ISSN 0233-7657. Биополимеры и клетка. 1997. Т. 13. № 5

Interaction of cyanine dyes with nucleic acids. 3. The use of new cyanine dyes Cyan 13 and Cyan 40 for detection of nucleic acids in agarose gel

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Detection of double-stranded DNA (dsDNA) and single-stranded DNA (ssDNA) and RNA with two new cyanine dyes Cyan 13 and Cyan 40 is reported. Cyan 13 and Cyan 40 bind to nucleic acids to form a stable fluorescent complexes and can be used for the detection of DNA and RNA samples separated by gel electrophoresis. Sensitivity of detection is comparable to that for ethidium bromide (EtBr), a common nucleic acid staining dye.

Materials and Methods *DNA and RNA preparation.* Double-stranded plasmid DNAs *pBR322* and *pUC18* were isolated by standard alkaline lysis method of Birnboim [1]. Linear plasmid DNA *pUC18* was obtained by digestion with *BamHI* restrictase. Closed circular single-stranded M13mp1 DNA (M13 ssDNA) was obtained from USB (USA). Total RNA was extracted from rat liver tissue by guanidinium thiocyanate-phenol method according to Chomczynski et al. [2].

Agarose gel electrophoresis and staining of nucleic acids. 0.8 % Agarose gels were prepared in standard TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) [3]. DNA and RNA samples in TAE buffer were loaded on the gel after adding tracking dyes xylene cyanole and bromophenole blue in 30 % glycerol to concentration 0.025 %. Separations were carried out at 10 V/cm. DNA and RNA gels were stained after electrophoresis in the solution of Cyan 13, Cyan 40 or EtBr (1 μg/ml) for 30 min.

Fluorescence detection of DNA and RNA in agarose gels. Detection of DNA-dye and RNA-dye complexes was performed with UV-transilluminator 2011 Macrovue («LKB», Sweden) at 524 nm. The gels were photographed using orange filter.

Results and Discussion. The use of non-isotopic detection techniques in biology and medicine is an attractive alternative to radioactive detection methods. Cyanine dyes are now widely used as fluorescence detection probes. Monomethyne cyanine dyes are weakly fluorescent in free state but show strong fluorescence upon binding to DNA or RNA, and some of these reagents are the most sensitive nucleic acid stains currently available [4, 5]. A series of new fluorescent cyanine dyes with improved spectroscopic properties including Cyan 13 [6] and Cyan 40 [7] has been recently developed in our laboratory (Fig. 1).

In this communication we demonstrate that Cyan 13 and Cyan 40 can be successfully used for DNA and RNA detection in the standard procedures. Dyes bind efficiently to different forms of DNA — covalently closed circular and linear dsDNA and ssDNA, as well as to various RNA types — ribosomal RNA, mRNA and tRNA, forming stable fluorescent complexes.

In Fig. 2 is shown the comparison of DNA staining in agarose gel with Cyan 13, Cyan 40 and EtBr. It was shown that fluorescence of Cyan 13 and Cyan 40 complexes with linear dsDNA and circular M13 ssDNA (lanes *1* and *3*, respectively) was comparable wit that of EtBr.

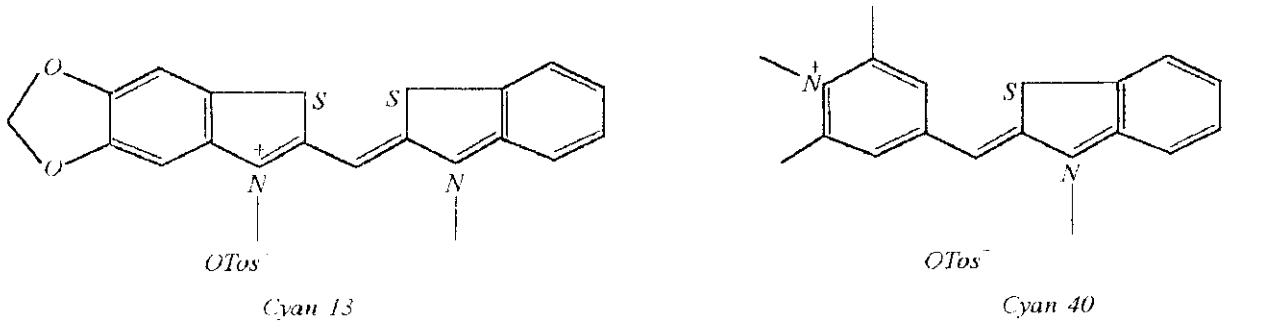

Fig. 1. The chemical structure of cyanine dyes

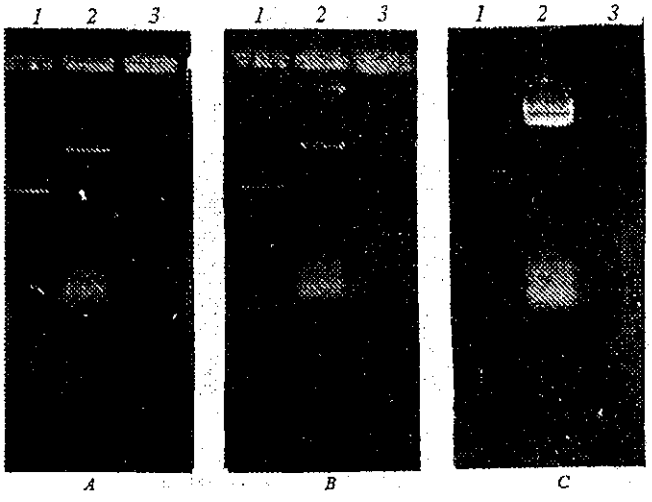

Fig. 2. Detection of DNA in agarose gels after electrophoresis:. lane *1* — 200 ng of dsDNA *pUC18* linearized with *BamHI* restrictase; lane *2* — 300 ng of intact *pBR322* dsDNA; lane *3* — 200 ng of M13 ssDNA. Staining was made with Cyan 13 (*A*), with Cyan 40 (*B*) and EtBr (*C*)

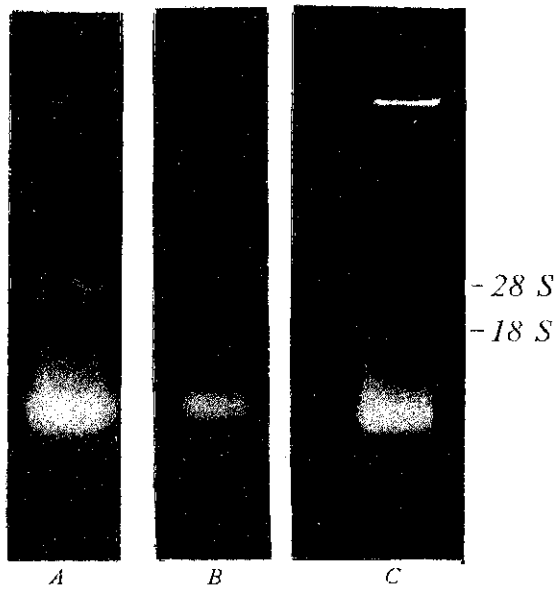

Fig. 3. Staining of RNA in agarose gels after electrophoresis with Cyan 13 (*A*), Cyan 40 (*B*) and EtBr (*C*). Each lane contains 5 μg of total rat liver RNA. Positions of ribosomal 28S and 18S RNA are indicated

But the intensity of fluorescence of EtBr complex with circular closed dsDNA (lane *2*) was higher than those for Cyan 13 and Cyan 40. It is necessary to note that the contamination of bacterial tRNAs in samples of circular closed plasmid DNAs shined with almost equal intensity.

The fluorescence intensity of Cyan 13 and Cyan 40 complexes with RNA is enough high. As is shown in Fig. 3, all types of RNA — ribosomal RNA, mRNA and tRNA — can be detected in total RNA samples. Both 28S and 18S rRNAs were detectable in the gels stained with Cyan 13 and Cyan 40, as well as stained with EtBr. The fluorescence of RNA complexes with Cyan 40 was lower as compared with two other dyes.

The colours of fluorescence of RNA and DNA complexes with Cyan 13 and Cyan 40 were from blue to light green. The colour of Cyan 13 — DNA complexes was shifted to red by increasing dye concentration or time of staining. It is possible that such a change off luorescence spectrum was caused by formation of H-aggregates (unpublished results).

It can be concluded therefore that two new cyanine dyes Cyan 13 and Cyan 40 can be used for DNA and RNA detection in agarose gels.

Acknowledgements. We are indebted to Dr. Yu. Kovtun for providing the dyes and thank Ms. V. Koval'ska for technical assistance.

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Взаємодія ціанінових барвників з нуклеїновими кислотами. 3. Застосування нових ціанінових барвників Cyan 13 та Cyan 40 для визначення нуклеїнових кислот в агарозних гелях

Резюме

Два нових ціанінових барвники Cyan 13 та Cyan 40 було застосовано для флюоресцентної детекції двоспіральної ДНК та односпіральних ДНК і РНК в електрофорезних гелях. Чутливість детекції нуклеїнових кислот цими барвниками схожа з такою бромистого етидію, що звичайно використовується для цієї мети.

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Взаимодействие цианиновых красителей с нуклеиновыми кислотами. 3. Применение новых цианиновых красителей Cyan 13 и Cyan 40 для определения нуклеиновых кислот в агарозных гелях

Резюме

Два новых цианиновых красителя Cyan 13 и Cyan 40 применены для флюоресцентной детекции двуспиральной ДНК и односпиральных ДНК и РНК в электрофорезных гелях. Чувствительность детекции нуклеиновых кислот этими красителями подобна таковой бромистого этидия, который обычно используется для этой цели.

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Received 18.02.97