THE INTERACTION OF HELA CELL HEAT DENATURED DNA WITH POLYGLUCOSE

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The aqueous phase in phenol extracts of HeLa cells contain deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and polyglucose (CHO), (Segovia et al., 1965). RNA reacts with the CHO and they coprecipitate in 20% ethanol-saline solutions (Graves et al., 1967). Native DNA precipitates when the ethanol concentration is increased to 50%. Although native DNA and CHO have equal buoyant densities in CsCl (Segovia et al., 1965) and both precipitate in 50% ethanol, the present data from analytical centrifugation indicate that these findings were not due to intermolecular bonding. In contrast, the following experiments demonstrate that heat denatured DNA which is soluble in 50% ethanol precipitates from this solution in the presence of CHO when the ratio of CHO/DNA approaches 2.5. The coprecipitation of CHO and DNA indicates that denatured DNA reacts with CHO, thus decreasing the solubility of the denatured DNA.

MATERIALS AND METHODS

HeLa cells were grown in monolayer cultures using Eagle's (Eagle, 1955) medium supplemented with 10% heat-inactivated calf serum. The cells were extracted with phenol (Colter et al., 1962). The RNA-CHO and DNA were successively precipitated in 20% and 50% ethanol solutions (Segovia et al., 1965). The RNA was removed from the CHO by precipitation with trichloroacetic acid (Graves et al., 1967) and the acid-soluble CHO was recovered by precipitating it with ethanol (66%). DNA and CHO were dissolved in phosphate buffered saline (PBS) 0.005 M, pH 7.2.

The DNA was quantitatively determined with the Burton (Burton, 1956) method and CHO with a diphenylamine reagent (Snell and Snell, 1953; Ackermann et al., 1964).

In analytical ultracentrifugation experiments the CHO, DNA and

CHO-DNA were dissolved in PBS, pH 7.2, centrifuged at 20,000 RPM at 20°C in a standard cell using schlieren optics.

RESULTS AND DISCUSSION

CHO was added to native DNA and to DNA which was previously heat denatured (100°C for 15 min.) in PBS and rapidly cooled. The mixtures (total volume = 1.5 ml.) were incubated 1 hour at 37°. Ethanol was carefully added to a final concentration of 50% and the solutions were held at 5°C. After centrifugation at 1000 g for 10 min. at 5°C, supernatant fluids were removed, precipitates dissolved in PBS and both analyzed for CHO and DNA. The total amounts of each polymer recovered from these two fractions are listed on Table 1. The CHO decreased the solubility of denatured DNA, whereas native DNA and CHO alone or in combination precipitated in 50% ethanol solutions. Velocity sedimentation measurements were conducted to assess whether the precipitation of the CHO-DNA was due to intermolecular bonding.

TABLE 1

THE PRECIPITABILITY OF DENATURED DNA, NATIVE DNA, CHO AND COMBINATIONS THEREOF WITH 50% ETHANOL[a]

	Supernatant		Precipitate	
Sample	CHO	DNA	CHO	DNA
CHO	45	--	429	--
$_{\Delta}$DNA[b]	--	12.4	--	0
DNA	--	2.9	--	14.7
$_{\Delta}$DNA[b]-CHO	34.2	5.1	465	16.2
DNA-CHO	51.3	0	408	22.4

a. All values are total ug present.

b. DNA heated 100°C/15 min. Fraction increase 260 mu absorption $A_{\Delta}/A_{o} = 1.3$.

When centrifuged independently the CHO at 600 ug/ml and DNA at 370 ug/ml formed single peaks with $S_{20,W}$ values of 11.6 and 11.1

respectively. When the CHO and DNA were combined and then centrifuged, two distinct peaks formed with $S_{20,W}$ values of 118 and 10.1. These values are not considered significantly different from the values for the added components and indicate that no intermolecular bonding occurred.

Regarding denatured DNA, the amount of CHO required to precipitate it was determined by adding increasing amounts of CHO (0 to 120 ug) to 25 ug of DNA. An equal volume of ethanol was added to the polymers which were dissolved in 1.5 ml. PBS. The solutions were held for 15 hours at 5°C, centrifuged, and the precipitates and supernatant fluids analyzed for their content of DNA and CHO. Figure 1 illustrates the percent DNA precipitated and the ratio of CHO/DNA in the precipitates as affected by the ratios of CHO/DNA in the mixtures.

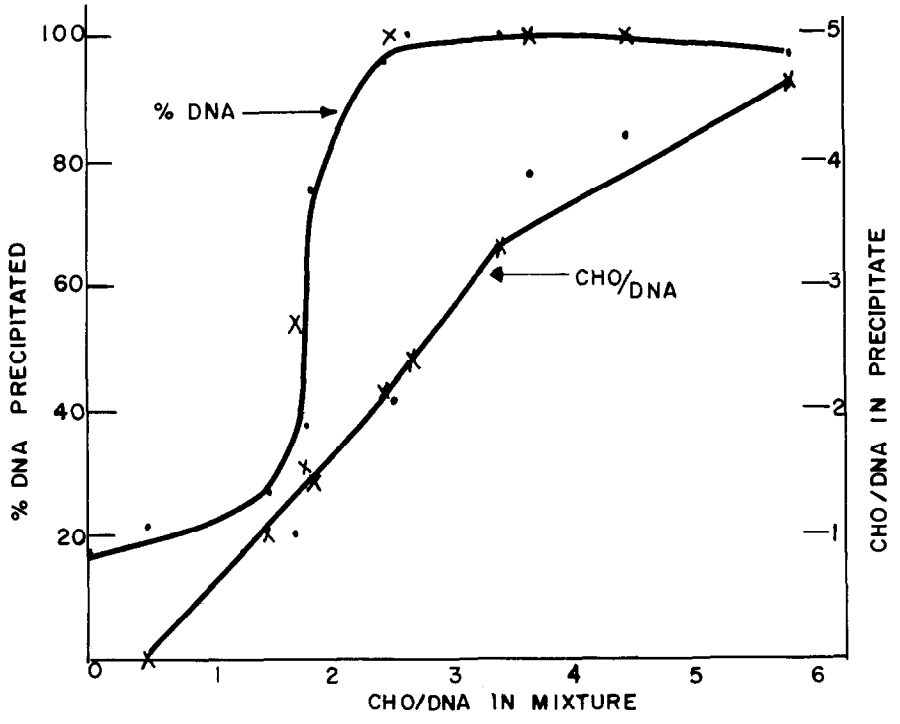

Figure 1. The solubility of heat denatured DNA in 50% ethanol solutions as affected by increasing amounts of polyglucose. Results from two experiments are distinguished by crosses and dots.

To precipitate each ug of DNA, 2.5 ug CHO was required. Since the molecular weight of CHO from HeLa cells has been determined to be about 3×10^6 (Sokol et al., 1966), each molecule of CHO precipitated an amount of denatured DNA equivalent to an average molecular weight of approximately 1.2×10^6.

The intermolecular reaction of CHO-DNA may be explained on the basis of the availability of hydrogen bonding sites in denatured DNA. Most of these sites in native DNA are internally occupied in its helical structure thus reducing the capacity of the DNA to bond with the CHO. In contrast, denatured, single stranded DNA in heat denatured preparations would have reactive sites for bonding with the CHO. Of interest is the parallel finding that HeLa cell RNA reacts with CHO (Graves et al., 1967).

In a biological context these interactions of CHO with nucleic acids may contribute to their intercellular protection, conformation, or regulation of expression.

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