HYBRIDIZATION OF DNA MODIFIED BY INTERACTION WITH A METABOLIC FRAGMENT FROM S-(1,2-DICHLOROVINYL)-L-CYSTEINE

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SUMMARY: DNA isolated from E. coli B cells was allowed to react in vitro with S-(1,2-dichlorovinyl)-L-cysteine and a bovine kidney lyase in order to introduce the alkylating thiovinyl fragment into the DNA [Arch. Biochem. Biophys., 153, 105 (1972)]. This modified DNA showed less complementary base interaction with normal DNA and RNA from the same organism. Competition experiments revealed that unmodified DNA is preferentially hybridized with its counterpart and that the modified DNA did not compete with normal DNA for complementary sites on unmodified but otherwise homologous DNA. Loss of hybridization capability of DNA was progressive with the enzymatic cleavage of DCVC and with concurrent alkylation of DNA; maximum loss (65% for DNA x DNA and 45% for RNA x DNA) was observed after about 4 minutes of reaction.

INTRODUCTION: We observed previously that DNA isolated from hemopoietic tissues of calves treated with a single intravenous dose of S-(1,2-dichlorovinyl)-L-cysteine (DCVC) had abnormal physical (1) and biological (2,3) properties. These abnormalities could also be produced when DNA from various sources were treated in vitro with DCVC and a lyase from calf kidney (4). This enzyme cleaves DCVC and produces a highly reactive, alkylating fragment (AF) containing the vinyl carbon atoms and sulfur (5). AF also reacted with a variety of synthetic polynucleotides (6) and histones (7). Analyses of AF-substituted DNA and polynucleotides by sequential enzymatic hydrolysis revealed that the AF moiety linked two adjacent bases of the same chain. Such reaction with a homopolymer markedly affected its ability to combine with complementary chain (6). Synthetic double stranded nucleotide heteropolymers, like DNA, after AF-substitution, were thermally more stable and, upon cooling of the heat-denatured solution did not show the normal complete renaturation (6).

It is likely that changes in biological properties of DNA as observed

coincident with DCVC toxicity are due to such loss of complementary base interaction between DNA strands. The results of our present studies on DNA x DNA and DNA x RNA hybridization strengthen this view.

MATERIALS AND METHODS: To prepare ^{3}H-DNA and ^{3}H-RNA, E coli B cells were grown in Fraser's medium (8). During the log phase a sterile aqueous solution of thymidine-methyl ^{3}H (5 μc) or ^{3}H uracil (100 μc) purchased from New England Nuclear Corporation was added to each 100 ml medium, respectively. For DNA labeling 250 μg/ml of uridine was also added to the growth medium (9). The cells were harvested after 90 mins. Labeled and unlabeled DNA were isolated by the procedure of Marmur (10). Labeled RNA was purified by adaptation of the method described by Girard (11).

MODIFICATION OF DNA: Native or heat-denatured DNA was modified in vitro in presence of DCVC and lyase as described earlier (4) so that about 1% of nucleotides in native DNA and about 50% in denatured DNA were substituted with AF.

IMMOBILIZATION OF DNA ON FILTERS: Schleicher and Schuell Type B-6 nitrocellulose membrane filters were used. The SSC solution contained 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0. DNA was denatured at a concentration of 200 μg/ml in 0.1 x SSC by heating at 100° for 10 mins. and quick cooling. Cold denatured DNA solution was brought to 6 x SSC and was passed (5 ml/min) through a filter presoaked in 6 x SSC for at least 4 hours. The filter was washed with 15 ml of 6 x SSC, dried overnight at room temperature in a vacuum dessicator, then placed in an oven at 80° for 4 hours (12). Control experiments in which the input DNA or RNA was unlabeled and immobilized DNA was radioactive were used for the quantitative measurement of immobilized DNA retained by the filter (13). More than 95% of the DNA was retained by the filter after exposure to the hybridization conditions used.

HYBRIDIZATION: For hybridization and competition experiments the general procedures described by Gillespie (14) was followed. For DNA x DNA hybridization experiments, preincubation at 65° in 0.1% bovine serum albumin in 3 x SSC

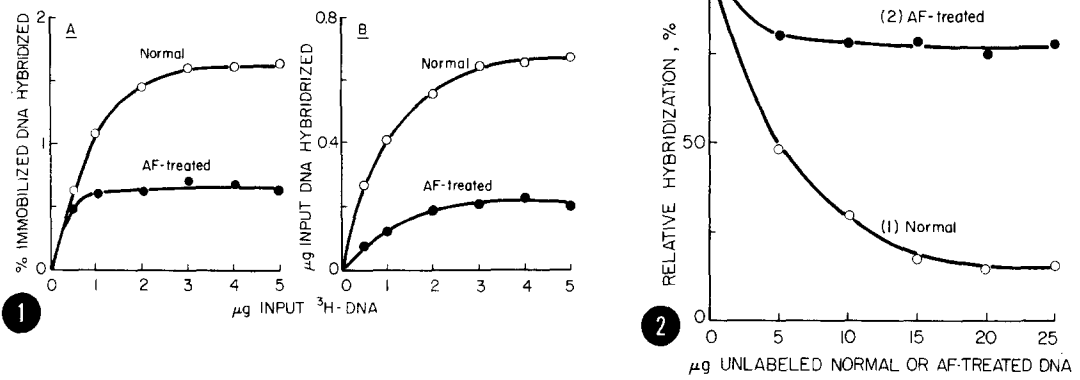


Fig. 1: Saturation curve of DNA x DNA

A. Hybridization of input normal ^{3}H-DNA (850 CPM/ µg) with immobilized normal or AF-treated DNA.

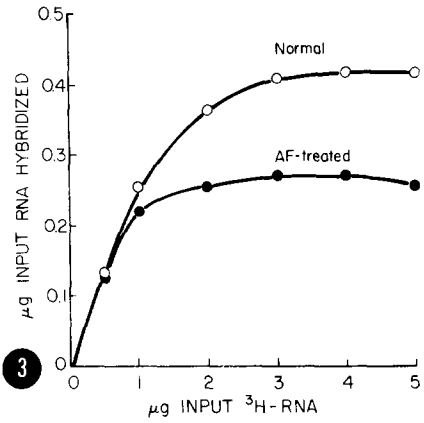
B. Annealing of input normal or AF-treated ^{3}H-DNA with immobilized normal DNA. Each filter contained 50 µg DNA.

Fig. 2: Competition of normal ^{3}H-DNA with unlabeled normal or AF-treated DNA for hybridization sites on immobilized normal DNA. Each filter contained 50 µg of normal DNA and was incubated with 5 µg of normal ^{3}H-DNA (4,250 CPM) and with increasing amounts of either normal (Curve 1) or AF-treated (Curve 2) unlabeled DNA.

by the method of Denhardt (15) was used to reduce background binding during hybridization. Annealing was carried out for 12 hrs at 65° in 6 x SSC containing increasing amounts of denatured ^{3}H-DNA or ^{3}H-RNA. The filters were then washed in 3 x SSC and in the case of DNA x RNA hybridization each filter was subjected to RNase treatment (100 µg in 5 ml 3 x SSC) and final washings in 3 x SSC (12). Appropriate blanks were set up to measure non-specific DNA and RNA retention. Radioactivity of the filters was measured in 10 ml Bray's solution using a Nuclear-Chicago liquid scintillation spectrometer.

RESULTS AND DISCUSSION: Preliminary experiments showed that optimum hybridization was obtained by keeping the ratio of DNA on the filter to DNA or RNA in solution at as high a value as possible. High salt concentration and low concentration of input DNA prevented its renaturation.

Modification of DNA by *in vitro* treatment with DCVC and lyase did not diminish its retention on the filters. However immobilized AF-treated DNA



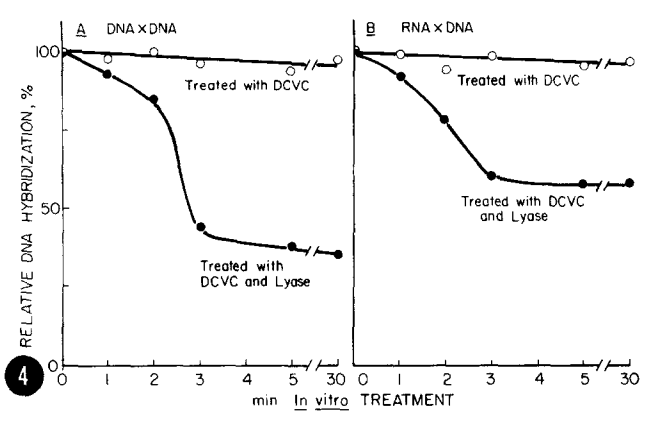


Fig. 3: Saturation curve of RNA x DNA. Hybridization of input ^{3}H-RNA (2,840 CPM/ μg) with immobilized normal or AF-treated DNA. Each filter contained 50 μg DNA.

Fig. 4: Progressive inactivation of the hybridization rate of DNA treated in vitro with DCVC and lyase. One hundred micrograms of control or AF-treated DNA was immobilized on each filter and incubated with 4 μg of ^{3}H-DNA (A) or ^{3}H-RNA (B).

showed a reduced ability to hybridize with normal input DNA as shown in Fig. 1 (A). These data also showed that much less normal DNA was needed to saturate binding sites on immobilized AF-treated DNA. If AF-treated input DNA was allowed to hybridize with normal DNA on the filter, much less was annealed as compared to homologous, normal input DNA (Fig. 1 (B)). These results indicated impairment of hydrogen bonding between normal and AF-substituted DNA. This concept was tested by competition experiments in which unlabeled normal or AF-treated DNA was allowed to compete with labeled normal DNA for hybridization sites on immobilized DNA. As expected addition of excess unlabeled, normal DNA reduced the hybridization of labeled DNA (Fig. 2, Curve (1)). However a constant amount of labeled DNA was hybridized with undiminished efficiency in the presence of excess, unlabeled AF-substituted competitor (Fig. 2, Curve (2)). Although such observation may indicate less nucleotide sequence homology between normal and AF-treated DNA, our results can be interpreted as a loss of complementary base interaction between normal and AF-modified DNA. Our previous observation also showed that AF-substitution in a single stranded

nucleotide homopolymer significantly reduced its capacity to form a double stranded complex when mixed with a complementary polynucleotide (6).

The ability of AF-treated DNA to hybridize homologous RNA was also diminished as seen in Fig. 3. Less RNA was needed to saturate AF-substituted DNA than normal DNA. Apparently there were fewer hybridization sites on the alkylated DNA. Such anomaly also indicates an impairment of base pair formation between AF-substituted DNA and normal RNA.

The reduced ability of DNA to hybridize was dependent on the extent to which it became AF-substituted by the action of DCVC lyase on DCVC. During modification of DNA in vitro with DCVC and lyase, maximum alkylation of DNA occurred after about 5 mins. (4), during which time the lyase activity was also at its maximum (5). Under similar conditions of DCVC and lyase treatment hybridization capability of DNA was quickly and progressively lost in about 4 mins. of reaction (Fig. 4). Thereafter the activity remained constant. Maximum loss for DNA x DNA hybridization was about 65% which is more than that observed for DNA x RNA hybridization (45%). We have previously shown that after 4-5 mins. of in vitro reaction with DCVC and lyase DNA also lost its template activity for DNA and RNA synthesis (4).

Molecular hybridization methods are important techniques to detect nucleotide sequence homology in nucleic acids. We have successfully used this tool to study complementary base interaction between homologous nucleic acids after they are subjected to modification. DNA modified by in vitro treatment with DCVC and lyase does not interact properly to form complementary base pairs with normal DNA or RNA. Hybrid formation in the cells is apparently a normal phenomenon linked to replication and transcription. Any impairment in this process may result in the inhibition of DNA and RNA synthesis. This is substantiated by the fact that modified DNA either obtained in vitro (4) or from the hemopoietic tissues of DCVC-treated calf (2,3) has lost its primer-template activity. It is possible that such a loss of interaction, as observed here between nucleic acid strands, also occurs in bone marrow and other target organs of the calf after treatment with DCVC.

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REFERENCES:

1. Bhattacharya, R. K. and Schultze, M. O. (1971). Arch. Biochem. Biophys. 145, 565-574.
2. Bhattacharya, R. K. and Schultze, M. O. (1971). Arch. Biochem. Biophys. 145, 575-582.
3. Bhattacharya, R. K. and Schultze, M. O. (1971). Biochem. Biophys. Res. Commun. 45, 1526-1532.
4. Bhattacharya, R. K. and Schultze, M. O. (1972). Arch. Biochem. Biophys. 153, 105-115.
5. Bhattacharya, R. K. and Schultze, M. O. (1967). Comp. Biochem. Physiol. 22, 723-735.
6. Bhattacharya, R. K. (1973). Fed. Proc. 32, 546 (Abst. No. 1848); Biochem. Biophys. Res. Commun. In Press.
7. Bhattacharya, R. K. and Schultze, M. O. (1972). Fed. Proc. 31, 839 (Abst. No. 3530).
8. Fraser, D. and Jerrel, E. A. (1953). J. Biol. Chem. 205, 291-295.
9. Warner, H. R. Personal communication.
10. Marmur, J. (1961). J. Mol. Biol. 3, 208-218.
11. Girard, M. (1967) in Methods in Enzymology (Grossman, L. and Moldave, K. eds.), Vol. XIIA, p 581, Academic Press, N. Y.
12. Gillespie, D. and Spiegelman, S. (1965). J. Mol. Biol. 12, 829-842.
13. Baker, R. F. (1972). J. Cell. Sci. 11, 153-171.
14. Gillespie, D. (1968) in Methods in Enzymology (Grossman, L. and Moldave, K. eds.), Vol. XIIB, p 641, Academic Press, N. Y.
15. Denhardt, D. T. (1966). Biochem. Biophys. Res. Commun. 23, 641-646.