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Synthesis of oligonucleotide–peptide conjugates using hydrazone chemical ligation

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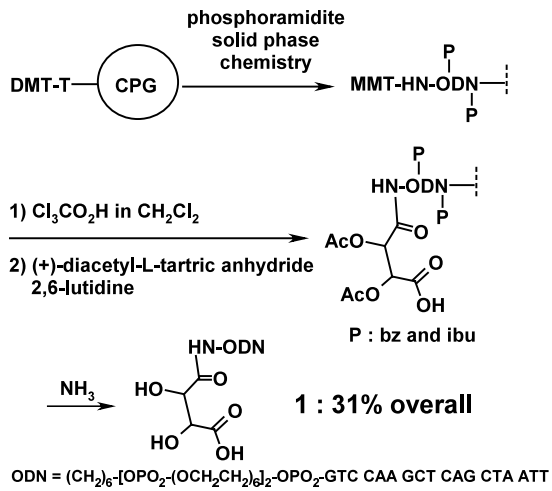
Abstract—An oligonucleotide was functionalized on the solid-phase by a tartaramide moiety, which could be converted efficiently in solution into a glyoxylyl group following a mild periodic oxidation. The glyoxylyl-oligonucleotide was found to be very stable upon storage and was successfully engaged in hydrazone ligation with an α-hydrazino acetyl peptide. © 2002 Elsevier Science Ltd. All rights reserved.

Oligonucleotides constitute a class of potential therapeutic agents. Improvement of certain properties, such as cell-specific delivery, cellular uptake efficiency, intracellular distribution, target specificity, can be realized by covalent association to peptides.[1] Rather than forming ionic oligonucleotide–peptide complexes, covalent association gives a more consistent product in which the desired properties can be controlled by structural variations. We present in this paper a novel methodology for the site specific ligation of deoxyoligonucleotides (ODN) to peptides through a hydrazone link, based on the synthesis of glyoxylyl-ODN[2] and their reaction with α-hydrazino acetyl peptides.[3] Hydrazone ligation has many advantages over thiol-based ligation chemistries which are often used for the construction of peptide–ODN conjugates.[1] Indeed, this reaction allows the use of fragments incorporating thiol functionalities, and does not require the exclusion of molecular oxygen. ODNs functionalized by an aliphatic or aromatic aldehyde group were described in literature.[4] The main problem associated with these ODN derivatives is their poor stability and the well-known propensity of the aldehyde group to react with purine bases.

The glyoxylyl group has been extensively employed in protein engineering.[5] This function reacts with hydrazines, hydroxylamines and β-amino thiols to give the corresponding hydrazones, oximes or thiazolidines in high yield. Interestingly, the glyoxylyl group is highly stable to air oxidation and to our knowledge was never found to form imine bonds with Lys side chains or α-amino groups during storage or ligation reactions. The need for an alternative aldehyde-ODN chemistry led us to evaluate the synthesis, stability and reactivity of glyoxylyl-ODNs.

Glyoxylyl-ODN **2** was synthesised as described in Schemes 1 and 2. Following standard phosphoramidite solid-phase elongation, the terminal amino group was deprotected with trichloroacetic acid and immediately reacted with an excess of (+)-diacetyl-L-tartric anhy-



Scheme 1.

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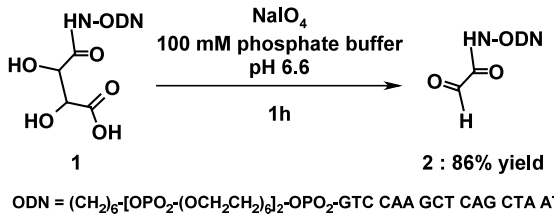


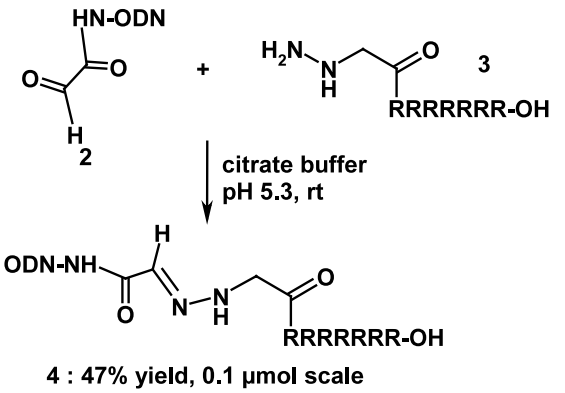
Scheme 2.

dride (commercially available) in the presence of 2,6-lutidine.[6] The tartaramide bond was found to be fully stable during the aminolysis step. Tartaramide-ODN **1** was isolated with a 31% yield following purification by C18 RP-HPLC.

Compound **1** in hand, we further examined its oxidative cleavage with periodate (Scheme 2). The reaction was performed using very mild conditions (1.7 equiv. of sodium periodate at pH 6.6) and resulted in the clean formation of glyoxylyl-ODN **2**, which was purified by C18 RP-HPLC using an eluent mixture composed of acetonitrile/pH 6.5 triethylamine acetate. However, while glyoxylyl-ODNs are stable for days in this eluent, lyophilization of the pure fractions resulted in a partial oxidation of the glyoxylyl group into oxalyl function.[7] This oxidation could be due to the presence of traces of an oxidant in the RP-HPLC buffer. Indeed, addition of minute amounts of $(n\text{-Bu})_3P$ to the glyoxylyl-ODN solution before lyophilization permitted us to suppress completely this side reaction. Once lyophilized, glyoxylyl-ODNs were found to be stable for months at −20°C.

We next examined the usefulness of glyoxylyl-ODN **2** for site specific hydrazone ligation using hydrazinopeptide **3** (Scheme 3).[8] Peptide **3** was synthesised on the solid phase using standard Fmoc/*t*-Bu chemistry. The α-hydrazino acetyl moiety was introduced using $Boc_2N\text{-}N(Boc)CH_2CO_2H$ and HBTU/HOBt[9] activation, as described elsewhere.[10] Peptide **3** was isolated with a 23% yield following purification by RP-HPLC.

The ligation was performed at rt in a pH 5.3 citrate buffer using 2 equiv. of hydrazinopeptide **3**. As shown



Scheme 3.

in Fig. 1(a), hydrazone formation was found to be very rapid, since few seconds after the addition of the peptide to glyoxylyl-ODN **2** the conversion was already close to 50%. Following 22 h at rt, the crude ligation product (90% by RP-HPLC, the remaining being **2**, see Fig. 1(b)) was purified and lyophilized to give the expected compound **4** in a 47% yield. The RP-HPLC of hydrazone **4** and the corresponding MALDI-TOF spectrum ($[M+H]^+$ calcd 7548.8, found 7544.8) are presented in Fig. 2(a) and (b), respectively, and highlight the efficiency of the ligation strategy. No aggregation of the peptide–ODN conjugate was observed despite the polycationic nature of the polyarginine sequence.

In conclusion, the data presented in this letter show that glyoxylyl-ODNs are easy to synthesize and stable ODN derivatives in the lyophilized form. Glyoxylyl-

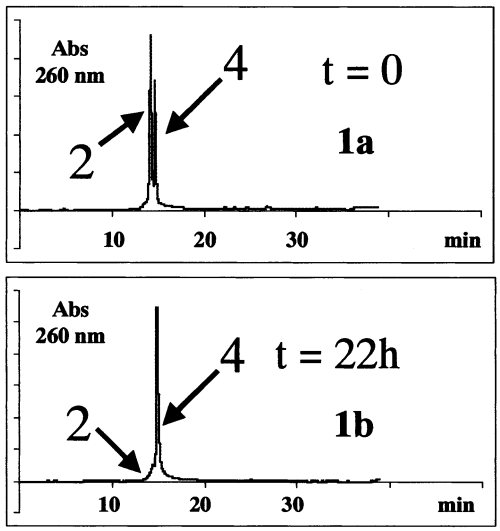


Figure 1. RP-HPLC of the ligation reaction. C18 hypersil 4.6×250 mm column, 260 nm A: 10 mM TEAA/CH_3CN 99/1 B: CH_3CN/water 95/5, 0–40% B in 28 min, 1 mL/min. (a) Just after the addition of peptide **3**. (b) Following 22 h of reaction at rt.

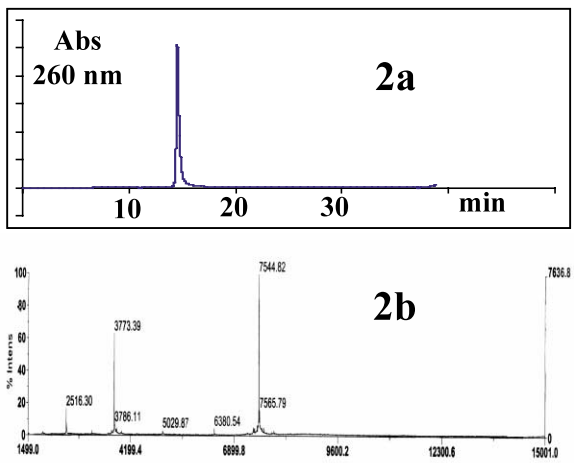


Figure 2. (a) RP-HPLC of purified hydrazone **4**, conditions as in Fig. 1. (b) MALDI-TOF spectrum of **4**.

ODNs react rapidly with α-hydrazino acetyl peptides and allow the efficient synthesis of peptide–ODN conjugates. This non-thiol based ligation chemistry allows, in principle, the use of cysteine-containing peptides.

Acknowledgements

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7. In the case of **2**, the formation of oxalyl-ODN resulted in the appearance in the MALDI-TOF spectrum of a peak at m/z 6400.3 ($[M+H]^+$ expected 6399.4).
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