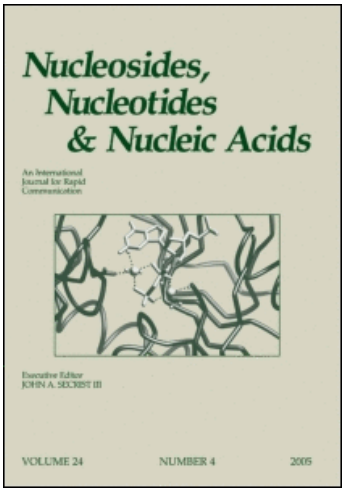

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Highly Efficient Synthesis of Peptide- and Carbohydrate-Oligonucleotide Conjugates Using Chemoselective Oxime and Thiazolidine Formation

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Severe limitations such as cellular uptake efficiency, cell-specific delivery, stability against nucleases have impeded the use of oligonucleotides in therapy. The design of delivery strategies that can improve the cellular targeting and uptake has emerged as a prerequisite for the therapeutic use of oligonucleotides. Anchoring peptides or carbohydrates to oligonucleotides represents a promising approach; indeed, properties such as cell penetration or targeting, affinity for DNA or RNA targets could be improved.[1] While a number of methods have been described for the preparation of such conjugates,[1] the development of a more efficient and selective ligation is still of great interest. The ideal conjugation reaction should take place between both unprotected moieties with minimal chemical manipulation and under quite physiological conditions.

We describe new methods for the preparation of peptide-oligonucleotide conjugates (POCs) and carbohydrate-oligonucleotide conjugates (COCs) that fulfilled these requirements (Fig. 1). The conjugation of a peptide to the 5′- or to the 3′-end of an oligonucleotide was achieved by reaction of an aldehyde moiety with an aminooxy or a 1,2-aminothiol via the oxime ether and the thiazolidine linkages formation, respectively.[2] The conjugation of carbohydrates was performed via oxime bond formation using aminooxy-sugar derivatives.[3]

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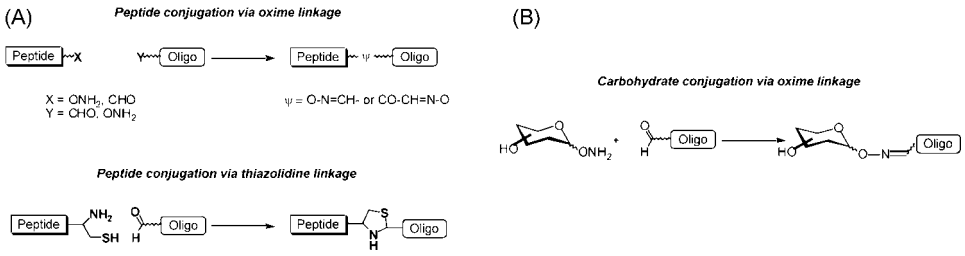

Figure 1. General strategy for the preparation of (A) POCs and (B) COCs.

The aldehydic function was generated at the 5′-end of oligonucleotides by the oxidation of a 1,2-diol or at the 3′-end via the oxidation of a 1,2-aminoalcohol. The 1,2-diol was incorporated at the 5′-extremity through a C_6 linker bearing the protected 1,2-diol using the corresponding phosphoramidite. For the 3′-functionalisation, a commercial support bearing a protected 1,2-aminoalcohol was used. The oxyamino moiety was included at the 5′-end of oligonucleotides as a trityl protected form.[4] The functionalisation of the peptide moiety was accomplished through the oxidation of a serine residue for the introduction of the aldehyde or through the coupling reaction with the activated ester of *N*-Boc-*O*-(carboxymethyl)-hydroxylamine for the incorporation of the oxyamino moiety. Two biologically relevant peptides were used: a cyclopenta-peptide containing an arginine-glycine-aspartic acid tri-peptide motif (RGD) known as a powerful and selective ligand of the $\alpha_V\beta_3$ integrin receptor and a nuclear localisation signal sequence (NLS): the basic peptide APKKKRKV. The conjugations with various carbohydrates (α-mannose, α-*N*-acetyl galactose, β-galactose and β-glucose) were performed by reaction with the carbohydrates bearing an aminooxy group at their anomeric position. The ligation via thiazolidine formation was achieved by coupling the peptide acylated with a cysteine residue to the oligonucleotide derivatised by the aldehyde function.

The coupling reactions were carried out in water at pH = 4.6 for oxime formation and pH = 5.5 for thiazolidine formation using a slight excess (2 eq.) of the peptide or of the sugar moiety. In all cases, the chemical ligation was found very efficient and selective affording the corresponding conjugates in good yield. Moreover, the oxime ligation proved useful to conjugate directly duplex oligonucleotide.

The chemical stability of the oxime and the thiazolidine linkage at pH = 4 and pH = 7 at 37°C was studied. Both linkage were found stable at pH = 7 even after 72 h of incubation. In the other hand, the thiazolidine was shown less stable than the oxime at pH = 4 as 20% of hydrolysis was observed after 48 hours of incubation in these conditions.

The stability in biological media (RPMI-1640 and calf foetal serum (20%)) was also studied. As anticipated, the 3′-modified oligonucleotide was found much more stable. In fact, degradation up to 50% was observed after 3 h of incubation of the 5′-modified and of the unmodified oligonucleotide, while the 3′-conjugate remained stable in these conditions. These results are in agreement with the protecting effect against nucleases observed with 3′-capped oligonucleotides.[5]

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Together the high efficiency and versatility of this strategy over conventional conjugation method is of great interest to devise new molecular system based on ODN.[6] Furthermore, the conjugation using oxime bond formation could also be performed on duplexes secondary structure.

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