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Synthesis of oligonucleotide 2′-conjugates via amide bond formation in solution

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Abstract—An efficient method for synthesis of 2′-*O*-carboxymethyl oligonucleotides is described. Fully deprotected oligonucleotides containing a carboxymethyl group at the 2′-position of sugar residue were obtained by a two-step procedure by periodate cleavage of an oligonucleotide containing 1,2-diol group followed by oxidation of the 2′-aldehyde resulted with sodium chlorite. 2′-*O*-Carboxymethyl oligonucleotides prepared were efficiently coupled in aqueous solution in the presence of a water-soluble carbodiimide to a number of amino acid derivatives or short peptides to afford novel 2′-conjugates of high purity in good yield. The method is thus shown to be suitable in principle for preparation of oligonucleotide–peptide conjugates containing an amide linkage between the 2′-carboxy group of a modified oligonucleotide and the amino terminus of a peptide.

1. Introduction

Conjugation of antisense oligonucleotides to amino acids, peptides and basic polymers may enhance their cellular permeability, target specific tissues, or alter their intracellular localization.[1–5] In these ways, chemical functionalization of synthetic oligonucleotides has found wide applications with rapid growth of molecular biology and biotechnology.[6,7]

Functionalization of the sugar moiety of oligonucleotides has encouraged the development of nucleoside phosphoramidite derivatives that are suitable for the incorporation of various electrophilic groups into a nucleic acid sequence.[8,9] Oligonucleotides containing a carboxylic acid group can be used for covalent attachment to other nucleic acids, peptides, reporter groups or for preparation of other conjugates.[10,11] Modification of the 2′-position of the sugar moiety allows the introduction of this modification via a corresponding phosphoramidite into any preselected site of an oligonucleotide chain.[12] That leaves both 3′- and 5′-ends free for a label introduction or an enzymatic reaction. Moreover, the relevant 2′-*O*-carboxamide modifications[13] are shown to increase the thermal stability of the corresponding duplexes with a complementary RNA, which is essential for antisense inhibition.[8]

Recently, we proposed an efficient method for synthesis of oligonucleotides containing a 1,2-diol as a masked aldehyde function at the 2-position of the ribose residue.[14] The resulting 2′-aldehyde oligonucleotides, generated by periodate oxidation, were successfully used for conjugation studies with N-terminally-modified peptides and small molecules.[15] Here we would like to report the preparation of 2′-carboxymethyl oligonucleotides, where a carboxylic acid group is generated after oxidation of the 2′-aldehyde with sodium chlorite.[16]

2. Results and discussion

To obtain an oligonucleotide modified at the 2′-position with a carboxylic acid group, we synthesized a fully deprotected purified model dodecamer (**I**) or pentadecamer (**II**) containing a 2′-diol group (Scheme 1)[17] by use of the phosphoramidite described by us earlier.[14] Conversion of the 2′-diol group into an aldehyde was carried out in a standard manner using 5 mM sodium metaperiodate solution.[15] Subsequent oxidation of (**I**) or (**II**) 2′-aldehyde by sodium chlorite in the presence of L-methionine produced the corresponding 2′-*O*-carboxymethyl oligonucleotides (**I**) and (**II**) in good yield without further purification (Fig. 1).[18]

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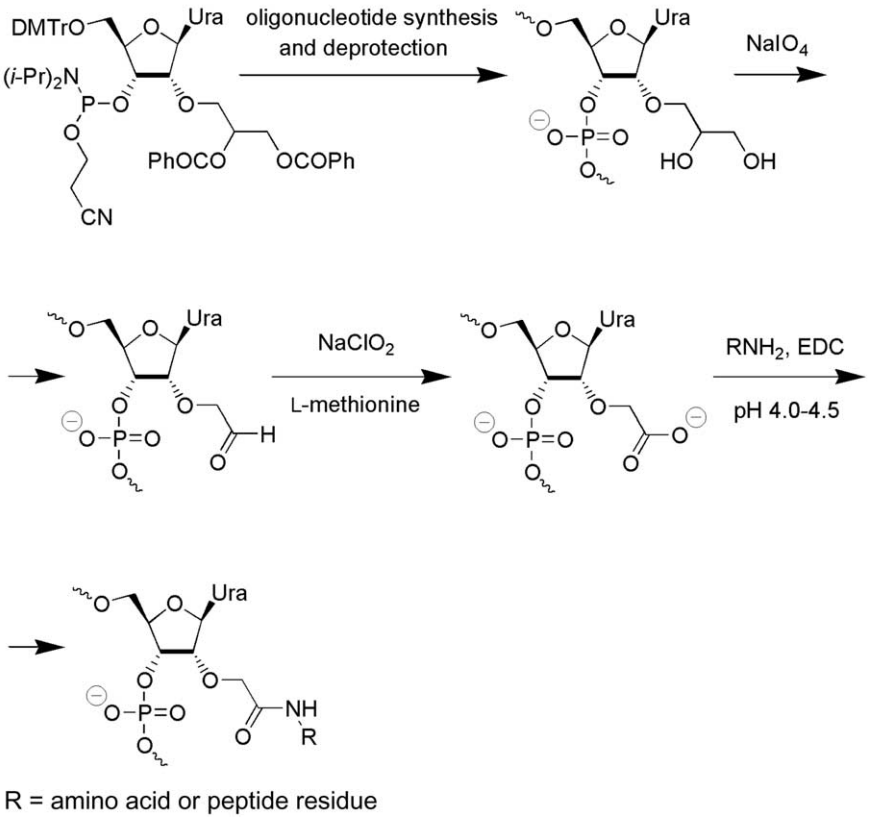

Scheme 1. Preparation of 2′-*O*-carboxymethyl oligonucleotides and their conjugates.

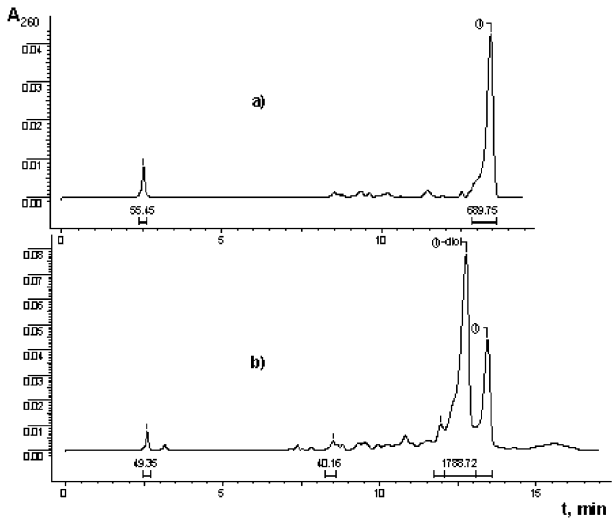

Figure 1. HPLC traces (ion pair-mode) of two-step oxidation of 2′-diol to carboxylic acid group: (a) crude reaction mixture; (b) co-injection of the above mixture with purified oligonucleotide (**I**) diol.

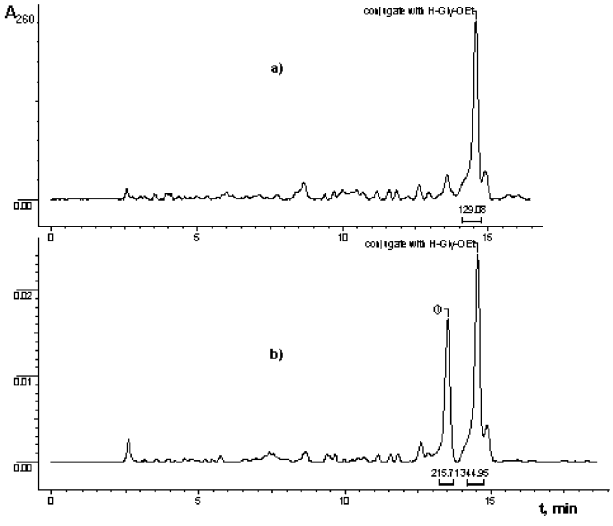

Figure 2. HPLC traces (ion pair-mode) of EDC-induced amidation of 2′-*O*-carboxymethyl oligonucleotide (**I**) with H-Gly-OEt (**1**): (a) crude conjugate (**I.1**); (b) co-injection of the above conjugate with purified oligonucleotide (**I**).

2′-*O*-Carboxymethyl oligonucleotide (**I**) was conjugated with a number of amino acids and short peptides in the presence of a water-soluble carbodiimide (EDC) and a catalytic amounts of imidazole using a coupling method suggested by Kremsky et al.[10] The reactions were carried out in MES buffer, pH 4.0–4.5, overnight at ambient temperature.[19] Concentrations of 0.7M of amino acid or peptide were used throughout.

Conversion yields of the conjugates were good, as seen from the corresponding HPLC pictures (Figs. 2 and 3). Only in the cases of conjugates (**I.2**) and (**I.3**) some of the starting 2′-*O*-carboxymethyl oligonucleotide (**I**) were still present after overnight reaction. MALDI-TOF mass-spectroscopic data of oligonucleotides (**I**) and (**II**) and conjugates (**I.1**–**I.6**) are in agreement with the proposed structures (see Table 1).

Table 1. MALDI-TOF mass spectra of model oligonucleotides (**I**) and (**II**) and conjugates (**I.1**–**I.6**)

No.	Oligonucleotide, 5′ to 3′, or conjugated molecule[a]	MALDI-TOF MS, calc./found	Conversion yields, %[b]
I	CTCCCAGGCU*[c]CA (**I**)	3626.35/3623.44	—
II	GCU*[c]CCCAGGCTCAAA (**II**)	4581.90/4579.64	—
I.1	H-Gly-OEt (**1**)	3711.45/3708.14	99
I.2	H-Leu-NH_2 (**2**)	3738.52/3741.04	70
I.3	H-Phe-NH_2 (**3**)	3772.54/3777.25	85
I.4	H-Gly-Gly-NH_2 (**4**)	3739.47/3736.31	92
I.5	H-Gly-Leu-Met-NH_2 (**5**)	3926.77/3926.92	90
I.6	H-Asn-Arg-Asn-Phe-Leu-Arg-Phe-NH_2 (**6**)	4580.45/4581.90	95

[a] Peptides **4**–**6** all have their N-termini free and are C-terminal amides.
[b] Calculated from the respective HPLC traces.
[c] **U*** = 2′-*O*-carboxymethyluridine.

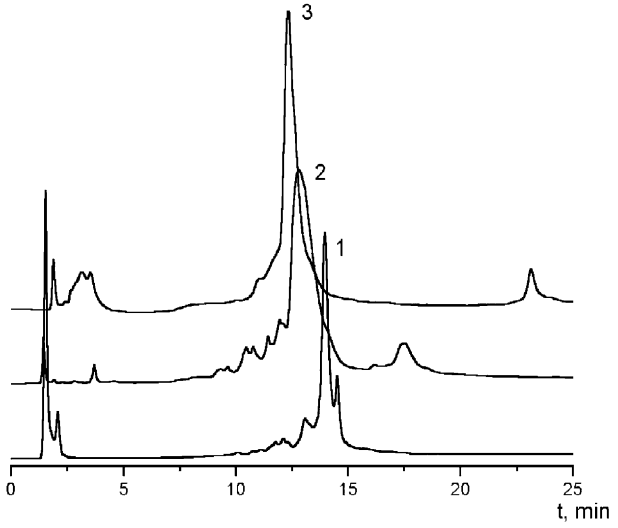

Figure 3. Reversed-phase HPLC traces of parent 2′-*O*-carboxymethyl oligonucleotide (**I**) (1), its conjugate (**I.5**) with H-Gly-Leu-Met-NH_2 (2), and its conjugate (**I.6**) with H-Asn-Arg-Asn-Phe-Leu-Arg-Phe-NH_2 (3).

Thus, the method described allows facile preparation of 2′-*O*-carboxymethyl oligonucleotides by use of simple chemical transformations and inexpensive reagents. The procedure afforded the 2′-amide-linked peptide-oligonucleotide conjugates that were synthesized and characterized for the first time. The method should be applicable to other peptide sequences as long as they contain a single N-terminal amino group. Any side-chain amino group, for example, that of lysine residue(s) has to be transiently protected, for example, by trifluoroacetylation.[20] Conjugation studies with various types of amines and longer peptides are currently underway and will be reported in due course.

3. Conclusions

We have presented here an efficient and reliable method for preparation of 2′-*O*-carboxymethyl oligonucleotides based on two-step oxidation of the 1,2-diol side chain of a 2′-modified oligonucleotide by sodium periodate followed by sodium chlorite. The resulting 2′-*O*-carboxymethyl oligonucleotides were coupled successfully to various amino acid derivatives and oligopeptides by a water-soluble carbodiimide-promoted reaction in aqueous solution.

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17. Oligonucleotide Synthesis. Oligodeoxynucleotide was assembled on ABI 380B DNA Synthesizer by the cyanoethyl phosphoramidite method following manufacturer recommendations. Deblocking and purification of 1,2-diol-containing oligonucleotide was carried out using conditions described.[14] MALDI-TOF mass spectra were run on a Voyager DE workstation (PE Biosystems) in a freshly prepared 1:1 v/v mixture of 2,6-dihydroxyacetophenone (40 mg/mL in MeOH), and aqueous diammonium hydrogen citrate (80 mg/mL) as a matrix. Conditions of reversed-phase HPLC: Phenomenex Bondclone 10 C_{18} column (3.9×300 mm), dual wavelength detection (215 and 254 nm); buffer A: 5% of MeCN (v/v) in 0.1 M triethylammonium acetate, buffer B: MeCN; flow rate 1 mL/min, gradient of B in A: 0–5%, 5 min, 5–15%, 10 min, 15–40, 30 min, 40–80%.
18. Oxidation of model oligonucleotide (**I**) 1,2-diol to carboxy group: The dried oligonucleotide (3.0 A_{260} units) was dissolved in mixture of 0.4 M sodium acetate (10 μL) and water (10 μL). Then, 5 mM $NaIO_4$ solution (10 μL) was added, and the reaction mixture was incubated at ambient temperature for 1 h. To that 0.2 M methionine solution was added and further incubation was carried out for 30 min followed by treatment by 20 mM sodium chlorite (10 μL) for 5 h. The reaction was quenched by addition of 20 mM sodium sulfite (10 μL) and then precipitated with 4 M sodium acetate solution (20 μL) and ethanol (1.5 mL) and then analysed using reversed-phase HPLC in ion pair mode.[11]
19. Conjugation of 2′-*O*-carboxymethyl oligonucleotide (**I**) with amino acid derivatives or peptides: 0.7 M solution (50 μL) of either amino acid or peptide derivative in MES buffer, pH 4.0–4.5, was added to the dried pellet of modified oligonucleotide **I** (0.5 A_{260} unit) followed by solid 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (5 mg) and imidazole (ca. 1 mg). The reaction mixtures were incubated overnight at ambient temperature, precipitated by addition of 2M $LiClO_4$ solution (0.2 mL) followed by acetone (1.5 mL) and analyzed using reversed-phase or ion-pair HPLC and MALDI-TOF mass spectroscopy. Oligonucleotide **II** was used to optimize the conjugation conditions (data not shown).
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