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LITERATURE REVIEW: OLIGONUCLEOTIDE CROSS-LINKING

INTRODUCTION

One of the most common requests at Glen Research over many years has been for procedures for cross-linking oligonucleotides with DNA, RNA, and proteins for a variety of purposes. Unfortunately, the response has been that many methods have been described and each probably works well for a given set of circumstances but no method works well in general. Basically, cross-linking is of interest to stabilize the interaction of two weakly bound biomolecules. This includes affinity labels of DNA- and RNA-binding proteins, interstrand cross-links between DNA and/or RNA, and capturing any molecule's approach to DNA or RNA. Cross-linking may be initiated by a variety of chemical reactions, for example, disulfide formation, but the predominant procedure is photo-induced cross-linking. The literature covering cross-linking is substantial so we have chosen a few examples of personal interest, which are supported from our product range.

HALOGENATED NUCLEOSIDES

Halogenated nucleoside phosphoramidites are the veterans of modified bases and some of the earliest to be commercially available. Oligonucleotides containing the bromo and iodo pyrimidine analogues found early use in crystallography studies. However, these products are also photolabile and were also used for photo cross-linking studies.^{1,2} Their limitations were that the cross-linking reactions could be very low yielding and were not reversible.

THIONUCLEOSIDES

The use of 4-thio-dU, 4-thio-dT and 6-thio-dG phosphoramidites has been described for template directed chemical cross-linking once incorporated into oligonucleotides. Subsequent post-synthetic modification of the thiocarbonyl group can lead to cross-linking by S-alkylation or disulfide formation. The initial studies were done in the early 1990s³⁻⁷ but this strategy has seen a more recent resurgence.⁸⁻¹⁰

DISULFIDE LINKAGES

Formation of disulfide linkages is readily accomplished by mild oxidation and linkage can be reversed by mild reductive cleavage. Disulfide exchange can be used to form cross-links between nucleic acid strands¹¹⁻¹³ and also between DNA templates and their binding proteins.¹⁴⁻¹⁶

CLICK CHEMISTRY

As one would expect, the great utility of click chemistry has also been applied to interstrand cross-linking and some fine examples have been reported.¹⁷⁻²⁰ Since the azide and alkyne groups required for click chemistry are not naturally occurring, click chemistry is very specific with virtually no side reactions. Although we expect examples of cross-linking with click chemistry to increase rapidly, there is a downside in that the reaction is not reversible.

PSORALEN

Interstrand cross-linking of oligonucleotides using psoralen has been a popular method for many years. Oligonucleotides containing psoralen are relatively simple to prepare and cross-linking to the complementary strand using UV irradiation is straightforward. Psoralen C2 is used to cross-link with the complementary strand in duplexes and psoralen C6 is used to cross-link to the third strand of triplex DNA.^{21, 22} There are significant limitations to the use of psoralen for cross-linking since psoralen reacts preferentially with thymidine, thereby restricting the sequences that can be used effectively to 5'-AT... or 5'-TA... Reversal of the photo-induced cross-link is possible but requires irradiation at 254nm, which may cause some UV-induced damage to the DNA by pyrimidine dimer formation.

NUCLEOSIDE ANALOGUES

Photo Cross-Linking

Recent developments in cross-linking have involved the use of specially designed nucleosides or nucleoside analogues. One of the most promising analogues is a cyanovinylcarbazole nucleoside analogue (C^{NV}K).²³

This product can be incorporated into oligonucleotides with no change to normal coupling and deprotection protocols. Irradiation at 366nm for 1 second leads to efficient interstrand cross-linking with adjacent pyrimidine bases. Reversal of the cross-link occurs in 3 minutes at 312nm. In a more recent publication, the authors describe that the same photo-reversible cross-linking reaction with the addition of heat also leads to C → U conversion.²⁴ This opens up potential uses in mutagenesis studies.

Oxidative Cross-Linking

The phenylselenide (PhSe) group in nucleoside analogues has been described as a cross-linker by generating methyl radicals induced by photo-irradiation²⁵ and also, interestingly, by an oxidative mechanism using sodium periodate.²⁶ Work has been carried out in both duplex and triplex DNA.²⁶

Cross-Linking by Hydrogen Bond Formation

When cross-linking is triggered by photochemical or chemical means, there is always the opportunity for side reactions to occur. A recent review article has highlighted work done on nucleoside analogues that cross-link only when hydrogen bonding to the target molecule is formed. The authors describe²⁷ the cross-linking behavior of 2-amino-6-vinylpurine (AVP) and 4-amino-6-oxo-2-vinylpyrimidine (AOVPY). Both of these nucleoside analogues exhibited efficient and specific cross-linking with thymidine in the complementary strand. These findings offer significant promise for the development of cross-linking systems that do not require the use of photochemical or chemical stimuli.

CONCLUSION

This very selective literature review of cross-linking demonstrates that a significant effort has been expended on the development of cross-linking techniques over a period of at least 15 years. As a result, a variety of useful techniques has been developed, some of which are in routine use. However, there is clearly still scope for improvement. And, it may never be possible to find the one cross-linking strategy that fits all needs.

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