Rapid and Selective Selenium-Mediated Autoligation of DNA Strands

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Methods for joining strands of DNA are widely used in chemistry, molecular biology, and biomedicine.[1−3] One of the most promising newer uses of enzymatic ligations has been the detection of disease-related DNA sequence polymorphisms, which is possible because of the enzymes' high sensitivity to DNA sequence complementarity.[4]

Nonenzymatic ligations might be of particular use in this context. Removing the need for enzyme can lower the cost and increase the generality of ligations. Several DNA ligation chemistries have been described in the literature;[5,6] a few of these are autoligations, in which the chemistry for reaction is incorporated into the DNA itself.[6] In many applications, the ideal ligation chemistry (1) would require no added reagents to carry out the reaction, (2) would require no postsynthesis modification of the DNA prior to reaction, (3) could also be carried out on an RNA template, and (4) would create a junction that causes little perturbation to the DNA structure.

Here we describe convenient and efficient new chemistry for the joining of DNA ends, and its use in detection of RNA and DNA sequences. This autoligation approach involves the reaction of a phosphoroselenoate anion on one strand with a 5′-carbon carrying an iodide leaving group on another. Selenium has previously been incorporated into DNA at nonbridging positions in the phosphodiester linkage;[7] however, the monosubstituted selenium was unstable. Prior to the present work, bridging selenium esters were unknown in nucleic acids.

We incorporated selenium into short synthetic DNA strands (WARNING: some forms of selenium are acutely toxic!)[8] by methods analogous to those used for sulfur.[9] We found KSeCN to be most convenient as a selenizing reagent.[9b,c,7b] The end-selenated oligonucleotides were removed from the solid support and deprotected using standard ammonia conditions. Because of uncertainties as to the long-term stability of the terminal phosphoroselenoate anion,[10] we used this modified DNA without further purification in subsequent ligations. For reaction with this nucleophile we prepared 5′-iodinated oligonucleotides by incorporation of 5′-iodothymidine phosphoramidite.[6d]

As with other DNA ligations, this chemistry requires that the reacting ends be bound at adjacent sites on a longer complementary templating strand, which serves to raise effective concentrations of the reactive groups markedly. Ligations were therefore carried out in the presence of complementary DNA or RNA target strands. The sequences were taken from the H-*ras* protooncogene ("WT") and the activated H-*ras* oncogene ("MUT"), which has a C→A point mutation in codon 12 (Figure 1).[11] We used a 13mer iodinated probe that is fully complementary both to normal and oncogene sequences. Combined with this were used either of two shorter (7mer) selenium-containing probes, one complementary to the normal codon 12 and the other to the mutant sequence. The reactions were carried out at 1.3 μM DNA concentration in a pH 7.0 Tris-borate buffer containing 10 mM $MgCl_2$ and were quantitated by fluorescence imaging of gels separating ligated products.

Experiments showed that the selenium reaction proceeds more rapidly than previous sulfur chemistry (Figure 2). Analysis of the initial slopes of product yields as a function of time shows a difference of 3.7-fold in rate when MUT probes were used on the MUT target DNA. Similarly, we carried out the same reaction on the MUT target RNA, and we again found that the selenium chemistry proceeded more rapidly (a 3.5-fold difference). For both sulfur and selenium cases, the ligation on the RNA was somewhat slower (by a factor of ∼2) than on the DNA. It is not yet clear whether this difference is general in the selenium case.[12] Control reactions with (all-oxygen) phosphate showed no ligation, confirming that selenium is necessary for reaction to occur. Overall conversion was relatively high (>∼70%), but appeared to be less than the sulfur case, possibly due to a small amount of loss of the selenium in the phosphate monoester prior to ligation.

Importantly, we found that the selenium/iodide autoligation is highly sensitive to the sequence of the target nucleic acid. We

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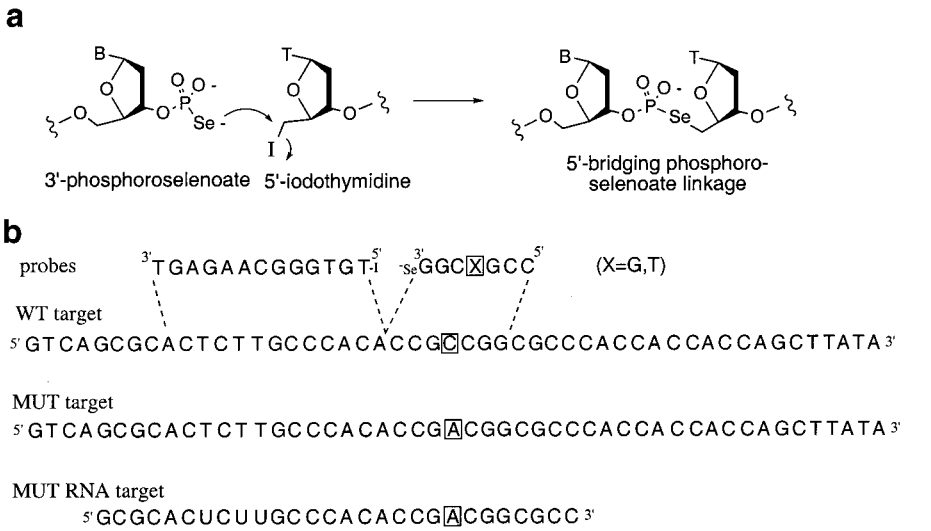

Figure 1. (A) Chemistry of phosphoroselenoate autoligation of DNA strands. Reaction is rapid only when the two strands are bound at adjacent sites on a fully complementary templating strand of DNA or RNA. (B) Sequences used for ligation studies. The target DNAs correspond to the H-*ras* gene sequence including codon 12.[11]

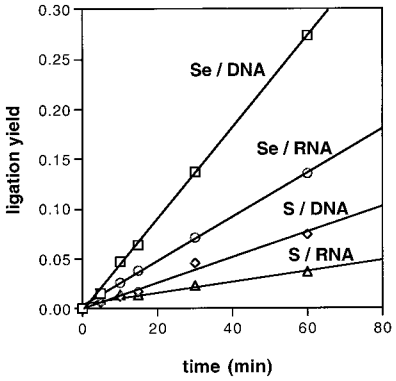

Figure 2. Time course of phosphoroselenoate autoligation on DNA and RNA template strands, with comparison to sulfur-mediated ligations with the same sequences. Lines represent fits to the early data points for initial rates analysis. Conditions: 1.3 μM in each DNA strand, 10 mM $MgCl_2$, 70 mM Tris·borate (pH 7.0) at 37 °C. Sequences are given in Figure 1 (MUT targets).

carried out a reaction using the 13mer iodo probe with the 7mer MUT selenium-containing probe and followed the course of the reaction with fully complementary (MUT) or singly mismatched (WT) DNAs. The mismatch in the latter case is T−C. While the fully complementary ligation proceeded to 27% yield in 60 min, the mismatched case showed no ligated product at this short time. After 24 h, we detected a trace of ligation product (data not shown); quantitation of this product suggests a 190-fold slower rate for ligation based on the initial slopes. This is greater selectivity than reported for T4 DNA ligase,[4g] the enzyme most widely used for ligations.

Because bridging selenium esters were previously unknown in DNA, we characterized the product, a 5′-bridging phosphoroselenoester, produced in an autoligation reaction. Electrospray mass spectrometry confirmed the presence of the selenium in a 17mer DNA strand (data not shown), clearly distinguishing it from sulfur- and oxygen-containing strands of the same sequence. The hydrolytic stability of this joined DNA was also tested at 23 °C over the pH range 5−9 (see supporting material). We found no measurable degradation, allowing us to estimate a lower limit of one year for the half-life for hydrolysis of this junction.

Finally, we tested the ability of the selenium-bridged DNA to hybridize to a complementary strand of DNA in the presence of 10 mM Mg^{2+}. The selenium-bridged DNA was found to bind with a T_m value (free energy (70 °C)) of 73 °C (−11.3 kcal/mol) for selenium and 76 °C (−13.1 kcal/mol) for oxygen. The sulfur case falls between the two at 74 °C (−12.0 kcal/mol). Overall, the selenium does not appear to be strongly destabilizing to the double helix.

The present results show that the use of selenium as a nucleophile allows for a substantial increase in ligation rate over previous sulfur chemistry,[6h,11] and is carried out with equal ease. Importantly, the selenium reaction can be carried out on RNA strands as readily as DNA strands and shows very high selectivity against point mutations. Thus, the selenium autoligation may prove useful in diagnostic strategies for direct analysis of RNAs. It may also find utility in a number of other applications for which previous ligation chemistries may not be well suited. For example, the selenium may be employed for probing mechanisms of catalyzed nucleic acid hydrolysis, as a complement to commonly used sulfur substitution.[13] In addition, selenium incorporation in DNAs may find use in structural biology, since heavy atom replacement is widely used as an aid in solving X-ray crystal structures of large biomolecules and complexes. It has not previously been possible to stably incorporate selenium into DNA, in analogy to the use of selenomethionine in proteins.[14]

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Supporting Information Available: Experimental details of oligonucleotide synthesis, characterization, ligation reactions, and thermal denaturation studies (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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