Incorporation of side-chain groups of basic amino acids into oligonucleotides via the 2'-position of uridine

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ABSTRACT

Certain amino acids and short peptides are known to act as enhancers of ribozyme-mediated RNA cleavage at low concentration of magnesium ion. Thus, covalent conjugates of oligonucleotides and some amino acids may have a potential for development as sequence-specific artificial ribonucleases. Here we would like to report an incorporation of basic amino acids, lysine and histidine, into oligonucleotides at the 2'-position of a uridine residue. The approach involves the 2'-O-alkylation and attachment of the corresponding amino acid by the α-amino group through a urethane-type linker.

INTRODUCTION

Chemically modified oligonucleotides and their conjugates represent versatile and potent tools to block gene expression within cells in culture, and are being studied as potential therapeutic agents in humans.¹ Design of different types of oligonucleotide-based artificial ribonucleases has been attracting a significant interest, mainly because these compounds may target mRNA or viral RNA.² Synthetic chemistry offers wide design opportunities for the construction of novel nucleic acid-based enzymes. A number of attempts have been made to chemically synthesise artificial RNases consisting of oligonucleotides that carry imidazole and amino functionalities.³,⁴,³ We have shown recently that basic amino acid and small peptide additives can stimulate hairpin ribozyme cleavage significantly at low magnesium ion concentration.⁵ Studies we present here were motivated by the need to develop a convenient method for the introduction of specific amino acid functional groups, e.g. those of histidine and lysine, into oligonucleotides. Since nucleobases in the core regions of ribozymes are often involved in various non-covalent interactions important for maintaining structure and function, the sugar part of nucleosides, particularly its 2'-position, seems to be an attractive site for ligand attachment. A promising 2'-functionalisation approach is via a carbamate, or urethane, group, easily generated from primary or secondary alcohols by the successive treatment with 1,1-carbonyldiimidazole (CDI)⁶ or N,N'-disuccinimidyl carbonate (DSC)⁷ and an aliphatic amine. Initially, this chemistry has been used successfully for the synthesis of oligonucleotides containing a number of N-substituted carbamates directly attached to the 2'-position of uridine.⁸ However, it was shown that the presence of a 2'-carbamate group in an oligonucleotide decreases the stability of the corresponding duplex with a complementary DNA or RNA.⁹ To maintain satisfactory duplex stability and, furthermore, to be able to modulate linker length, another approach was explored recently that is based on alkylation of the 2'-hydroxyl.

Our general scheme of preparation of the 2'-amino acid-functionalised uridine 3'-phosphoramidites involves 2'-O-alkylation by a benzoylcarbonylmethyl group,⁹ which can then be reduced to the corresponding 2-alkoxyethanol and attached to the α-amino group of an amino acid via a carbamate linkage. After solid-phase assembly and deprotection, the resultant oligonucleotides display pendant 2'-imidazole and 2'-aminoalkyl groups.

RESULTS AND DISCUSSION

A general route to 2'-amino acid-functionalised phosphoramidites is outlined in Scheme 1. 2'-Modified nucleoside phosphoramidites were used in machine-assisted solid-phase oligonucleotide synthesis by the standard 2-cyanoethyl phosphoramidite chemistry. The average coupling efficiency of the novel monomers at 0.2 M concentration in dry MeCN and 30 min reaction time was found to be above 97%. Several model oligo-2'-O-methylribonucleotides were assembled, cleaved from their solid supports and deprotected by conc. aq. ammonia treatment at 55°C overnight. Removal of the trityl group from histidine was accomplished by treatment with 80% acetic acid for several days at 20°C. Reaction mixtures obtained were analysed by ion-pair reverse-phased HPLC and MALDI-TOF mass spectroscopy.

CONCLUSION

In conclusion, we present here an efficient and reliable method for the preparation of 2'-amino acid-functionalised
uridine 3′-phosphoramidites and oligo-2′-O-methylribonucleotides thereof. The approach described could prove valuable in the design of sequence-specific artificial ribonucleases.

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REFERENCES