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Facile determination of DNA-binding nuclear factor-κB by chemiluminescence detection

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Abstract

A simple, rapid, and sensitive method for the assay of a sequence-specific DNA-binding protein, nuclear factor-κB (NF-κB), has been developed by using a DNA-detectable chemiluminogenic reagent and a centrifugal filter that distinguishes different molecular sizes. After the formation of a complex between NF-κB and DNA, the unbound DNA is separated from the complex by the centrifugal filter. The amount of the bound NF-κB is estimated by chemiluminescence detection of the bound DNA. This detection is performed within 2 min at room temperature by the use of a chemiluminogenic reagent, 3′,4′,5′-trimethoxyphenylglyoxal, which selectively recognizes guanine moiety in oligonucleotides or DNAs. This method does not require any labeled probes or antibodies and can determine a concentration as low as 5 nM of DNA-binding NF-κB. The sensitivity is nearly the same as that of other methods such as gel shift assay using fluorescence-labeled probes and enzyme-linked immunosorbent assay. Therefore, the current method provides a convenient tool for surveying various DNA-binding proteins.

Keywords: NF-κB; Chemiluminescence detection; DNA-binding protein; TMPG

Sequence-specific DNA-binding proteins play critical roles in the biology of cells. These proteins are involved in the regulation of gene transcription and DNA replication, recombination, repair, and restriction [1–4]. Among the various sequence-specific DNA-binding proteins, transcription factors have been estimated to constitute 6 to 7% of all proteins expressed by eukaryotic genomes, which are responsible for the transfer of biological information from genes to proteins [5,6]. These transcription factors have become potential targets in medical diagnosis and drug development because of their pivotal roles in the pathways and networks involving the regulation of gene expression [7].

A prominent protocol for the determination of various sequence-specific DNA-binding proteins has attracted considerable attention from many research fields such as genomics and proteomics. However, the conventional methods for the detection of those proteins, including gel shift assay, DNA footprinting assay, and enzyme-linked immunosorbent assay (ELISA[1]), usually are time-consuming. In addition, they require labeled probes and/or specific antibodies for the detection of the DNA-binding protein in combination with electrophoresis for separation.

In this article, we describe a convenient method for the determination of sequence-specific DNA-binding nuclear factor-κB (NF-κB). NF-κB is a ubiquitous redox-sensitive transcription factor that responds to pro-inflammation

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[1] *Abbreviations used:* ELISA, enzyme-linked immunosorbent assay; NF-κB, nuclear factor-κB; TNF-α, tumor necrosis factor-α; TMPG, 3′,4′,5′-trimethoxyphenylglyoxal; dsDNA, double-stranded DNA; SDS, sodium dodecyl sulfate; ChRE, carbohydrate response element; dI–dC, poly(deoxyinosinic deoxycytidylic) acid; NMWL, nominal molecular weight limit; TPA, tetra-*n*-propyl ammonium phosphate; DMF, *N*,*N*-dimethylformamide; EDTA, ethylenediaminetetraacetic acid; FITC, fluorescein isothiocyanate; DMSO, dimethyl sulfoxide; MW, molecular weight; BSA, bovine serum albumin; FRET, fluorescence resonance energy transfer.

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caused by cytokines and oxidative stress [8,9]. By the activation with tumor necrosis factor-α (TNF-α), NF-κB translocates into the nucleus, where it binds to specific nucleotide sequences [8,9]. The cellular response of NF-κB to inflammatory and stress signals has been implicated in disease conditions such as atherosclerosis, cancer, diabetes, and Alzheimer's disease [10]. For the sensitive and facile detection of NF-κB bound to DNA, we employed 3′,4′,5′-trimethoxyphenylglyoxal (TMPG) as the chemiluminogenic reagent. TMPG reacts specifically with guanine moiety in DNA to form a chemiluminescent product [11]. The reaction conditions were first improved for the rapid detection of DNA with TMPG within 2 min in a neutral medium, and then a facile method was developed for the quantitative and sensitive determination of the DNA-binding NF-κB by the chemiluminescent detection with the TMPG reaction.

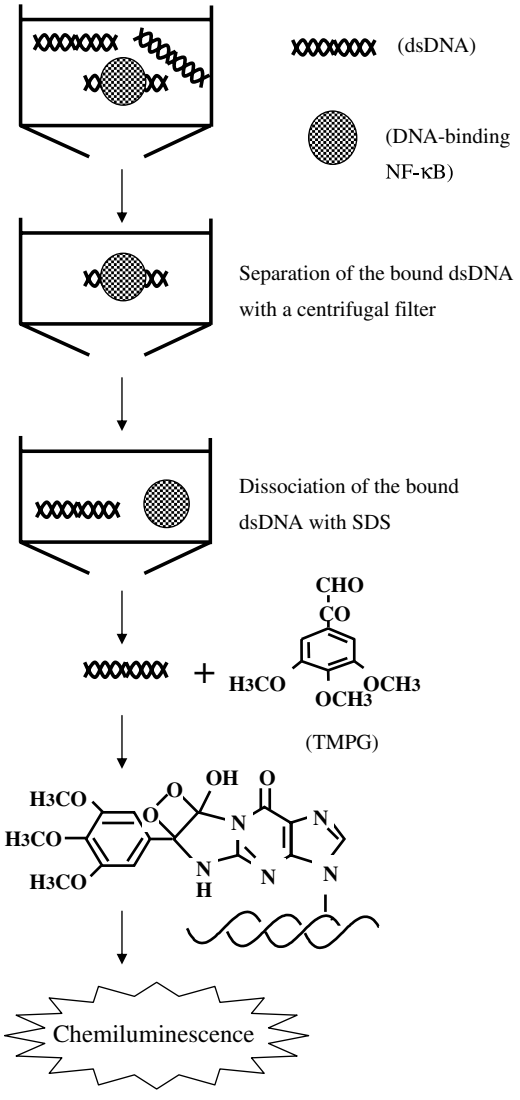

Fig. 1. Schematic protocol for the assay of DNA-binding NF-κB by means of chemiluminescence reaction with TMPG.

Fig. 1 illustrates our protocol proposed for the determination of the DNA-binding NF-κB. In this assay, the bound and unbound double-stranded DNAs (dsDNAs) that contain 14 guanine bases after the formation of a complex between NF-κB and dsDNA were readily separated with a centrifugal filter that was able to remove molecular sizes lower than 100,000 Da. The bound dsDNA, which was dissociated with sodium dodecyl sulfate (SDS), was filtrated. The amount of the DNA in the filtrate was then detected by the chemiluminescence reaction with TMPG. The proposed method does not require any labeled probes or specific antibodies, and it allows the analysis of the interaction between DNAs and proteins by using conventional inexpensive instruments.

Materials and methods

Materials and reagents

Purified recombinant human NF-κB p50 was purchased from Promega (Madison, WI, USA). A dsDNA, dsDNA/NF-κB with the sequences 5′-AGTTGAGGGGACTTTCCCAACTAGGAATCT-3′ and 3′-TCAACTCCCCTGAAAGGGTTGATCCTTAGA-5′, was used for binding to the protein, NF-κB. The underlined sequence represents the protein-binding site [8]. Another dsDNA, dsDNA/ChRE with the sequences 5′-GGGCGCACGTGGCACTCACGTGGTTCC-3′ and 3′-CCCGCGTGCACCGTGAGTGCACCAAGG-5′ (which binds to a carbohydrate response element (ChRE)-binding protein [12]), was used as the negative control for dsDNA/NF-κB. Those single-stranded oligonucleotides and a 60-mer oligonucleotides, 5′-$(TTAGGG)_{10}$-3′, were products obtained from Sigma–Genosys Japan (Ishikari, Japan). Poly(deoxyinosinic deoxycytidylic) acid (dI–dC) was purchased from Roche (Mannheim, Germany) and was used to suppress nonspecific binding of the protein. Nuclear extracts of HeLa cells were purchased from Active Motif (Carlsbad, CA, USA). The concentration of whole proteins in each extract was 2.5 g/L based on information provided by the supplier. Centrifugal filter devices (Ultra-free MC 100,000 nominal molecular weight limit [NMWL] filter units) were purchased from Millipore (Bedford, MA, USA).

TMPG was synthesized according to previously reported conditions [13], but with a slight modification as follows. To a stirred solution of selenium dioxide (45 mmol) in dioxane (40 ml) was added 3,4,5-trimethoxyacetophenone (50 mmol) at 40 °C. The mixture was refluxed for 2 h, and selenium dioxide (45 mmol) was then added to the reaction mixture. After being refluxed for 3 h, the mixture was filtrated to remove an insoluble selenium. The filtrate was mixed with 240 ml of H_2O and then kept at 4 °C for approximately 15 h. The formed precipitates were recrystallized from water to give colorless needles (melting point 101–102 °C, yield 60–70%).