A Novel Multifunctionally Labelled DNA Probe Bearing an Intercalator and a Fluorophore

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A 15-mer DNA labelled with a novel multifunctional fluorescent agent bearing acridine and fluorescein moieties is synthesized and the intensity of fluorescein-based fluorescence detected by the excitation of acridine is shown to be strongly affected by the DNA's secondary structure.

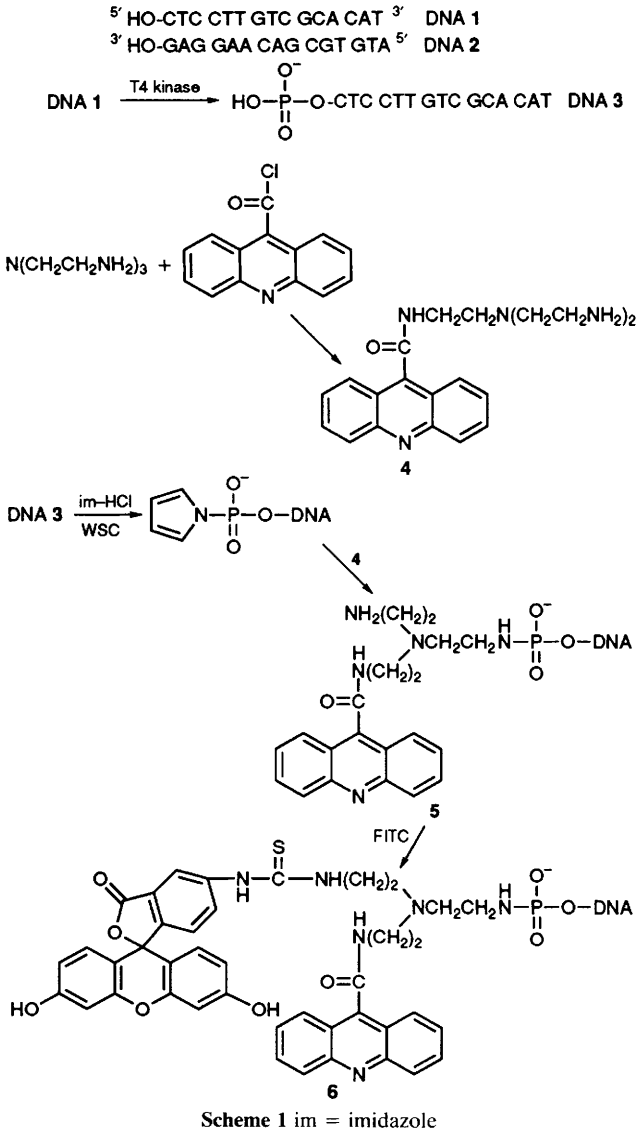
It is well known that the reversible association of oligonucleotides with complementary sequences is strongly related to the expression of the function of oligonucleotides. To detect these phenomena in solution, however, it usually requires tedious hypochromic measurement using the UV photometric method over a range of temperatures.[1] It is obvious that this method is not suitable for analysis of the structure and the thermodynamic profile of complicated nucleotide molecules, for example, those possessing several selfcomplementary regions, in solution.

With the objective of developing a new methodology and a novel DNA probe to obtain information about reversible association of oligonucleotides in solution,[2] we have synthesized a 15-mer DNA probe labelled with a tris-amine derived novel multifunctional fluorescent labelling agent[3] bearing acridine and fluorescein moieties. The hybridization ability and fluorescent properties, particularly the fluorescence intensity, of the labelled DNA were also examined. We present evidence here that the labelled DNA binds to complementary DNA strands more effectively than does unlabelled DNA. The fluorescein based fluorescence of the labelled DNA can be detected by UV irradiation at a wavelength which corresponds to the excitation maximum of acridine and is presumably due to intramolecular energy transfer.[4] Furthermore, the fluorescence intensity is strongly affected by the formation of a double strand so that the reversible association of oligonucleotides can be monitored by examination of the fluorescence intensity.

The oligomers **1** and **2** were synthesized using Model 381-B ABI DNA synthesizer. The oligomer **1** was phosphorylated on its 5′-terminus by T4 kinase[5] to give oligomer **3**. 9-Acridinecarbonyl chloride obtained from thionyl chloride and 9-acridinecarboxylic acid was dissolved in CH_2Cl_2 and the solution was added dropwise to a stirred solution of tris(2-aminoethyl)amine (5 equiv. of 9-acridinecarbonyl chloride) to yield the acridine conjugate of the tris-amine **4** (73% from acridine-9-carboxylic acid). A mixture of oligomer **3** and the conjugate **4** was reacted in imidazole–HCl buffer (0.1 mol dm^{-3}, adjusted to pH 8.0 with NaOH) in the presence of water-soluble carbodiimide (WSC).[6] The product, acridine-tagged DNA **5** was then treated with fluorescein isothiocyanate (FITC) (10 equiv. of **5**) in carbonate buffer (0.5 mol dm^{-3}, pH 9.2). After gel filtration (Sephadex G-25) and C-18 reverse phase HPLC purification, multifunctionally labelled DNA **6**† was isolated in 50.5% yield. The reactions are summarized in Scheme 1.

The hybridization ability of both unlabelled DNA **1** and labelled DNA **6** to their complement **2** were assayed by hypochromic changes in A_{260} as the function of temperature.‡ Under near physiological conditions (0.15 mol dm^{-3} NaCl, 0.1 mol dm^{-3} sodium phosphate buffer, pH 7.2) the *Tm* values estimated for **2** and **6** were 62.0 and 64.0 °C respectively. The higher *Tm* value, which is advantageous for a probe, obtained for **6** indicates that the duplex is stabilized by the intercalative action of the acridine moiety of **6**.[7]

The fluorescence intensity of DNA **6** was measured§ by irradiation at 360 nm, which corresponds to the excitation maximum of the acridine moiety, as the function of temperature in the same buffer solution as above. In the absence of complement **2**, the fluorescein-based fluorescence was easily detected at low temperature, presumably arising from intramolecular energy transfer from the acridine moiety to the fluorescein moiety attached on DNA **6**. On the other hand, the intensity was reduced at higher temperatures (Fig. 1). This type of thermal quenching of fluorescence is usual for fluorescent compounds. However, we have found that in the presence of **2**, fluorescence of DNA **6** was markedly quenched, being almost one-third of that of DNA **6** alone, at low temperatures. However, the intensity was enhanced upon increasing the temperature and reached a maximum at 75 °C giving nearly the same level of fluorescence as that for DNA **6** alone at the same temperature (Fig. 1). The change of fluorescence intensity also well reflects the thermal transition of the complex. No shift of the emission maximum under the conditions used was found.



Scheme 1 im = imidazole

Based on the UV photometric study, the acridine moiety of **6** is believed to intercalate to base-pairs of the complex **6–2**. Thus, the observed characteristic change in fluorescence intensity of complex **6–2** can be interpreted as follows; under the condition that DNA **6** forms a complex with DNA **2**, the acridine moiety exists in an intercalated status. It is, therefore, less capable of efficient energy transfer[4] resulting in reduction of fluorescence. On the other hand, the complex is resolved at higher temperatures enabling the acridine to be freed from its *trapped* status in the base-pairs causing the enhancement of the fluorescence intensity.

The findings presented here will make the current multi-functionally labelled DNA probe a possible new tool for the direct detection of complementary oligonucleotides in solution[2] and for the analysis of regional structure of complicated oligonucleotide molecules having several selfcomplementary regions such as ^{t}RNA.[8]

This work was partially supported by Grant-in-Aid for Scientific Research from The Ministry of Education Japan and a grant from Gilead Sciences.

Received, 28th February 1994; Com. 4/01184H

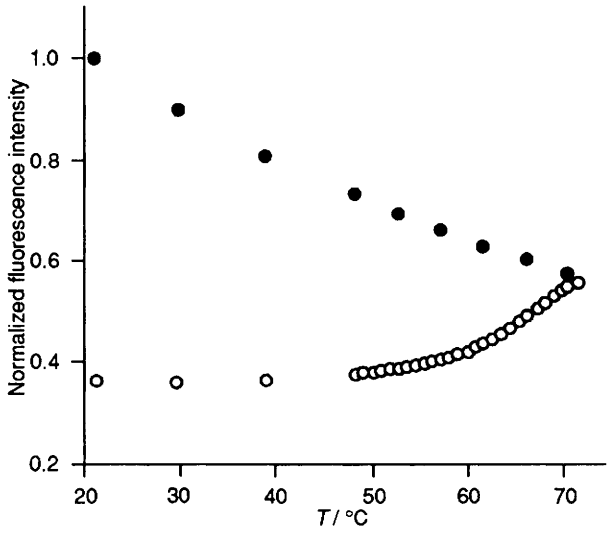


Fig. 1 Temperature dependence of fluorescein-based fluorescence intensity; (●) DNA **6** alone, (○) DNA **6** in the presence of the complementary DNA **2**

Footnotes

† Attempts to prepare DNA **6** by the direct reaction of DNA **1** and a tris-amine derivative doubly conjugated with acridine and fluorescein by the same reaction process was unsuccessful due to the low reactivity of the conjugate.

‡ The melting curves of the duplexes were recorded using a Hitachi 200-10 spectrophotometer connected to a Tokyo Rikaki NCB-221 water-bath thermocontroller. Cuvettes were 1 mm pathlength quartz cells and nitrogen gas was continuously circulated through the cuvette compartments at a temperature below 20 °C. All samples formed 1 : 1 mixtures of a strand with its complement in a buffer solution and were pre-melted at 80 °C and allowed to thermally equilibrate before recording. Total strand concentration was 10.4 μmol dm^{-3} for each sample

§ The fluorescence was recorded using Hitachi 850-M fluorimeter connected with Tokyo Rikaki NCB-221 water-bath thermocontroller under the same conditions as the UV photometric study.

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