THE CHEMICAL BASIS FOR DNA DIAGNOSTICS USING BORONATED DNA

Bernard Spielvogel*, Walda Powell, and Anup Sood

Boron Biologicals, Inc., 620 Hutton Street, Suite 104, Raleigh, N.C. 27606-1490, USA

Abstract

Substitution of a borane group (BH_3) for a non-bridging oxygen in the phosphodiester backbone of DNA gives rise to new therapeutic and diagnostic applications. One potential diagnostic application relies upon the utilization of the reducing ability of the boron-hydrogen bond toward certain metal cations. The reduction process produces highly catalytic metal deposits which can be further amplified by additional metallic deposition. This communication presents a feasibility study using a boronated TpT and a normal TpT dimer as models for boronated and normal DNA. The study shows the potential for detecting boronated DNA in the presence of normal DNA and points the way toward a new non-radioactive technique for DNA diagnostics.

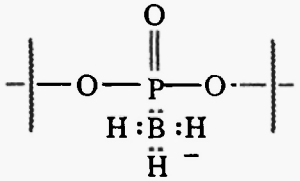
Introduction

We have been involved in the synthesis and biological activity of boron analogues of biomolecules such as amino acids, peptides, nucleic acids and others. Such analogues have been found to possess a wide variety of pharmacological activity, including anticancer, hypolipidemic, anti-inflammatory, antiviral, etc. [1,2]

A key factor of these analogues is that they are based upon 4 coordinate boron and possess sufficient hydrolytic stability such that they can be used under physiological conditions. Most of these analogues also contain one or more B-H bonds and gives rise to the possibility of detection systems based upon the proclivity of BH to reduce certain metal cations and produce a metallic deposit.

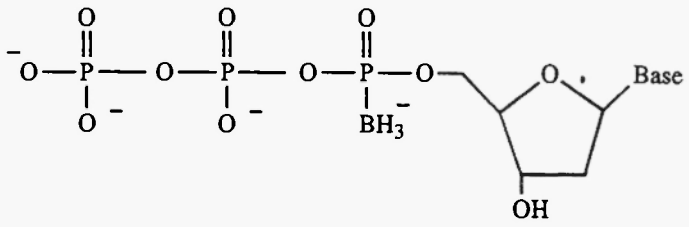
One area of considerable potential is in DNA or RNA diagnostics. This application rests upon the demonstrated capability of replacing a nonbridging oxygen atom in the phosphodiester backbone with an isoelectronic BH_3 group [3] to give a boranophosphate backbone **1**.

Boranophosphate



1

This placement can be effected either chemically [3] or enzymatically using an α-boranonucleoside triphosphate. [4]



2

To test the feasibility of this concept, a boronated TpT dimer and a normal TpT dimer were used as models. The experiment was simply to spot solutions of varying, concentration on a plate and the contact with a $AgNO_3$ solution to provide (in the case of the Borano phosphate) nucleation sites. The plate was then placed in a developer solution.

Experimental

Dithymidylyl boranophosphate (TpBT) was prepared as described previously. [5] Dithymidylyl phosphate (TpT) was purchased from Sigma Chemical Company. All other reagents were purchased from Aldrich Chemical Company.

Developer solutions were prepared as described in U.S. Patent 5,206,122. [6] Solution A contained citric acid (1.79g), sodium citrate (0.71g), imidazole (4.00g), and silver nitrate (0.19g) in 50ml water. Solution B contained sodium citrate (3.29g), sodium sulfite (1.00g), hydroquinone (0.06g), and citric acid (1.53g) in 100ml of water. The two solutions were mixed immediately before development in the ratio of 1:2 (Solution A:Solution B).

Typical Staining Experiment

One μl each of 2 μM, 20; μM, 200 μM and 2 mM TpT and 2 μM, 20 μM, 200 μM and 2 mM TpBT were spotted individually onto a thin layer silica gel chromatography plate and allowed to dry. The plate was placed in 1% $AgNO_3$ solution for 15 minutes and then placed in the developer mix for 15 minutes. The plate was examined by naked eye for staining of spots. (Fig. 1)

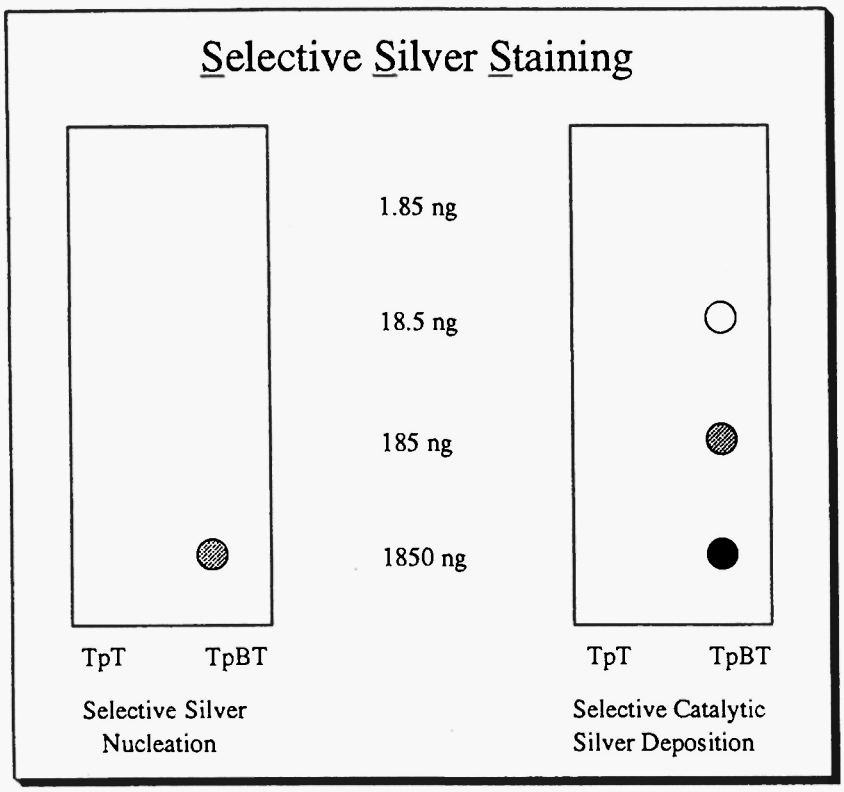


Fig. 1. A representation Showing Selective Staining using Nucleoside-α-boranotriphosphates

Results

Staining was performed in two steps, first involving nucleation and second, catalytic development. In the first step nucleation was performed using a solution of silver nitrate in water. The 2 mM spot of TpBT turned dark while the other spots remained unstained. After exposure to the developer, spots corresponding to 200 μM TpBT also turned black while 20 μM TpBT spot turned light brown. No other spots were stained as observed by naked eye. (Fig. 1)

Discussion

The basic principle being used in this method is the same as used in photographic film development [7] and electroless plating [8] in that a large amount of metal (silver or other) can be selectively deposited at a catalytic site. These catalytic sites (also called nucleation sites) are generally small metal deposits of a select product that are obtained by different techniques including chemical reduction, photochemical deposition, and thermodecomposition.

This principle has been applied for decades for the staining of proteins and more recently DNA. [9] The process again involves deposition of a small amount of silver or

gold as nucleating agent and a complexing agent that prevents reduction of silver or gold salt in the absence of protein or DNA. Thus, protein or DNA is stained exclusively without staining the background substrate. This method, however, cannot distinguish between different DNA or RNA molecules as is required for diagnostic DNA or RNA probes, which have to be selectively identified in the presence of non-target DNA or RNA molecules. The reason for that is simply that during nucleation, both a metal salt and a reducing agent are used together in a solution to deposit the metal wherever DNA is present (Fig. 2).

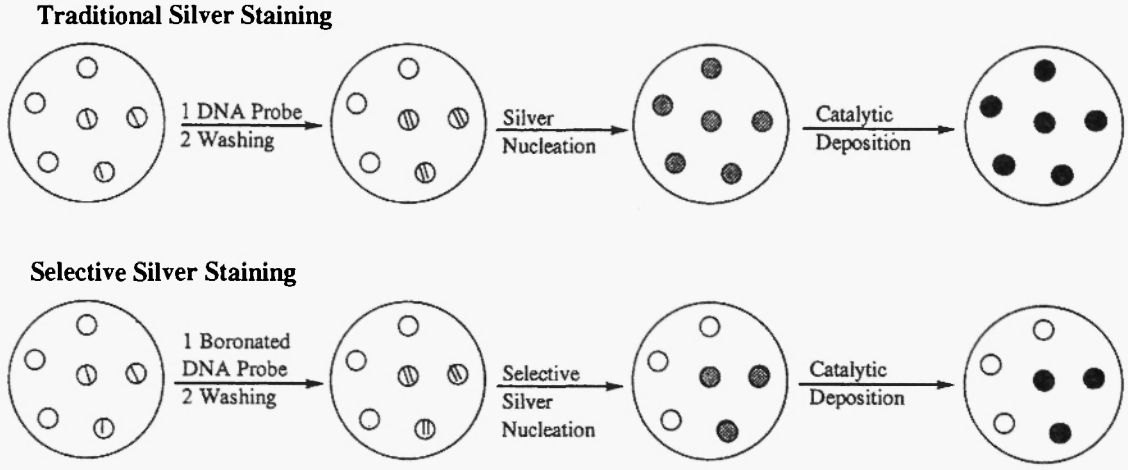


Fig. 2. Selective Silver Staining VS Traditional Silver Staining

The selectivity of current method which can distinguish between a boronated DNA probe and non-boronated DNA, lies in that the reducing agent is built into the probe in the form of borane groups. So, during nucleation, only a silver salt solution is used which deposits silver at the site of the boronated probe by reacting with the borane group (Figure Xb), while no nucleation takes place at sites without the probe.

One of the most attractive features of incorporating hydrolytically very stable, yet powerfully reducing boron-hydrogen containing labels into DNA probes is the great versatility that is imparted. With the BH label a variety of metals, magnetically active borides, catalytically very active borides, and colored substances can be produced and deposited. [10]

It is well known in boron hydride chemistry that the BH bond is reactive with a number of heavy metal cations producing either the free metal or metal boride. Metals reduced by sodium borohydride include Cd, Co, Cu, Au, Ir, Pb, Hg, Ni, Pd, Pt, Rh, and Ag. [10] Complexes containing BH such as Lewis Base-BH_3 can also reduce many of the above depending upon the Lewis Base. The reduction is often quantitative, thereby giving rise to potentially quantitative assays. Additionally, the reducing action of BH can also produce color species, e.g. WO_4^{2-} can be reduced to tungsten blue.

In our preliminary feasibility studies, we used a boronated TpT and a normal TpT as models for boronated and normal DNA respectively. With these models, we were able to demonstrate the selectivity of this process (Figure 2). Nucleation (using $AgNO_3$ solution, as nucleating agent), is selective, as would be expected, because the only reducing agent present in the system is the borane group of boronated TpT (Figure 2a). The normal TpT is not stained.

This model system, although useful initially, is not an ideal system and does not truly represent DNA for several reasons. First of all, this model only contained thymine base. In a DNA oligomer suitable as probe, all bases would most likely be present. Since silver ions are expected to coordinate to guanine or adenine base, it still has to be demonstrated that selectivity can be preserved when using DNA.

Secondly, a dinucleotide is not an appropriate model as DNA conformation could also affect the staining process. For this method to work access to the borane group by the metal complex is essential and depending upon the size of metal complex, and the conformational state of the DNA, the access will vary.

Finally, for diagnostic use, our final target is a double stranded DNA in a gel matrix or pendant to a microbead or other surface. A dinucleotide is again not a good model for a double strandard DNA. Nevertheless, the chemical basis for DNA diagnostics using boronated DNA has been established in this model system.

Acknowledgments:

We thank the National Institutes of Health for support under the Small Business Innovation Research program. This paper is dedicated to Prof. Sheldon G. Shore on the occasion of his 65th birthday.

References:

1. B.F. Spielvogel, Phosphorus, Sulfur, and Silicon **87**, 267, 1994.

2. B.F. Spielvogel, A. Sood, B.R. Shaw, and I.H. Hall, Chemistry of Boron, Royal Society of Chemistry, 193, 1994.

3. A. Sood, B.R. Shaw, and B.F. Spielvogel, J. Am. Chem. Soc., **112**, 9000, 1990.

4. J. Tomasz, B.R. Shaw, K. Porter, B.F. Spielvogel, A. Sood, Angew. Chem. Int. Engl, **31**, 1373, 1992.

5. B.R. Shaw, J. Madison, A. Sood, B.F. Spielvogel in Methods in Molecular Biology, Vol. **20**: Protocols for Oligonucleotides and Analogs, S. Agarwal, ed., Humana Press Inc., Totowa, NJ, 225, 1993.

6. M.J.M. Noppo, L.A.M. Van Nuffel, "Light Stable Physical Developer," U.S. Patent 5,206,122, April 27, 1993.

7. "The Theory of the Photographic Process, III edition, T.H. James, ed., The MacMillan Company, NY, 1966.

8. C.R. Brummett, R.N. Shaak, and D.M. Andrews, U.S. Patent 3,937,857.

9. M. Gottlieb and M. Chavko, Analytical Biochemistry, 165, 33, 1987.

10. Sodium Borohydride Digest, Morton International, Inc. p. 38 and references cited therein.