating in CsCl. Thus, the dimer bonds are not broken previous to the initiation of DNA synthesis in E. coli; the rupture may occur rather as an integral part of the synthetic process. It is possible that the isolation of dimeric and monomeric DNA from different species is a consequence of different chronological relationships within the division cycles of different types of cells.

TABLE I

Sample	Isolation*	Treatment	Molecular Weight X 10^{-6}	Radius of Gyration Å
1 - E. coli B	-	None	11	1600
2 "	-	Heated in CsCl	5.6	1100
3 "	$CHCl_3$-octanol	None	2.4	1770
4 "	"	Heated in CsCl	1.3	430
5 "	"	Heated without CsCl	2.3	550
6 "	Chymotrypsin	None	2.6	1300
7 - E. coli 15_T-	$CHCl_3$-octanol	None	1.3	1340
8 "	"	Heated in CsCl	0.65	460
9 - Calf thymus	"	None	3.3	2300
10 "	"	Heated in CsCl	3.5	1340

* All samples were deproteinized by means of Duponol. Samples 3 - 10 were further deproteinized as indicated.

Experimental

DNA was originally isolated from the bacteria by stirring with Duponol in 1 M NaCl - 0.01 M EDTA, pH 6. Further deproteinization in some cases was accomplished either by treatment with chymotrypsin or by shaking with chloroform-octanol in 0.2 M or 2.0 M NaCl.

All molecular weights are weight averages and were determined by light scattering, usually in 0.2 M NaCl, repeated several times. Equilibrium sedimentations were carried out in 0.01 M tris - 7.7 molal CsCl (Maywood Co.), pH 8.5 - 9.5,at 25° and 44, 700 r. p. m. The dimer was split by heating to 100° for 30 minutes in the same medium, with the addition of 0.1 M EDTA. Following heating the medium was adjusted to 0.2 M NaCl - 0.01 M Na_3EDTA by dialysis before light-scattering measurements were carried out. The denatured DNA had a tendency to aggregate in the presence of small amounts of divalent metal ions. This was overcome by the use of EDTA, in some

cases warming for 4 minutes to 60° to accelerate chelation, and by shaking with chloroform-octanol before light scattering to remove any residual protein, which appears to be involved in the aggregation. Melting-point curves (optical density vs. temperature) were determined in 0.2 M NaCl.

Kinetic studies were carried out in 0.15 M NaOAc - 0.01 M EDTA, pH 5.0, at 34± 0.5°. Measurements of scattered light were made from 35° to 90° and extrapolated to 0° to calculate the ratio $M_w/(M_w)_o$. The error of the readings, limited by the galvanometer, is less than 0.5%. The number of strands calculated is correct to within about 0.3. DNase II was used in the form of a purified extract obtained from mitochondrial debris of mouse sarcoma 180. This was used since it requires no divalent metal ions as activators, thus avoiding aggregation of the denatured DNA. For these runs the DNA concentration was 0.065 mg/ml and the enzyme about 10^{-6} times as much.

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