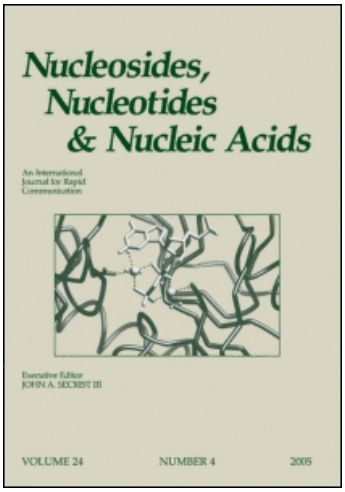

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CONSTRUCTION OF EUROPIUM (Eu^{3+})-LABELLED OLIGO DNA HYBRIDIZATION PROBES

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Abstract
Three different oligo DNA probes were synthesized and their value as hybridization probes was tested.

Hybridization techniques and labels that have been employed in these studies have been recently reviewed.[1] Europium or terbium are known to be useful as nonradioactive markers in immunoassays as they can be measured with high sensitivity by the time-resolved fluorometric procedure[2], which eliminates most of the background fluorescence. Recently published reports[3] show that lanthanide chelates can also be employed as markers in hybridization assays. In all of the above cases long DNA probes were used. Nowadays, however, there is a tendency to employ short oligonucleotides instead of longer poly-DNA fragments as hybridization probes.

They can be easily synthesized today even by non-chemists. An additional feature of the synthetic DNA fragments is the possibility of their specific derivatization at protected stage. The oligo-DNA-probes are characterized by very favourable kinetics of hybridization, allowing the assay to proceed at a low temperature and in a much shorter time period than those which are necessary for poly-DNA-probes.

A brief calculation of the fluorescence intensity using the probe labelled with only a few fluorescent markers showed that the sensitivity of such an assay will be insufficient. Therefore, an important feature of the present work is the use of water soluble, linear polymers as a carrier of a large number of covalently coupled lanthanide chelates.

A closely related system utilizing a modified nucleotide for the extinction of the oligo probe sequence[4] has also been constructed and its value as a hybridization probe demonstrated.

MATERIALS AND METHODS

Materials

Polyvinylamine x HCl[5] (30000) (1) and polyacrylic acid (50000, Polyscience INC) (9) were used as water soluble polymers.

Stabile chelates (2), (3) and (4), (5) are new products the synthesis of which will be described elsewhere. The modified nucleotide (11) has been prepared according to existing methodology.[6] The synthesis of DNA sequences was performed by using an automated Gen-Assembler (Pharmacia, Sweden) employing original solvents and reagents. Functionalized at 5'OH mercapto respective amino oligo DNA fragments were synthesized following the literature prescriptions.[7,8]

Oligo DNA probes complementary to known sequences of Adenovirus 2 or λ-phase DNA were of 50 base lenght through all experiments. All other reagents used were of the highest commercial quality.

Oligo DNA-polyvinylamine (basic polymer)-Eu^{3+} chelate (Fig. 1)

0.5 mg of polyvinylamine HCl in carbonate buffer (0.5 M, 200 µl, pH 10.5) was labelled using isothiocyanate chelate (2) (10 mg). The reaction mixture was incubated overnight at 20°C. The labelled polymer was separated by gel filtration through a Sephadex G-50 column (0.7 x 20 cm), desalted and concentrated.

The polymeric chelate (6) was dissolved in 100 µl of phosphate buffer pH 6.5, and a bifunctional coupling reagent (7) (as in Scheme 1) (0.27 mg) in ethanol (10 µl) was added. The reaction mixture was incubated for 6 hours with occasional shaking, and the product was isolated by precipitation from acetone.

5'-Mercapto oligo DNA probe (5 OD), complementary to a known sequence of Adenovirus 2 was mixed with the activated chelate at different ratios using phosphate buffer (pH 7.0, 100 µl). All mixtures were incubated overnight and separated using a Sephadex G-100 column. The column was equilibrated and eluted with PBS.

Oligo DNA-polyacrylic acid (acidic polymer)-Eu^{3+} chelate (Fig. 2)

Amino-functionalized 50-mer (10), (5 OD), complementary to the λ-phage DNA was coupled to polyacrylic acid (9) (1.5 mg) with the help of water soluble carbodiimide (EDAC) (5 mg) in buffer (pH 7.5, 200 µl)

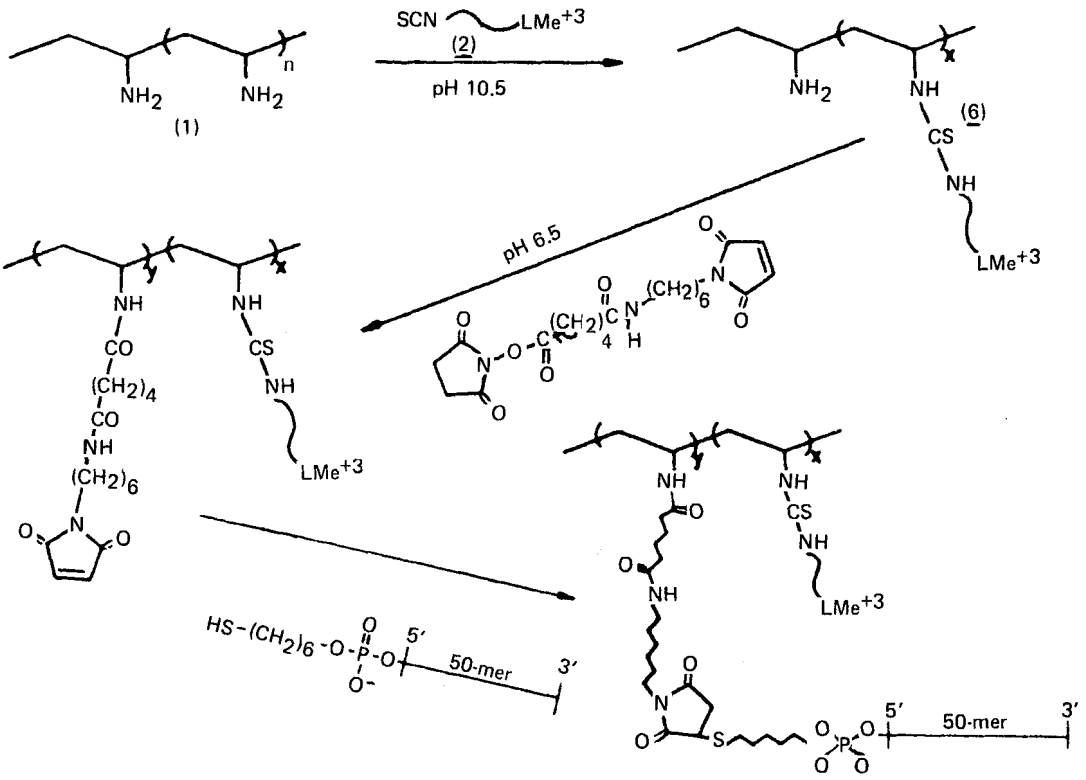
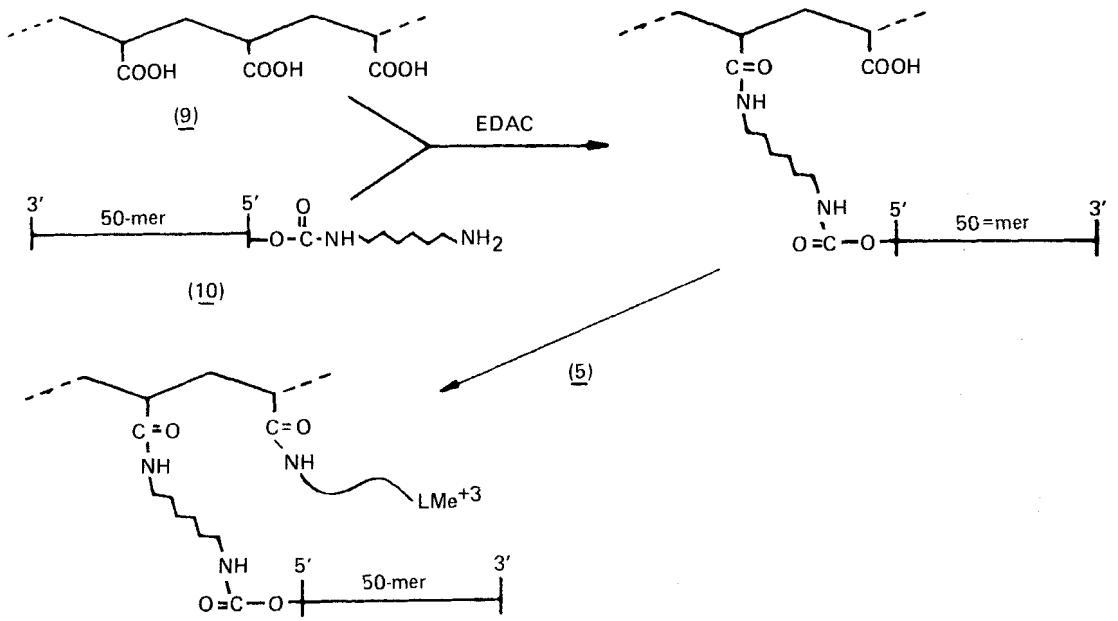


FIGURE 1



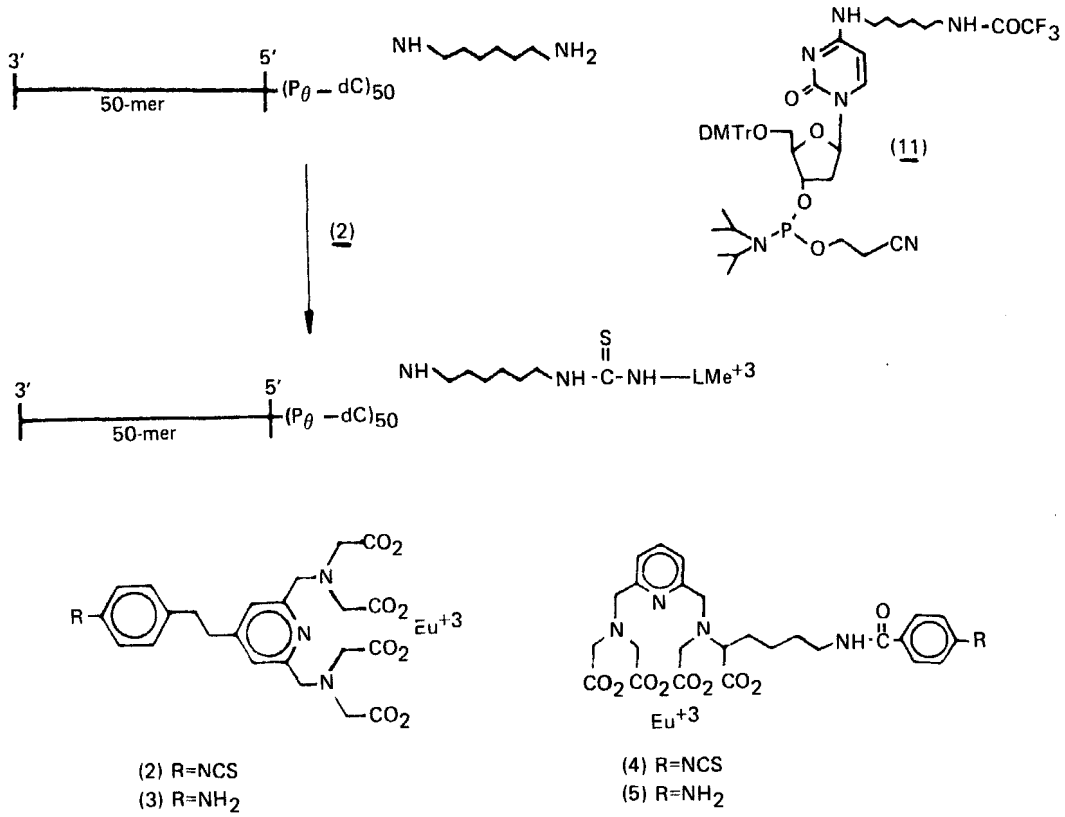


Fig. 3

consisting of MOPS (0.2 M) and imidazole (0.05 M). The mixture was incubated for 50 min at 20°C, and the high molecular product separated by gel filtration. The desalted product was dissolved in the above buffer again and carbodiimide coupling was repeated using aminochelate (5) (3 mg) for introduction of a label. The final product was separated by gel filtration after 3 hours incubation at 20°C.

Oligo DNA-base modified oligo nucleotide-Eu^{3+} chelate (Fig. 3)

Modified nucleotides (11) were incorporated at the 5'-end of the synthetic 50-mer complementary to the λ-phage DNA following the standard addition protocol for Gen-Assembler, but by ommitting the capping stage. The above cycle was repeated 50 times and the synthesized oligomer deprotected and purified under standard conditions.

Purified 100-mer (250 μg) in carbonate buffer (200 μl, pH 10.5) was extensively labelled using (2) (3.0 mg) by incubating the reaction mix-

ture at room temperature for 6 hours. The labelled oligomer was separated from the free chelate by filtration through a Sephadex G-25 column using phosphate (0.2 M, pH 7.5) as eluent.

Hybridization procedure

Target λ-phage DNA was coated onto polystyrene strips as described previously[9]. Strips were prehybridized in 0.9 M NaCl, 20 mM Tris-HCl, pH 7.5, 0.1 % SDS, 0.5 % BSA, 0.5 % polyvinyl pyrrolidone, 100 µg/ml sodium polyacrylate, 50 µM EDTA at 54°C for 30 min. Oligomeric probes (50 ng/ml) were hybridized in the same solution at 54°C for 3 hours. Posthybridization (3x10 min) washings were performed in 150 mM NaCl, 10 mM Tris-HCl, pH 7.5, 0.1 % SDS, 10 µM EDTA at 37°C. The hybridized Eu^{3+}-labelled probes were detected by adding 200 µl of enhancement solution and measuring the developed fluorescence in a time-resolved fluorometer (Arcus 1230, Wallac Oy).

RESULTS

Polyvinylamine-based oligo DNA probe could detect 200 pg of Adenovirus 2. The oligomer attached to polyacrylic acid gave a sensitivity of 500 pg of λ-DNA, while the dC analogue-tailed oligomeric probe could detect 200 pg of λ-phage DNA.

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