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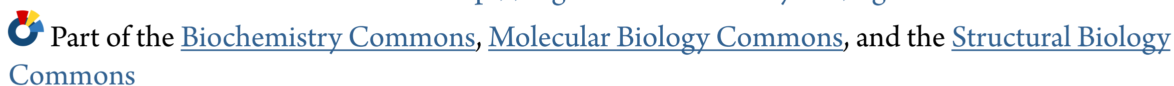
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# Epichlorohydrin Cross-Linking of Synthetic DNA Oligomers

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## Abstract

Epichlorohydrin (ECH), an important chemical in the synthetic polymer industry, is a bifunctional alkylating agent with the potential to form DNA interstrand cross-links. Occupational exposure to this suspect carcinogen leads to chromosomal aberrations, and ECH has been shown to undergo reaction with DNA *in vivo* and *in vitro*. We are using denaturing polyacrylamide gel electrophoresis to assess cross-linking of synthetic DNA oligomers by both ECH and the related compound, epibromohydrin (EBH). Both epihalohydrins produce a low-mobility band on denaturing gels consistent with an interstrand cross-link. Moreover, the efficiencies, sequence preferences, reaction kinetics, and pH dependence differ for the two compounds, suggesting different mechanisms of reaction. Understanding these alkylation reactions may help explain the role of the epihalohydrins in cancer development.

## Introduction

Cancer is the number two cause of death in the United States [1], motivating interest in DNA interstrand cross-linking reactions. Some cross-linking agents such as nitrogen mustards have been shown to have therapeutic effects on certain cancers [2]. Others, such as diepoxybutane (DEB), are powerful carcinogens [3]. Epichlorohydrin (ECH; Figure 1), an important raw material in the production of epoxy resins, pesticides, and plastics, is a suspect mutagen in both eukaryotes and prokaryotes [4]. The structurally related compound epibromohydrin (EBH; Figure 1) is also a suspect carcinogen.

There are no literature reports of DNA cross-linking by either ECH or EBH. However, our work shows that their reactions with synthetic DNA duplexes produce a low mobility band on denaturing polyacrylamide gel electrophoresis (dPAGE) consistent with interstrand cross-linking. This band is absent in the controls (where no cross-linking agents were used). This observation has motivated us to determine whether this band corresponds to ECH and EBH cross-links, and if so at what DNA sequence. Previous research in our laboratory has determined that the reaction kinetics and pH dependence of the ECH and EBH reactions with DNA differ, suggesting different mechanisms of reaction for the two compounds [5].

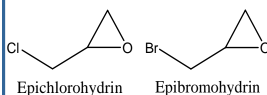


**Figure 1.** Structures of the cross-linking agents, ECH and EBH

## DNA Duplexes Used

**Duplex A:**
S1: 5'-CGTTTAA**GGCCC**TT**GGCCC**TA**GGCCC**ATGT-3'
S2: 3'-TT**CCGGG**AA**CCGGG**AT**CCGGG**TACA-5'
**Duplex B:**
S1: 5'-AATATAA**GC**TTTAAAT-3'
S2: 3'- TTATATT**CG**AAATTTA-5'
**Duplex C:**
S1: 5'-AATATA**GGC**TTTAAAT-3'
S2: 3'-TTATAT**CCG**AAATTTA-5'
**Duplex D:**
S1: 5'-AATATA**GGGC**TTAAAT-3'
S2: 3'-TTATAT**CCCG**AATTTA-5'
**Duplex E:**
S1: 5'-TATATATTTATA**GGC**TATATTTATATT-3'
S2: 3'-ATATATAAATAT**CCG**ATATAAATATAA-5'

## Results

Possible cross-linking of DNA by ECH and EBH was suggested by the presence of the low mobility band that appears on denaturing polyacyrlamide gels (Figure 2). The two strands of **Duplex A** were independently radiolabeled with P-32 and annealed to their complementary strand. These duplexes were then incubated with ECH and EBH at the previously-determined optimal reaction conditions.

Both ECH and EBH produced similar low-mobility bands on denaturing gels regardless of whether the long (S1) or the short strand (S2) was radiolabeled. This observation supports the assumption that this band corresponds to a cross-linked duplex rather than a single-stranded product (which appears further down on the gel) . However, the intensity of the putative cross-linked band differs. This suggests better reactivity of EBH as compared to ECH, possibly because bromide is a better leaving group than chloride. Furthermore, the established cross-linker DEB [6], used for comparison on this gel, produces a very intense cross-linking band relative to either epihalohydrin.

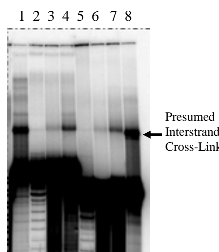


**Figure 2.** The dPAGE analysis of the cross-linked Duplex A. Lanes 1-4 are duplexes with S1 radiolabeled, whereas lanes 5-8 are duplexes with S2 radiolabeled. Lanes 1 and 8 are DEB products; Lanes 2 and 5 are controls (no cross-linking agent); Lanes 3 and 6 are ECH products; and Lanes 4 and 7 are DEB products.

## Acknowledgments

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Previous results in our laboratory [5] suggested alkylation at the N7 position of guanine by the epihalohydrins. However, the sequences at which cross-linking occurs remained unclear.

In order to determine the optimum core sequence for cross-linking, we used the radiolabeled **Duplexes B, C, and D**. These duplexes vary only in their central guanine-containing sequence. Duplexes were independently incubated with one of the three cross-linking agents (DEB was included for comparison). Products were analyzed via dPAGE (Figure 3) and quantified via phosphorimagery. The average results of three trials are shown in Table 1 below.

ECH had similar cross-linking efficiencies for the three duplexes (containing central GC, GGC, and GGGC sequences), suggesting little sequence preference for this agent. The same was true for EBH. On the other hand, DEB showed a preference for the 5'-GGC, as predicted by its established preference for the sequence 5'-GNC [6]. Again, the overall relative efficiency of cross-linking each duplex varied by agent, with DEB > EBH > ECH.

**Table 1.** Average percentages of cross-linking (% XL) of Duplex B (containing a central GC site), Duplex C (containing a central GGC site), and Duplex D (containing a central GGC site), with the three cross-linking agents.

| Lane | GC DEB | GC ECH | GC EBH | GGC DEB | GGC ECH | GGC EBH | GGGC DEB | GGGC ECH | GGGC EBH |
|---|---|---|---|---|---|---|---|---|---|
| Average % XL | 14.29 | 4.14 | 5.42 | 30.35 | 4.99 | 4.56 | 26.58 | 4.53 | 6.56 |
| Std. Dev. | 1.77 | 1.11 | 1.27 | 4.37 | 0.59 | 1.09 | 4.83 | 1.22 | 1.44 |

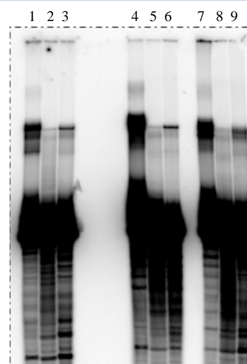


**Figure 3.** The dPAGE analysis of the reactions of Duplexes B, C, and D. Lanes 1-3 are Duplex B; Lanes 4-6 are Duplex C; Lanes 7-9 are Duplex D. Lanes 1, 4, and 7 are DEB products; Lanes 2, 5, and 8 are ECH products; and Lanes 3, 6, and 9 are EBH products.

In order to confirm interstrand cross-linking, mass spectrometry will be performed on **Duplex E**. This duplex contains the central 5'-GGC site preferred by DEB [6]. In order to optimize reaction conditions, a cross-linking time trial was performed with both ECH and EBH (Figure 4). Two low-mobility bands are observed that may correspond to cross-linked isomers [7]. We are currently verifying linkage sites through piperidine cleavage of each band individually followed by high-resoluation dPAGE analysis. We will then perform epihalohydrin reactions on cold duplexes and isolate individual low-mobility bands to analyze by mass spectrometry.

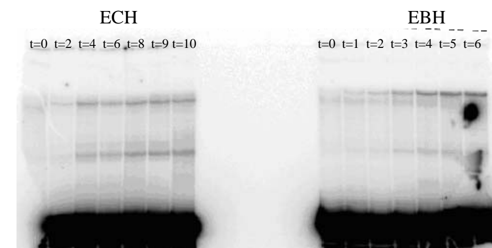


**Figure 4.** The time trial gel of the ECH and EBH reactions with Duplex E. Time points are in hours.

## Conclusions

Our data support the following conclusions:

- ECH and EBH form DNA interstrand cross-links.
- EBH is a more efficient cross-linker than ECH, but both agents are far less efficient than DEB.
- The epihalohydrins have far less sequence specificity for cross-linking than DEB.

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