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N-Hydroxysuccinimide ester labeling 5′-aminoalkyl DNA oligomers: reaction conditions and purification

Zhixian Wang, Nanying Bian, Xiaohua Qian, Roger W. Giese*

Department of Pharmaceutical Sciences in the Bouvé College of Pharmacy and Health Professions, Barnett Institute, and Chemistry Department, Northeastern University, Boston, MA 02115, USA

Abstract

Difficulties were encountered in labeling 5′-aminoalkyl DNA oligomers with glycolketo electrophore N-hydroxysuccinimide esters in aqueous sodium bicarbonate (a common base for this purpose), followed by C_{18}-silica reversed-phase high-performance liquid chromatography (HPLC) to achieve purification. The electrophore-labeled oligomers were not separated readily either from the hydrolyzed electrophore or from the starting oligomer. This problem was overcome by conducting the reaction with triethylamine as a base, organic washing the reaction mixtures after evaporation, and separating on a C_{18}-poly(styrene–divinylbenzene) HPLC packing. © 1998 Elsevier Science B.V.

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1. Introduction

DNA probes and primers are commonly prepared with a molecular label such as a fluorophore or biotin at the 5′-end. Typically a 5′-aminoalkyl oligomer is prepared first on a DNA synthesizer, and the oligomer then is coupled to an N-hydroxysuccinimide (NHS) ester of the label of interest. Precipitation, gel filtration, HPLC (reversed-phase or ion-exchange), or electrophoresis may be employed to purify the final product. Several similar procedures have been reported e.g. [1–4] and the overall subject has been reviewed [5,6]. DNA oligomers also can be end-labeled as part of their synthesis [7,8]. Here we report a method which was successful after difficulties were encountered with conventional techniques.

2. Experimental

Oligomer 2 (100 μg in 100 μl of water; Operon Technologies, Alameda, CA, USA) and electrophore NHS ester 1 (synthesized as described in [11]; 1 mg in 100 μl of tetrahydrofuran; THF) were mixed in a 1.7-ml Eppendorf tube and 25 μl of 10 mg/ml triethylamine in acetonitrile was added. After brief vortexing, the solution was rotated (rocking plate) for 2 days (arbitrary period; other times were not tested) and then evaporated in a Speedvac under vacuum. THF (1 ml) was added to the mixture which was centrifuged after 20 s vortexing. The supernatant was removed and the residue was similarly washed with acetonitrile and THF (1 ml each) followed by air drying, dissolution in 50 μl of water, and injection into a custom-packed ACT-1 HPLC column (packed in 20% rather than the usual 30% acetonitrile by InterAction Chromatography, San Jose, CA, USA). After a full day of use, the HPLC column is washed

*Corresponding author.

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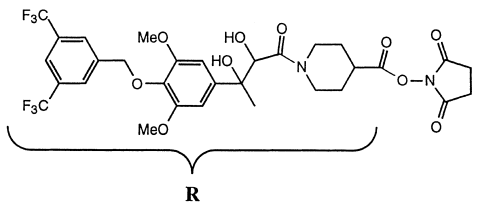
with 20 ml of 20% acetonitrile (acetonitrile–water, 20:80, v/v) and then a gradient over 30 min up to 70% acetonitrile.

3. Results and discussion

We are working on the development of glycolketo electrophores, such as the one shown in Fig. 1, as a new class of labels for DNA oligomers [9]. This type of label can be detected by laser desorption electron capture mass spectrometry since the glycolketo linkage undergoes a retro-aldol reaction to release a ketone electrophore when heated. DNA testing can potentially be highly multiplexed with such labels, where each electrophore possesses a different mass so that it can be distinguished in the mass spectrometer.

We encountered difficulties in using conventional methods to prepare and purify a 20-mer DNA containing a 5′-glycolketo electrophore starting with a 5′-aminoalkyl DNA and a glycolketo electrophore NHS ester. While the coupling reaction seemed to work in the ordinary way (e.g. with $NaHCO_3$ as a base), separation of the electrophore–DNA from the unlabeled oligomer, and also from the hydrolyzed electrophore NHS ester, was problematic. Part of the problem seemed to arise from the apparent detergent-like properties of the hydrolyzed electrophore. For example, once the hydrolyzed electrophore as a sodium salt was injected into a C_{18}-silica reversed-phase HPLC column, extensive washing of the column was necessary before eluted fractions no longer contained electrophore. Further, when a reaction mixture was subjected to HPLC (using C_{18}-silica columns either from Rainin Instruments or MAC-MOD Analytical), the labeled and unlabeled oligomers coeluted (or essentially so) under various mobile phase conditions. The latter type of problem was encountered by others in the similar preparation of DNA oligomers labeled with fluorescein or biotin, and was overcome by using HPLC with a C_{18}–poly(styrene–divinylbenzene) packing [4]. A poly-(styrene–divinylbenzene) packing also can be used [4,10], although ghosting was reported [4].

We overcame these problems by relying on a combination of two techniques. First, a volatile base (triethylamine) is used for the coupling reaction so that residual electrophore NHS ester (intact and hydrolyzed) and base can be efficiently removed from the electrophore-labeled oligomer by evaporation followed by organic washing of the residue. When a base like sodium bicarbonate is used, a salt residue is obtained after evaporation that interferes with the organic washing. Second, HPLC separation is performed similarly to the method cited above [4] with a C_{18}–poly(styrene–divinylbenzene) packing. This yields the chromatogram shown in Fig. 2, where the labeled oligomer is seen to elute much later than



2 5'-NH_2-$(CH_2)_6$-TGGTACCGGTCATGACAACG

3 R-NH-$(CH_2)_6$-TGGTACCGGTCATGACAACG

Fig. 1. Structures of (1) the electrophore NHS ester (2) starting DNA oligomer and (3) labeled DNA oligomer.

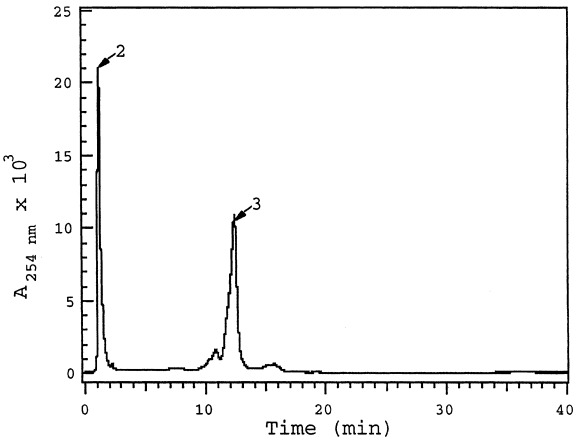


Fig. 2. HPLC chromatogram for the purification of an electrophore labeled DNA oligomer. Peak identity: see Fig. 1. HPLC column: custom-packed ACT-1. Mobile phase: A: 0.05 *M* Na_2HPO_4 in water; B: acetonitrile–water (70:30, v/v). Gradient profile: start with 70% A and 30% B, and ramp to 100% B in 30 min, hold at 100% B for 10 min and then return to 70% A and 30% B in 25 min; flow-rate: 0.6 ml/min.

the unlabeled oligomer. Other oligomers and their conjugates with other electrophores elute similarly. Labeled oligomer was collected, evaporated, redissolved in 100 μl of water, and desalted (the HPLC mobile phase contains sodium phosphate) on a NAP-10 column (Pharmacia, Uppsala, Sweden). The overall yield of electrophore-labeled oligomer, based on UV absorption of the final, collected product, ranged from 60 to 80% depending on the oligomer and the glycolketo electrophore.

Initially we used a conventional ACT-1 HPLC column for the separation that was packed by the manufacturer in 30% acetonitrile. While this gave essentially the same separation as that shown in Fig. 2, peak shape degraded markedly, and rather suddenly, after just 1 month of use. When the same problem repeated with a second ACT-1 column, we contacted the manufacturer. Apparently the bed was collapsing since our gradient begins at 20% acetonitrile, but the column is packed in 30% acetonitrile. The manufacturer ordinarily selects the latter condition for packing since it maximizes the plate count for the column. Nevertheless, a similar plate count is obtained by packing in 20% acetonitrile. The custom-packed column was found to be stable under our conditions.

4. Conclusion

Our somewhat new method for preparing a labeled DNA oligomer appears to be advantageous since the steps and conditions are simple, the resolution in the HPLC step is high, and the overall yield is good (60–80%).

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