Study of the characteristics of the fluorescent dye YO-Dam-1

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Abstract:*The absorption and emission characteristics of new fluorescent dyes were determined. YO-Dam-1 has an absorption maximum at 478nm.The complex YO-Dam-1-DNA has an emission maximum at 511nm and a higher fluorescent intensity compared with the dyes propidium iodide and YOYO 1. The rate of binding of the YO-Dam-1 DNA is very large. Equilibrium is achieved after 30s.*

Key words: *fluorescent dye, DNA, somatic cells*

INTRODUCTION

Fluorescent dyes are widely used and are especially suitable for the detection of biological objects. By binding to a specific biological ingredient in a sample, the fluorescent dye may be used to indicate the presence or quantify the specific ingredient. There are known various of fluorescent dyes for quantification of DNA and RNA [1-4].

The purpose of this study was to determine the absorption and emission characteristics of the synthesized dye-YO Dam-1 and examination of its ability to bind to DNA and somatic cells.

MATERIALS AND METHODS

Materials

The fluorescent dye YO-Dam-1 is provided by the company Milkotronik LTD. Fluorescent dyes YOYO 1 propidium iodide, dimethylformamide (DMF) and DNA were purchased from Sigma Aldrich.

Aparatus

The measurements are performed using a UV / Vis spectrophotometer model 6900 Jenway, equipped with quartz cuvettes with 10 mm and fluorescence spectrophotometer model Carry Eclipse, Varian.

Binding the fluorescent dye withDNA.

A stock solution of the YO-Dam-1 was prepared in DMF at a concentration of 1μg / ml.By dilution with 10 mM phosphate buffer, pH 7.4 containing 0.2% Triton 100 solution was prepared YO-Dam-1 at a concentration of 50 μg / ml. A solution of DNA in double distilled water was prepared at a concentration of 25μg / ml. The binding of YO-Dam-1 with DNA was carried out by mixing the two solutions at a ratio of dye: DNA = 1: 12.5. The resulting mixture was excited at 478nm in order to investigate the emission maximum of the resulting complex.

RESULTS AND DISCUSSION

To the resulting new fluorescent dye YO-Dam-1 (50μg / ml DMF) absorption spectrum analysiswas made with spectrophotometer JENWAY 6900. Fig. 1 shows that the dye has an absorption maximum at 478nm. Then emission spectrum analysiswas made to the dye with the spectrophotometer Eclipse. It was found that the pure dye shows no emission peak and the background signal is only 5 fluorescence units at a concentration of the dye- 50μg / ml. Fig. 2 shows that the addition of 12.5 parts of DNA at a concentration of 25μg / ml to 1 part dye at a concentration of 50μg / ml anemission maximum was manifested.

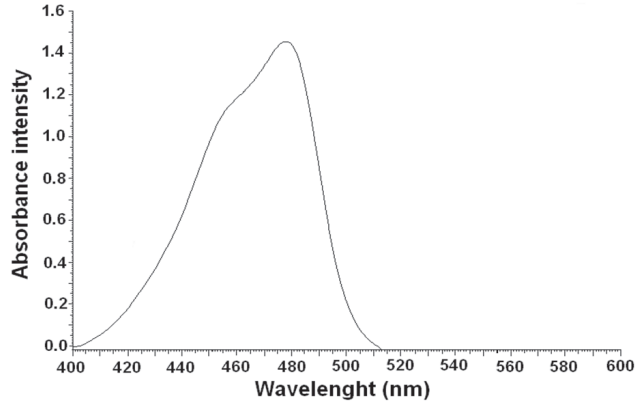

Fig. 1.Absorption spectra of the YO-Dam-1

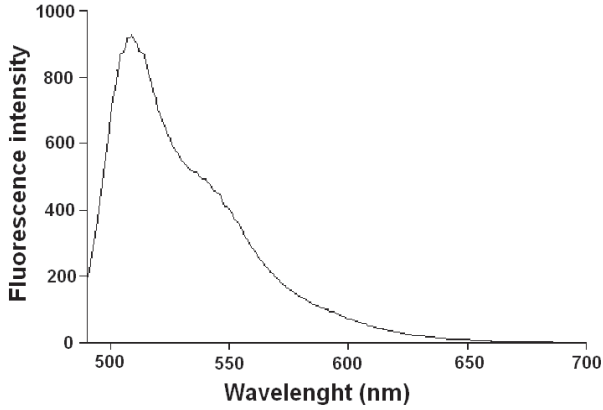

Fig.2.Fluorescence spectrum of YO-Dam-1 + DNA.
Ratio dye (50μg / ml), : DNA (25μg / ml) = 1: 12.5

A comparison was made of the time to reach equilibrium binding of the synthesized YO-Dam-1 dye with DNA with the equilibrium time of binding of two other fluorescent dyes - YOYO-1, and propidium iodide (PI). The results are presented in Fig. 3, 4 and 5.The experiments were carried out with aliquot concentrations of dye and DNA and the same mixing ratio. From the figures it can be seen that the rate of binding of the YO-Dam-1 DNA is very fast. Equilibrium was achieved after 30s. This speed was close to the speed of propidium iodide binding to DNA. The binding of DNA by YOYO-1 was the slowest. From Figure 4 it can be seen that even at 340 min still was not fully balanced. Its full balance was achieved after 6 hours. Moreover, at the 6^{-th} hour (under equilibrium occurred) the intensity of the fluorescent signal upon binding of YOYO-1 with DNA was 1.5 times higher than the fluorescence intensity of the YO-Dam-1 with DNA also at equilibrium occurred (30 s).

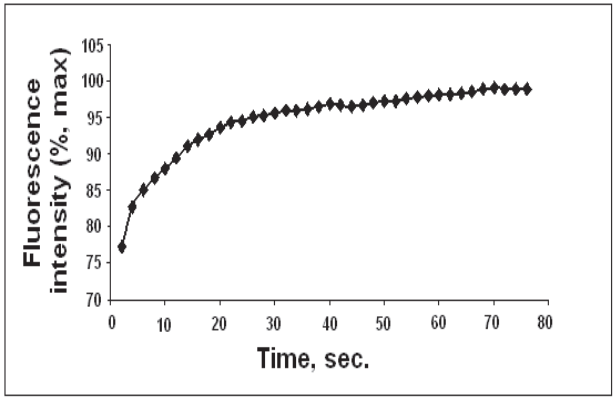

Fig. 3. Time influence on fluorescence intensity of bound dye YO-Dam-1with a DNA. Ratio dye (50μg / ml) : DNA (25μg / ml) = 1: 12.5

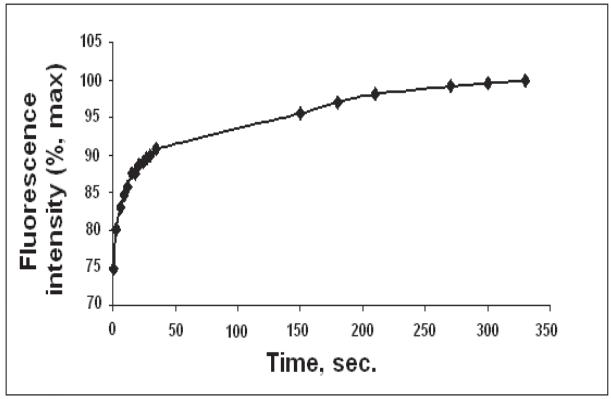

Fig. 4. Effect of time on the fluorescence intensity of the bound dye YOYO-1 with a DNA. Ratio dye (50μg / ml) : DNA (25μg / ml) = 1: 12.5

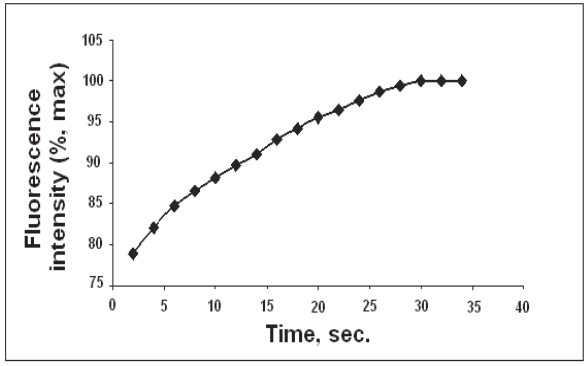

Fig. 5. Effect of time on the fluorescence intensity of bound propidium iodide with a DNA. Ratio dye (50μg / ml) : DNA (25μg / ml) = 1: 12.5

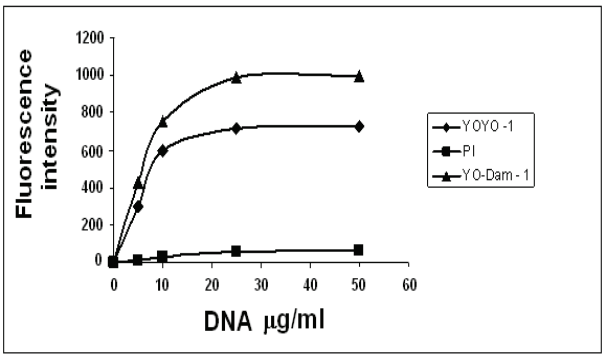

Fig. 6. Effect of DNA concentration on fluorescence intensity of the dyes bound with the DNA. Ratio dye (50μg / ml) : DNA (25μg / ml) = 1: 12.5

The effect of DNA concentration on the intensity of fluorescent signal from the three dyes was traced, measured at 30 s (Figure 6). Measurements were taken every 30 s, as the speed of dyeing of the cells with fluorescent dyes is a very important parameter for analysis. Experiments were carried out with aliquot dye concentrations and the same mixing ratio. The concentration of DNA was ranged from 5-50μg / ml. From Figure 6 it is obvious that the fluorescence intensity from propidium iodide was low at all concentrations of DNA, compared to the fluorescence intensity of the other two dyes. So compared to YO-Dam-1 the signal is about 20 times lower, compared to YOYO-1 about 1.5 times, measured at every 30 sec. The newly synthesized dye has the highest fluorescence intensity for all concentrations of DNA measured at every 30sec. In the three dyes is noticeable that after 25μg / ml DNA fluorescent intensity becomes constant. Therefore, the optimal DNA concentration is 25μg / ml.

The newly synthesized dye may be used in addition to binding to DNA and for coloring of dying or dead cells - somatic cells (SCC), neutrophils and macrophages. FIG. 7 shows microscopic images of colored dye macrophages isolated from mastitis sick cow with SCC 900 000 cells / mL of milk and healthy cow with SCC 80 000 cells / mL.

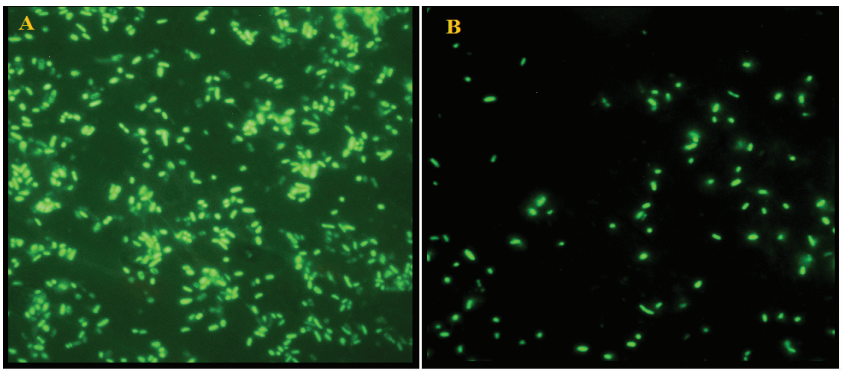

Fig. 7. Microscopic images of dyed with YO-Dam-1 izolated macrophages from somatic cells,zoom in x 1,000. (A) mastitis milk SCC 900 000 cells / mL; (B) Milk from healthy cows with SCC 80,000 cells / mL

Figure 7 shows that newly synthesized dye has a very good capacity for dyeing of dead cells. Thus, the synthesized dye can be used in combination with other dyes for the purpose of differential counting of dead and live cells.

CONCLUSION

1. YO-Dam-1 has an absorption maximum at 478nm.

2. The complex YO-Dam-1-DNA has an emission maximum at 511nm and a higher fluorescent intensity compared with the dyes propidium iodide and YOYO 1.

3. The speed of the binding YO-Dam-1 with DNA is very fast, and balance is achieved after 30s.

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This paper has been reviewed