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Analytica Chimica Acta 538 (2005) 151–158
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

A surfactant enhanced stopped-flow kinetic fluorimetric method for the determination of trace DNA

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Received 10 October 2004; received in revised form 26 January 2005; accepted 29 January 2005
Available online 2 March 2005

Abstract

A novel and high-sensitive stopped-flow kinetic fluorimetric method for determining the concentration of DNA (calf thymus DNA), in the presence of SDS, based on monitoring the variation of fluorescence within a very short period has been developed. The optimum conditions for various parameters, on which the binding of YOYO-1 to DNA depends, were investigated. It was found that the initial reaction rate increased linearly with increasing DNA concentration in the range from 8.0×10^{-10} to 9.0×10^{-8} M. The detection limit was calculated to be 3.0×10^{-10} M. This method was used for determining the concentration of DNA in synthetic samples and real samples extracted from leaves of *Arabidopsis thaliana*, tobacco and rice with satisfactory results. The kinetics of the interaction under pseudo first-order conditions was established by the aid of single-mixing stopped-flow techniques.

Keywords: Kinetic fluorometry; Stopped-flow; YOYO-1; DNA; Surfactants

1. Introduction

The analysis of DNA continues to be an important area in the fields of chemical and biochemical analysis, since it is often used as a reference for measurements of other biologically active components in biological fluids and genetic diagnosis. Till now, many approaches for DNA determination have been put forward such as spectrophotometry [1,2], voltammetry [3], spectrofluorimetry [4–12], chemiluminescence (CL) [13,14] and resonance light scattering (RLS) [15–19]. The common CL method for DNA determination is based on the activation of luminol CL by the antibody-horseradish peroxidase system. Recently, Ma et al. [14] presented a flow-injection CL method for DNA, which provided a wider linear range and direct determination for low-level DNA concentration in synthetic samples. However, the activation of DNA by imidazole–HCl buffer solution needed one hour prior to the determination, which makes the method time-consuming.

At present, spectrofluorimetry and RLS methods, based on the interaction between DNA and dyes, are the most widely used techniques for DNA determination. For example, Huang et al. had reported several methods for the determination of DNA based on its enhancement effect of RLS by Azur A [17], Azur B [16], Safranine T [18], and Methylene Blue [19], respectively. However, the RLS method suffers the disadvantages of low signal levels and lack of sensitivity unless laser facilities are employed. This technique, as a physical method, does not perform well in chemical recognition [13]. Furthermore, in some cases, the instrumental conditions are of importance to decide the RLS spectral characteristics, and affect the sensitivity and limit of determination of a sample [17].

The natural fluorescence intensity of nucleic acids is so weak that direct use of the fluorescence emission of nucleic acids, to study their biological properties, is limited [20]. Therefore, to detect DNA, it must be stained with suitable fluorescence dyes. Up to now, many fluorescent reagents have been widely used as DNA probes, by which many methods have been developed for the determination of DNA.

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0003-2670/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2005.01.068

Different probes react with the DNA in different ways. Among these methods, the fluorescence intensity of some probes is enhanced by DNA, such as ethidium bromide [4], 4,6-diamidino-2-phenylindole (DAPI) [5], the bis-benzimidazole dye Hoechst 33258 [6], cationic cyanine [7], etc., and the fluorescence of some other probes is quenched by DNA. For example, Xu et al. proposed several approaches based on the fluorescence quenching of 9,10-anthraquinone-2-sulfonate [8], hydrophobic thiacyanine [9], Nile Blue [10], Magdala Red [11], and Vitamin K_3 [12]. In recent years, the binding of Ru(II) complex to DNA has been studied by many researchers [21] and used for analyzing DNA with fluorescence, detecting with good sensitivity and little interference [22]. However, the preparation of the reagents is inconvenient.

Among fluorescence dyes, an intercalator YOYO-1 (1,1′-(4,4,7,7-tetramethyl -4,7-diazaundecamethylene)-bis-4-[3-methyl-2,3-dihydro-(benzo-1,3-oxazole)-2-methylidene]-quinolinium tetraiodide) [23] is often used for direct observation of DNA [24–26], and Rye [27], reported a steady-state fluorimetry for determining the concentration of DNA by utilizing YOYO-1. YOYO-1 is virtually non-fluorescent in its free form, but show very strong fluorescence when bound to double-stranded DNA, so it provides high fluorescence intensity specific to DNA and a low background. The rate of the YOYO-1 interacting with DNA is very high and it is difficult to determine the initial fast binding of YOYO-1 to DNA in a conventional fluorimeter, since mixing two solutions takes at least a second. Thus, the stopped-flow mixing technique is required in order to make kinetic measurements. So far, no kinetic fluorimetric application has been reported for the determination of DNA concentration.

Kinetic methods of analysis have become increasingly popular in many areas of analytical and bioanalytical chemistry. In combination with the stopped-flow mixing technique, kinetic methodology is highly suitable for this purpose as it allows sample and reagent solutions to be mixed automatically and rapidly, as well as measurements to be made shortly after mixing [28]. In recent years, stopped-flow technique has become popular in analytical biochemistry [29,30]. Furthermore, the stopped-flow technique is the automatic approach most commonly used when fast reactions are involved [31]. The rapid response of the stopped-flow apparatus ensures an experimental time window in the millisecond range, which allows equilibrium reactions to be studied even in cases where one or more of the components have transient lifetimes in aqueous buffers.

This paper describes a stopped-flow kinetic fluorimetric method for determining the concentration of DNA by using the interaction of YOYO-1 with DNA in the presence of SDS, which was applied to monitor the change of the fluorescence intensity and measured following excitation at 460 nm using a 495 nm cut-off filter within the original short period of time. It was found that the rate of increase near zero reaction time was linearly related to the initial concentration of DNA. The reaction rate was very high and therefore, the kinetics of the binding of YOYO-1 to DNA was established by the aid of single-mixing stopped-flow technique. Further, influence of various surfactants on this interaction was investigated and kinetic parameters in the presence or absence of SDS were reported. Results showed that with the low concentration of SDS, the enhancement of initial rate was observed, similar to the case with sodium deoxycholate when its concentration was low; whereas, with the high concentration of SDS, the interaction was inhibited. To our knowledge, this is the first successful application of the stopped-flow technique to determine the concentration of DNA in synthetic and real samples, and time required for analysis by the proposed method is the least in comparison with existing methods; the present work is considered to be a better alternative with respect to precision, reproducibility and speed.

2. Experimental

2.1. Reagents

Calf thymus DNA (ct DNA) and RNAse A purchased from Sigma. DNA stock solution was prepared by suspending ct DNA in water and occasionally gently shaking the solution was made for complete dissolution at 4 °C away from light. The concentration was determined according to the absorbance at 260 nm [32] after establishing that the absorbance ratio A_{260}:A_{280} was in the range 1.80–1.90. The molarities of ct DNA was calculated by using $\varepsilon = 6600\ \mathrm{L\ mol^{-1}\ cm^{-1}}$. The working solution of DNA was 1.0×10^{-5} M (3.2 μg/mL).

YOYO-1 was obtained as its iodide salt in DMSO from Molecular Probes. The stock dye solution was prepared by dissolving YOYO-1 in distilled water to a final concentration of 4.0×10^{-6} M and the working solutions were prepared by diluting the stock solutions with water. 0.2 M Tris–HCl (pH 7.2) was used as the buffer solution. The ionic strength control of the solution was kept with 1.0 M NaCl. Sodium dodecyl sulfate (SDS), sodium deoxycholate (SDC), cetyltrimethylammonium bromide (CTMAB), polyoxyethylene lauryl ether (Brij35), and Triton-100 were purchased from Sigma and stock solutions made in water. Unless otherwise specified, all other chemicals were of analytical-reagent grade. All aqueous solutions were prepared from de-ionized water, purified with a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Apparatus

Fluorescence spectra were measured with a Shimadzu RF-540 spectrofluorimeter with a 1.0 cm quartz cell, equipped with a xenon lamp and dual monochrometer. The temperature was controlled by using a Tb-85 thermostat bath (Shimadzu, Japan), and the pH was measured with a pHs-3C meter (Shanghai, China).