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Interaction of Heat-Denatured HeLa Cell DNA with Synthetic and Natural Polysaccharides

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Synopsis

Heat-denatured DNA from HeLa cells interacts with natural as well as synthetic polysaccharides. Glucose does not inhibit the interaction nor will it produce it. Polysaccharides with a molecular weight of 10 000 or greater are required before the interaction takes place.

Introduction

Native DNA and polysaccharide (CHO) polymers found in an extract of HeLa cells,[1] as well as the DNA and CHO in sea urchin mitochondrial preparations,[2] band at similar buoyant densities in CsCl. Another association of the two polymers is the interaction of heat-denatured HeLa cell DNA (ΔDNA) with a CHO from the same kind of cells.[3] This interaction may possibly be explained on the basis of hydrogen bonding. If hydrogen bonding accounts for the interaction, CHO polymers other than those isolated from the same source as the DNA should interact with ΔDNA.

This report contains a description of the following properties of HeLa cell DNA: $S_{w,20}$ values for native and ΔDNA, melting and annealing temperatures, rate of melting at 100°C, and solubility of ΔDNA in ethanol. Evidence for the interaction of ΔDNA with synthetic and natural CHO polymers in 50% ethanol is presented.

Experimental

The phenol extraction and quantitation methods of HeLa cell DNA, RNA, and CHO[1] and the method of separating CHO from the RNA–CHO complex[4] have been described. The mouse (C3H) liver CHO used here was extracted and purified by the same procedures. The following CHO's were a gift from Dr. I. J. Goldstein: synthetic linear polysaccharide,[5] yeast mannan, synthetic polyglucose (Merck L524023-0), dextran from *Leuconostoc mesenteroides* (B-1355-S), and dextran 10 having a molecular weight of 10000 (Pharmacia Fine Chemicals). Oyster glycogen and melezitose were purchased from Nutritional Biochemical Co. and Difco, respectively. The CHO's and DNA used in all of the following experiments were dissolved in phosphate (0.005*M*) buffered (pH 7.2) NaCl (0.05*M*).

Results and Discussion

Sedimentation coefficients of native and ΔDNA were determined in a Beckman model E analytical centrifuge. The native DNA (400 μg/ml) $S_{w,20}$ value was 10.4 S, while that for ΔDNA (400 μg/ml) was 24 S. The melting (T_m = 77°C) and annealing curve for DNA is presented in Figure 1. Rapid cooling of ΔDNA stopped the annealing process. The increase in the 260 mμ optical density (OD)/min was determined by heating (100°C) the DNA solution, cooling it, and then measuring the OD. The T/T_0 ratios following 2, 5, 10, and 15 min of heating were 1.21, 1.24, 1.27, and 1.32, respectively. The ΔDNA used here was heated (100°C) for 15 min, then rapidly cooled. The solubility of ΔDNA in increasing concentrations of ethanol was determined by measuring the ΔDNA found in the precipitate and supernatant fluids by use of the Burton[6] test and 260 mμ absorption. The percentage of the total amount of ΔDNA found in the precipitate at ethanol concentrations (v/v) of 55%, 60%, 65%, 70%, and 75% was 0%, 55%, 75%, 98.6%, and 100%, respectively. Ethanol (50%) was used thereafter.

The CHO polymers were investigated, using glucose as a control, by adding each of them in increasing amounts to individual tubes containing 20 μg of ΔDNA. The solutions were held at 37°C for 1 hr, and an equal volume of absolute ethanol (1.5 ml) was added while mixing. The tubes were held at 5°C for 15 hr and centrifuged at 800g for 10 min at 5°C, after which the supernatant fluids were removed and their OD at 260 mμ measured. The resulting data in Figure 2 show that when the CHO/ΔDNA ratio approached 2.5, most of the ΔDNA precipitated. However, when

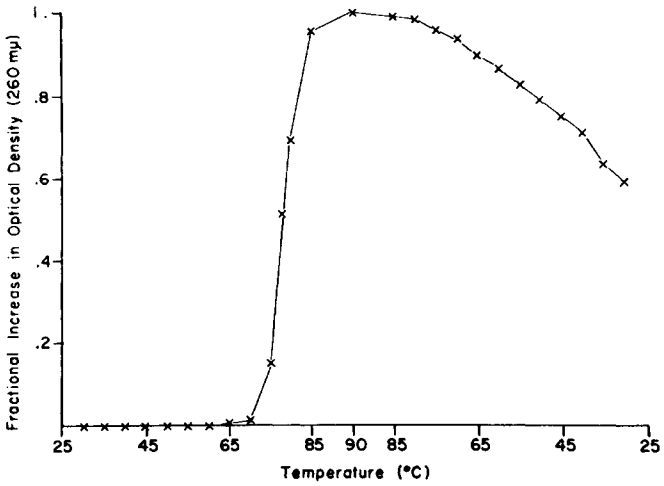

Fig. 1. Melting and annealing temperatures of HeLa cell DNA. The increase in OD 260 mμ ($T\Delta/T_0$) was 1.3. The DNA (20 μg/ml) was dissolved in phosphate-buffered saline, as described in the text, and heated at 1°C/min in a Gilford instrument. The melting temperature (T_m) was 77°C.

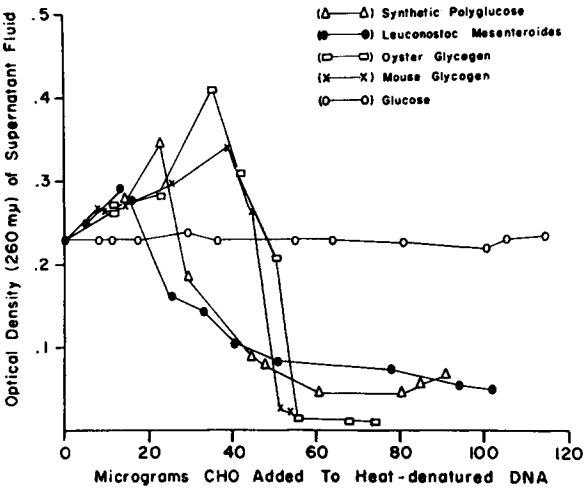

Fig. 2. Hyperchromic and precipitating effects of natural and synthetic polysaccharides on heat-denatured HeLa cell DNA. The polysaccharides and glucose were added to heat-denatured DNA and held at 37°C for 1 hr. Ethanol was added and the solutions were held at 5°C for 15 hr, centrifuged, and the OD 260 mμ of the supernatant fluids is recorded above. When the CHO/ΔDNA ratios approached 2.5, the ΔDNA precipitated, whereas at lower ratios, a hyperchromicity was seen.

this ratio was less than 2.5, the OD was greater than that of ΔDNA alone (hyperchromicity). With glucose, the precipitating and hyperchromic effects were absent. This result demonstrates that the interaction of ΔDNA–CHO was dependent on the polymeric nature of the CHO rather than on the properties of the simple sugar.

Additional evidence that glucose *per se* had no effect on ΔDNA is that attempts to inhibit the precipitation of ΔDNA in a three-component system (ΔDNA, glucose, and CHO added in that order at glucose/CHO weight ratios up to 4) have failed. The molecular weight of CHO that is required to precipitate ΔDNA is 10000 (dextran 10). Melezitose (molecular weight = 504) failed to precipitate the ΔDNA.

The ultraviolet spectrum of the supernatant fluids in which the hyperchromic effect was demonstrated was compared with the spectrum of ΔDNA (Fig. 3). The 260/280 ratio of CHO–ΔDNA was 1.2 as compared with 2 for ΔDNA. An interpretation of these changes is that the ΔDNA molecules interact with the CHO and form the CHO–ΔDNA complex which scatters light.

The solubility of the CHO in the presence, and in the absence, of ΔDNA also indicated that the ΔDNA and CHO interact. The percentage of the total CHO which precipitated, as the CHO per tube was increased, is plotted on Figure 4. Note that the points at which the percentage of CHO in the precipitates was low also correspond to those points at which the hyperchromic effect was seen (Fig. 2). The ΔDNA, then, increased the solubility of the CHO, and the CHO decreased the solubility of ΔDNA.

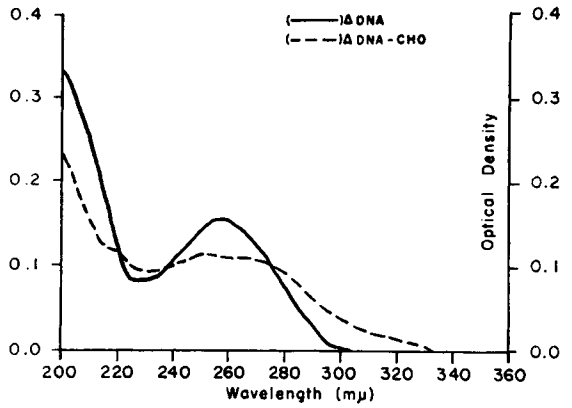

Fig. 3. Change in the ultraviolet spectrum of heat-denatured DNA following its interaction with polysaccharides. The supernatant fluid, in which the hyperchromic effect was seen (see Fig. 2), was compared with the spectrum of ΔDNA. The measurements were conducted in a Cary spectrophotometer using 1-cm silica cells. Note that the 260/280 ratio of the CHO–ΔDNA preparation is less (1.2) than that of the ΔDNA alone (2.0).

depending upon the amount of CHO present in the reacting mixtures. Controls without ΔDNA but containing the same amounts of CHO as the experiment which showed the hyperchromic effect (see Fig. 2) failed to give a 260 mμ OD absorption following ethanol precipitation. In addition, a greater percentage of CHO precipitated from these controls. The hyperchromic effect, then, is not attributable to the additive sums of CHO and ΔDNA, but rather to the ultraviolet absorption and the light scattering due to the CHO–ΔDNA complex. Two CHO's which interacted with ΔDNA but did not exhibit a pronounced hyperchromic effect were linear synthetic CHO and yeast mannan.

All CHO polymers examined have the capacity to interact with ΔDNA from HeLa cells. The universality of the reaction supports the thesis that hydrogen bonds form between the ΔDNA and the neutral CHO's. In the Watson-Crick model[7] of ordered, double-stranded DNA, the hydrogen bonds are primarily internally occupied, whereas ΔDNA[8] is disordered and undoubtedly contains reactive sites such as single strands which are capable of bonding with the CHO in a manner similar to RNA.[4] If the nucleic acid–CHO interactions are present *in vivo* and intermolecular specificity exists, the disordered characteristics of ΔDNA would probably eliminate any specificity detectable in the *in vitro* test system.

Some findings that initiated investigations of these *in vitro* CHO–nucleic acid interactions were: (*1*) a stimulation of CHO synthesis occurring in polio virus-infected HeLa cells[9] at a time when other macromolecules are inhibited[10] and (*2*) the interaction of the polycation DEAE-dextran with polio virus RNA[11] which may[12] contribute to the observed increased infectivity. Findings which may suggest *in vivo* CHO–DNA interactions

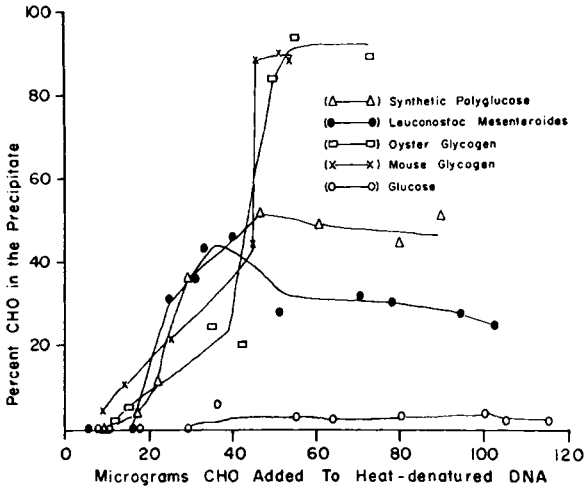

Fig. 4. Precipitability is 50% ethanol of polysaccharides in the presence of heat-denatured DNA. The percentages of the total amounts of polysaccharides (CHO) found in the precipitates following centrifugation (see Fig. 2), are plotted above. Note that the ΔDNA increased the solubility of the CHO and CHO decreased the solubility of the ΔDNA depending upon the amount of CHO present in the reacting mixtures. However, the precipitability of glucose was not influenced in ΔDNA (see above), nor did glucose affect the solubility of ΔDNA (see Fig. 2).

are the accumulation of CHO prior to vigorous mitotic activity in regenerating human epithelial cells[13] and the disappearance of CHO during cell division.[14] The CHO's functioning as a reservoir of energy may only be a partial explanation for these observations. That the ΔDNA and synthetic as well as natural CHO's have the capacity to interact *in vitro* is evidenced here by: (*1*) the hyperchromic effect with the associated increase in solubility of the CHO and the change in the ultraviolet spectrum of ΔDNA after reaction with the CHO, and (*2*) the precipitation of ΔDNA when sufficient CHO is present in the reaction mixture. Any *in vivo* interaction awaits demonstration.

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