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A rapid and sensitive chemiluminescent DNA probe system (HPA) for detection of amplified HIV and HBV DNA

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Acridinium esters are highly chemiluminescent molecules with high quantum yield and rapid reaction kinetics [5]. DNA probes can be labeled with acridinium esters using alkylamine linker arms to approximately the same specific activity as the free ester. We have identified conditions in which acridinium ester linked to unhybridized probe is hydrolyzed to a non-chemiluminescent form, while ester linked to hybridized probe is protected [1]. We have incorporated chemiluminescent labeled probes into a homogeneous DNA probe assay referred to as the Hybridization Protection Assay (HPA) and applied the assay to the detection of Hepatitis B (HBV) and Human Immunodeficiency Virus (HIV) DNA sequences. Rapid and sensitive detection methods for screening large numbers of samples in a simple format are needed for clinical diagnoses as well as basic research endeavors, particularly in clinical syndromes in which currently available tests or serological tests cannot be used to follow the course of infection. HIV and HBV are present in levels too low to allow consistent detection by direct methods. Detection of these low levels of virus is made possible by specific amplification of viral nucleic acids by enzymatic methods including the polymerase chain reaction or PCR [4] or transcription-based amplification methods [2]. Current methods for detection of the specific amplification products include visualization after gel electrophoresis and hybridization in solution or to immobilized targets. These methods require many steps and several hours to days to complete, and often involve radioisotopic DNA probes. We demonstrate that HPA is a rapid and sensitive method for detection of HIV and HBV DNA amplified by PCR.

Materials and methods

DNA probes were labeled as described in Arnold et al. [1]. Chemiluminescence was detected following the addition of hydrogen peroxide under basic conditions. The reaction proceeds through a cyclodioxetane-like intermediate with the production of an excited acridone which emits light upon collapse to ground state. Purified cloned DNA was amplified with Taq polymerase under conditions recommended by the enzyme supplier (Cetus) for 30 or 35 cycles in a Perkin-Elmer Cetus thermocycler. Ten microliters of the PCR reaction were denatured at 95° C and hybridized to AE-labeled probe at 60° C, followed by a differential hydrolysis step at the same temperature. After differential hydrolysis, remaining chemiluminescence was a direct measure of the amount of hybrid formed. Detection of chemiluminescence was performed with a Leader I luminometer. The results were given as a numerical reading in Relative Light Units (RLU), allowing quantitation of the amount of target present. The hybridization assay required less than 30 min to complete.

Results and discussion

The differential hydrolysis of hybridized and unhybridized probe forms the basis of a homogeneous DNA probe assay referred to as the Hybridization Protection Assay (HPA). The HPA format was used to detect HIV and HBV DNA amplified by PCR. Purified cloned HBV DNA was amplified by PCR with primers from conserved regions within the HBV genome. A single band was seen on EtBr-stained agarose gels, confirming the specificity of the primer sequences. When dilutions of HBV cloned DNA were amplified and then analyzed by HPA, reactions containing as few as 10 copies of input HBV DNA gave chemiluminescent signals significantly above background, even when only 10% of the sample was analyzed. The quantitative capabilities of HPA were demonstrated by analyzing serial dilutions of HBV+ serum. The assay showed a linear response over three logs of target dilution. One HBsAg+ serum was positive by HPA even when diluted 10^7-fold prior to amplification. We have also applied HPA to specifically detect HIV-1 DNA amplified by PCR using gag-region primers described in Ou et al. [3]. Amplifications containing less than 10 copies of input HIV-1 DNA gave signals significantly above background. HPA provides a rapid and sensitive technique, which should be useful in studies involving epidemiology, diagnosis, prevention and treatment of viral diseases.

References

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