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Sequence specific recognition of ssDNA by fluorophore 3-hydroxyflavone

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ABSTRACT

A fully water soluble 3-hydroxyflavone (3HF) derivative, N-(3-hydroxy-4′-flavonyl)-N,N,N-trimethylammonium sulfate (3HFNMe3) was synthesized. Investigation of its emissions at varying wavelengths revealed that it had three emission bands of normal (N^*), anionic (A^*) and tautomeric (T^*), in ultrapure water. Recognition of single-stranded ten ssDNA chains, having different nucleotide sequences was studied, using the ratiometric change of the intensities of the two bands (A^*/T^*), depending upon the varying environment of the 3HFNMe3 with different ssDNA chains. Addition of the ssDNA chains to the 3HFNMe3 solution caused gradual quenching of the A^* band and had almost no effect on the T^* band. As the ratios of the two bands (A^*/T^*) *vs* increasing amount of the ssDNAs generated characteristic curves for each ssDNA chain, it became possible to identify the chains with their characteristic curves.

1. Introduction

Designing DNA probes for detecting DNA chains, having varying sequences is a great challenge [1]. The motivation for DNA detection is due to its widespread applications in various fields such as DNA diagnostics, gene analysis and detection of genetic mutations, which may lead to the gene analysis before symptom(s) of a disease appears.

Majority of the probes synthesized so far depends on designing complementary probes for targeted DNA sequences to allow them to hybridize with the probe. Such a process could be performed in solution or probe could be presented on a solid support. Their detection could be electrochemical [2,3] or optical [4–8]. However, in majority of the cases, nucleic acid is covalently labeled by a fluorescent probe and the detection is studied by fluorescence resonance energy transfer (FRET) or fluorescence quenching. On the other hand, detection of DNA through the interactions of positively charged fluorophore with negative charges of DNA is rare [9].

3-Hydroxyflavones (3HF) **1** (Scheme 1) have unique photophysical properties, which exhibit well-resolved and intense two fluorescence bands in the visible region, resulting from excited-state intramolecular proton transfer (ESIPT) (Scheme 2), *i.e.* the initially excited normal form (N^*) and the tautomeric form (T^*) [10–13]. Their ratios are highly sensitive to the environment such as polarity and hydrogen bonding perturbations of the solvents. As the ratio between two bands depends on the microenvironment of 3HF, not on its concentration or the instrument settings, this property provides 3HF with an important advantage over conventional single-band dyes [14–16]. Information could be obtained about the microenvironment of 3HF with the change of the ratios of its two emission bands (I_{N^*}/I_{T^*}) and this ratio could be specific for each environment. Examples have already been reported for sensing lipid bilayers, cell membranes, proteins and peptides using 3HF as a sensor [17–19]. Concerning the use of 3HF as a two-band switchable fluorescence sensor for the interactions of DNA, 3HF as a nucleobase mimic has been demonstrated [4]. Double- and single-stranded DNAs were sensed through their characteristic effects on binding to 3HF, possessing polycationic spermine [20]. It was disclosed that while on binding to a double-stranded DNA, emission bands of the 3HF changed up to 16-fold, only moderate changes in the dual emission on binding to a single-stranded DNA was observed. In a separate study, ratiometric change in two emission bands of 3HF was used as a tool to distinguish different peptide–ODN complexes, [21] and using the similar technology, a series of 3HFs were synthesized and successfully applied to identify various peptide-ODN complexes [22]. Moreover, fisetin, which is a 3HF derivative, was employed for recognition of DNA nucleotide based on selective abasic site binding [23].

In this study, a novel fully water soluble 3-hydroxyflavone (3HF) derivative, N-(3-hydroxy-4′-flavonyl)-N,N,N-trimethylammonium sulfate (3HFNMe3) **2** (Scheme 1), which had emission bands of normal (N^*), anionic (A^*) and tautomeric (T^*), in ultrapure water, was employed as

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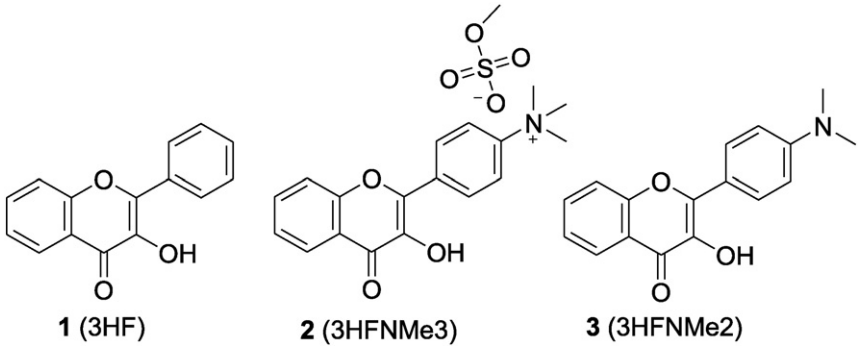


Scheme 1. Structures of 3-hydroxyflavone and its derivatives 3HFNMe3 **2** and 3HFNMe2 **3**.

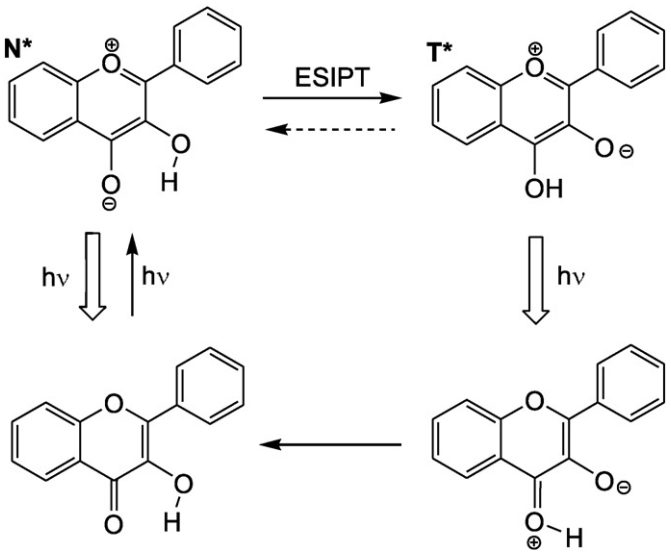
a fluorophore for recognition of single-stranded DNAs (ssDNA), having varying nucleotide chains. The study discloses that gradual addition of ssDNA to the solution of the fluorophore, 3HFNMe3, resulted in obtaining characteristic I_{A^*}/I_{T^*} curve, rather than conventional I_{N^*}/I_{T^*} ratio, for each single-stranded DNA chain.

2. Experimental

All reagents were purchased from Aldrich, Acros and Merck, and were used without further purification. All the solvents used in the syntheses were technical grade and freshly distilled prior to use. The water used for the measurements had an ultrapure grade (distilled from Millipore Milli-Q Academic). The single-stranded DNA chains were obtained from Metabion International AG, Germany. UV–Vis and fluorescence measurements were studied on HITACHI U-0080D and HITACHI F-4500, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a Varian 600 spectrometer. Proton and carbon chemical shifts were reported in parts per million downfield from tetramethylsilane, TMS. Mass spectra were recorded on ThermoLCQ-Deca ion trap mass instruments.

*2.1. Synthesis of N-(3-hydroxy-4′-flavonyl)-N,N,N-trimethylammonium sulfate **2** (3HFNMe3)*

Dimethylamine 3-hydroxyflavone (3HFNMe2) **3** was synthesized following the literature procedure [24]. A solution of **3** (0.37 g, 1.3 mmol) and dimethylsulfate (6.55 mmol, 0.62 ml) in THF was refluxed for 24 h. The precipitate was filtered after the mixture was reached room temperature. The crude solid was purified by Soxhlet extraction, using dichloromethane as solvent, which yielded *N*-(3-hydroxy-4′-flavonyl)-*N*,*N*,*N*-trimethylammonium sulfate (3HFNMe3) **2** as a white solid (0.122 g, 23%).^{1}H NMR (600 MHz, D_2O) δ (ppm) 8.11 (d, 2 H, $J = 8.4$ Hz), 7.82 (d, 2 H, $J = 7.6$ Hz), 7.71 (d, 2 H, $J = 8.4$ Hz), 7.61 (t, 1 H, $J = 7.6$ Hz, $J = 7.5$ Hz), 7.44 (d, 1 H, $J = 7.6$ Hz), 7.25 (t, 1 H, $J = 7.6$ Hz, $J = 7.5$ Hz), 5.32 (s, 9 H), 3.61 (s, 1 H), 3.55 (s, 3 H); ^{13}C NMR (150 MHz, D_2O) δ (ppm) 157.2, 134.5, 132.1, 129.5, 129.4, 129.3, 125.1, 124.9, 124.4, 124.3, 119.8, 119.7, 118.3, 56.8, 53.8; Found C, 55.78, H, 5.35, N, 3.60, Calculated C, 56.03, H, 5.22, N, 3.44; m/z 296 M^+.



Scheme 2. ESIPT reactions of 3HF.

2.2. Fluorescence Measurements

The single-stranded DNA chains comprising 25 Cytosines (d(C)25), 25 Guanines (d(G)25), 25 Adenines (d(A)25), 25 Thymines (d(T)25), 15 Cytosines and 5 Adenines (d(C)15-d(A)5), 10 Cytosines and 10 Adenines (d(C)10-d(A)10), 5 Cytosines and 15 Adenines (d(C)5-d(A)15), 15 Thymines and 5 Guanines (d(T)15-d(G)5), 10 Thymines and 10 Guanines (d(T)10-d(G)10) and 5 Thymines and 15 Guanines (d(T)5-d(G)15) were applied for the measurements.

From a stock solution of **2** (3HFNMe3), dissolved in ultrapure water (2.1 mg, 2 ml, 2.5×10^{-3} M), was transferred 200 μl into a quartz cell ($1 \times 1 \times 3$ cm) and diluted to 2 ml with ultrapure water. Fluorescence emission was then recorded after each addition the ssDNA solution (2 μl, 20 nmol/ml). The ratio of A* and T* bands was calculated and a graph, having A*/T* ratio *vs* DNA concentration was plotted. Each experiment was repeated five times to understand the repeatability of the results, which gave the same results.

3. Results and Discussion

3.1. Synthesis

The precursor, dimethylamino-3-hydroxyflavone (HFNMe2) **3**, to obtain its salt form *N*-(3-hydroxy-4′-flavonyl)-*N*,*N*,*N*-trimethylammonium sulfate (3HFNMe3) **2**, was synthesized applying the literature procedure (Scheme 3) [24]. Its methylation with dimethylsulfate and then purification of the product with Soxhlet extraction successfully gave the product **2** in 23% yield.

3.2. Spectroscopic Measurements

UV measurement of 3HFNMe3 **2**, methyl salt of 3HFNMe2 **3**, having maximum at 400 nm in dichloromethane, [24] displayed two maxima at 305 and 345 nm in ultra-pure water (Fig. 1). While 3HFNMe2 had a broad peak between 330 and 450 nm, its methyl salt 3HFNMe3 had two maxima covering a shorter area of 280–390 nm. Excitation of 3HFNMe3 at maximum 345 nm produced two emissions at 405 (weak) and 520 nm (strong) as N* and T* bands (Fig. 1, A). As for ratiometric study, such an emission was not suitable since the intensity