SUBUNIT FORM OF CALF THYMUS DNA*

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The persistent occurrence of amino acid residues in preparations of human leucocyte deoxyribonucleic acid (DNA) and degradation of the DNA into uniform subunits by 0.1 N sodium hydroxide and hydroxylamine have led Bendich (1963, 1964) to postulate that DNA is composed of polynucleotide subunits. These subunits appear to have an approximate molecular weight of 500,000 and they are believed to be interlinked by amino acids or simple peptides. The above evidence, plus the isolation of O-phosphoserine from human leucocyte DNA upon mild acid hydrolysis, enabled Bendich to suggest the hypothetical linkage depicted in Figure 1. The linkers between polynucleotide units were suggested to be hydroxyamino acids or simple peptides thereof.

Welsh (1962) has described the isolation of non-fibrous DNA from calf thymus nuclei with a molecular weight of 500,000. Furthermore, he has described the extraction of an acid-soluble, phosphate-containing polypeptide from calf thymus nuclei which may link the DNA subunits to cause their polymerization.

In this paper we will present evidence to show that calf thymus DNA is degraded into subunits by hydroxylamine at pH 9 and that papain will reduce the sedimentation constant to a considerable extent, thus lending additional evidence to the proposed existence of peptide linkers.

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METHODS AND MATERIALS

DNA was prepared from calf thymus glands obtained immediately after slaughter by the procedure of Kay et al (1952). When hydrolyzed in strong acid, this DNA preparation was shown to contain approximately 0.3% amino acid residues by weight.

One ml of 0.2 M citrate buffer (pH 6), one ml of 0.05 M cysteine, one ml of 0.01 M EDTA and two mg of papain (General Biochemicals) were dissolved in 10 ml of water and incubated at 45° C for 30 minutes. Ten ml of a 0.1% DNA solution were added to the above preincubated mixture and the entire mixture was incubated for three hours at 37° C. The incubation mixture was kept at 4° C from two to seven days. A DNA control which did not contain papain was treated in exactly the same manner. A second control which contained inactive papain (since cysteine and EDTA were not added) was also treated in the same manner. The incubated mixtures were analyzed in a Spinco Model E analytical ultracentrifuge using schlieren optics.

To 12 mg of DNA dissolved in water were added four ml of 0.2 M Tris buffer (pH 9) and four ml of neutralized 2 N hydroxylamine. The solution was incubated at 37° C for 24 hours. A DNA control which did not contain hydroxylamine was treated in exactly the same manner. Potassium chloride (0.6 gm) was added to the control to equalize its salt concentration with that of the hydroxylamine-treated solution. Four ml aliquots of the incubation mixtures were dialyzed overnight in the cold against 0.15 N NaCl-0.015 M sodium citrate. The dialyzed solutions were analyzed in a Spinco Model E analytical ultracentrifuge using schlieren optics. Aliquots that had remained at room temperature for 120 hours after the initial 24 hours incubation at 37° C were also examined in the same manner.

RESULTS AND DISCUSSION

A small reduction in the sedimentation constant of calf thymus DNA from 21.7 to 16.3 S (8,130,000 M.W. to 3,570,000 M.W.) was observed when it was incubated for approximately a week with the proteolytic enzyme papain. This

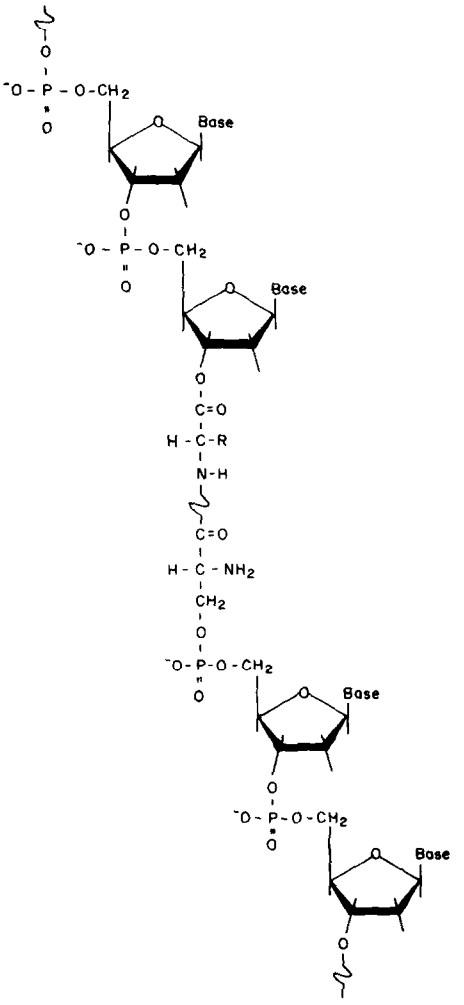


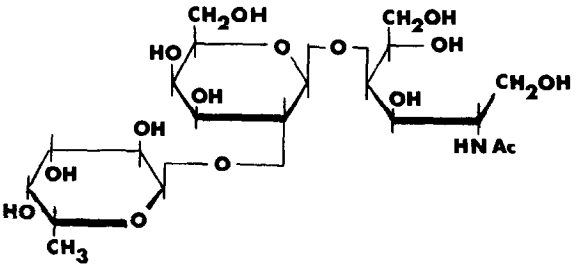
Figure 1. Proposed chemical linkages of amino acid residues in DNA. This formulation was originally postulated by Bendich (1963, 1964).

reduction in S value could not be due to an artifact of papain complexing with DNA, thus resulting in a change in molecular configuration and S value. This conclusion can be drawn, since a control using inactive papain was found to have no effect on the S value of the DNA. The S^{o}_{20} obtained from a plot of $1/S_{20}$ against concentration is shown in Figure 2. These results suggest that calf thymus DNA may be composed of long polynucleotide subunits interlinked by peptides. Butler, Phillips and Shooter (1957) reported that chymotrypsin,

of theoretical). These data provide strong evidence for the linkage of the fucose to the galactose at position 2, rather than 6. In addition they rule out the possibility of a branched structure (in which fucose and galactose are both linked to the N-acetylgalactosaminitol), for in this case the galactose should react quantitatively (Avigad et al, 1962).

Methylation. Approximately 5 mg each of the purified material and 2'fucosyllactose as standard were subjected to Haworth methylation at 4°. The reaction mixtures were neutralized, deionized with Amberlite MB-3, and lyophilized. The residues were dried in vacuo over P_2O_5 and further methylated (Purdie technique) in dimethylformamide as described by Montgomery et al (1965). The reaction mixtures were again neutralized, deionized and lyophilized, and following hydrolysis in 1 N HCl at 100° for 2.5 hours, examined by paper chromatography with Solvents A, D, E, and F. With each solvent a component was derived from both starting materials which co-chromatographed with authentic 3,4,6-tri-O-methylgalactose. The 2,3,4- and 2,4,6-tri-O-methylgalactose derivatives moved slightly ahead of the 3,4,6- derivative in all 4 solvents. Only traces of galactose and fucose were observed, indicating nearly complete reaction in both cases. These data establish conclusively the linkage of fucose to galactose at position 2.

Conclusions. These results lead us to the conclusion that the trisaccharide derived from porcine submaxillary glycoprotein has the structure:



α-L-fucopyranosyl-(1→ 2)-β-D-galactopyranosyl-(1→ 4)-N-acetyl-D-galactosaminitol.

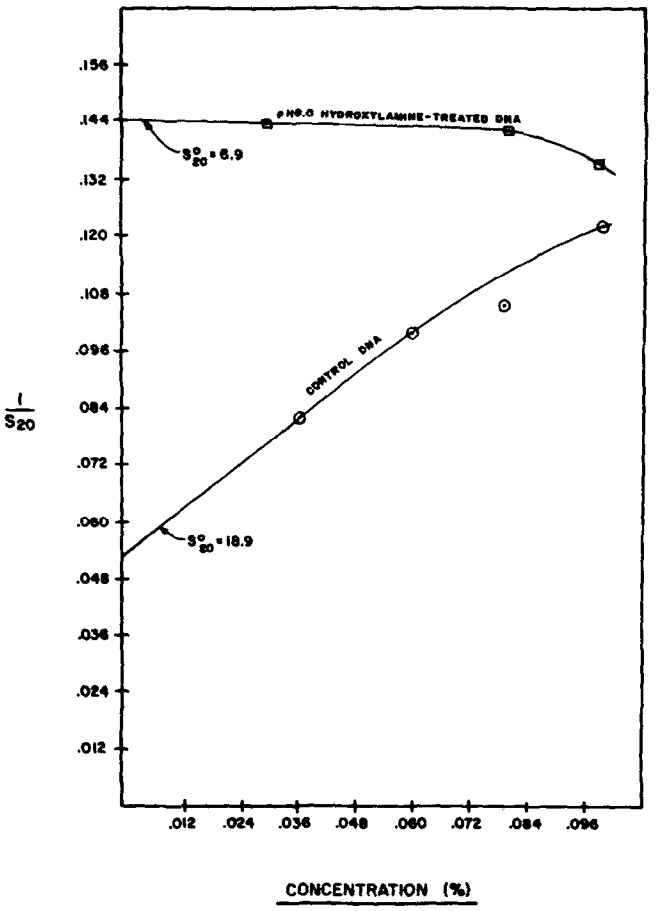


Figure 3. Determination of the sedimentation constants of normal and hydroxylamine-treated DNA. See "Methods and Materials" for details of the hydroxylamine treatment.

1955; Lipmann and Tuttle, 1954). Figure 3 shows that the sedimentation constant of DNA is reduced from 18.9 to 6.9 S (5,440,000 M.W. to 296,000 M.W.) by hydroxylamine at pH 9. No reduction was observed at pH 5.4 or 7.7. This indicates that the postulated peptides could be linked through a C-terminal carboxyl group to the 3'-hydroxyl group of deoxyribose at one end of a polynucleotide subunit as shown in Figure 1. DNA that had been incubated for 144 hours with hydroxylamine did not show any further reduction in sedimentation constant when compared with the 24 hours incubation sample.

Since a portion of the non-extractable peptide material can be released

from DNA in the form of an insoluble precipitate when DNA is degraded by DNase I and snake venom phosphodiester (Lesko and Emery) and since Bendich (1963) has isolated O-phosphoserine from human leucocyte DNA, it would appear conceivable that a phosphodiester linkage may be involved in the binding of the postulated peptides to DNA. Such a bond is possible when one of the hydroxy-amino acids, serine or threonine, is esterified to the 5'-phosphate group at one end of a polynucleotide subunit as suggested by Bendich and shown in Figure 1.

Our amino acid analyses of calf thymus DNA show a distribution of one serine or threonine residue per 775 nucleotide residues (Lesko and Emery). This permits one to calculate a molecular weight of approximately 250,000, assuming one serine or threonine molecule per peptide unit, for the postulated polynucleotide subunits and is similar in size to the subunits isolated from calf thymus nuclei by Welsh (1962) and to the fragments obtained by Bendich after hydroxylamine degradation of human leucocyte DNA.

The data reported here lend additional support to the DNA-peptide structure proposed by Bendich. Furthermore, it has been demonstrated that papain is capable of producing subunits of calf thymus DNA which have molecular weights one-half that of the purified DNA while hydroxylamine is capable of producing smaller subunits which have molecular weights approximately one-twentieth that of the purified DNA.

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