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Preparation of oligonucleotide microarrays on modified glass using a photoreactive heterobifunctional reagent, 1-*N*-(maleimidohexanoyl)-6-*N*-(anthraquinon-2-oyl) hexanediamine (MHAHD)

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ABSTRACT

A novel approach for the construction of oligonucleotide microarrays under the influence of light and microwaves is described. For that purpose, a new heterobifunctional crosslinking reagent, 1-*N*-(maleimidohexanoyl)-6-*N*-(anthraquinon-2-oyl) hexanediamine (MHAHD), possessing a photoactive anthraquinone moiety on one terminus and an electrophilic maleimide group on the other one, was developed. The immobilization of oligonucleotides using the reagent, MHAHD, was realized via two routes (A and B). In order to speed up the immobilization procedure, the reaction between 3′- or 5′-mercaptoalkylated oligonucleotides and maleimide moiety of MHAHD was carried out under microwaves in just 15 min. The oligonucleotide arrays produced by both the routes were analyzed by hybridization experiments (hybridization efficiency 30.13%) and subsequently used for the discrimination of base mismatches. The constructed microarrays were found to possess good thermal stability (only ~4.5% loss of fluorescence intensity observed after ten cycles).

1. Introduction

Chemical crosslinking reagents find a wide variety of applications such as preparation of affinity matrices, modification and stabilization of diverse structures, identification of ligands and receptor binding sites and structural studies and so forth [1]. The ability to link two proteins or molecules having different binding specificities or catalytic activities has opened the potential for creating a new universe of unique and powerful reagent systems for assay and targeting applications. Chemical crosslinking reagents, an outgrowth of protein modification chemistry, are designed to have specific reactivity for functional groups contained in each reactant. A large number of homo- and heterobifunctional reagents are known [2–8] in the literature. Since heterobifunctional reagents possess two selectively reactive groups that allow coupling to be carried out in a stepwise manner, better control of the conjugation chemistry is attainable. These reagents find a variety of applications in modern molecular biology and serve as molecular tools for attachment of biomolecules such as proteins, peptides, nucleic acids and enzymes on a variety of surfaces. Recently, these covalently immobilized biomolecules have yielded bio-surfaces for molecular analysis, separation, targeting, synthesis and diagnosis. Most commonly used reagents are based on the thermochemical reactive groups [1–8]. Light-dependent immobilization procedures have been introduced [3] to circumvent drawbacks imposed by thermochemical reactions, such as requirement of the nucleophilic functions (–SH, $-NH_2$) in the ligand molecules or on the polymer surface. A number of methods are currently available where modified oligonucleotides are immobilized on polymer surfaces for the construction of microarrays. Most of them utilize functionalized polymer surfaces for this purpose [9].

Recently, oligonucleotide arrays have emerged as a powerful tool for genetic analysis with higher throughput and reproducibility than the traditional gel-based methods. Arrays of DNA molecules, either as double stranded segments or short single-stranded oligonucleotides have been utilized for different molecular biology applications including mutation detection [10,11], gene expression monitoring [12,13], DNA sequencing [14], genome analysis [15,16], immunology [17] and medical diagnostic of genetic diseases [18]. Two general methods have established themselves for producing DNA microarrays, viz., (a) direct on-surface synthesis of oligonucleotides [19–22], and (b) the immobilization of presynthesized oligonucleotides, i.e. deposition method [23–30]. Concerning the direct synthesis of oligonucleotides on the surface, the best known method is photolithographic oligonucleotide

Abbreviations: MHAHD, 1-*N*-(maleimidohexanoyl)-6-*N*-(anthraquinon-2-oyl) hexanediamine; THF, tetrahydrofuran; NHS, *N*-hydroxysuccinimide; DCC, dicyclohexylcarbodiimide; TLC, thin layer chromatography; NMI, *N*-methylimidazole; DMSO, dimethylsulfoxide

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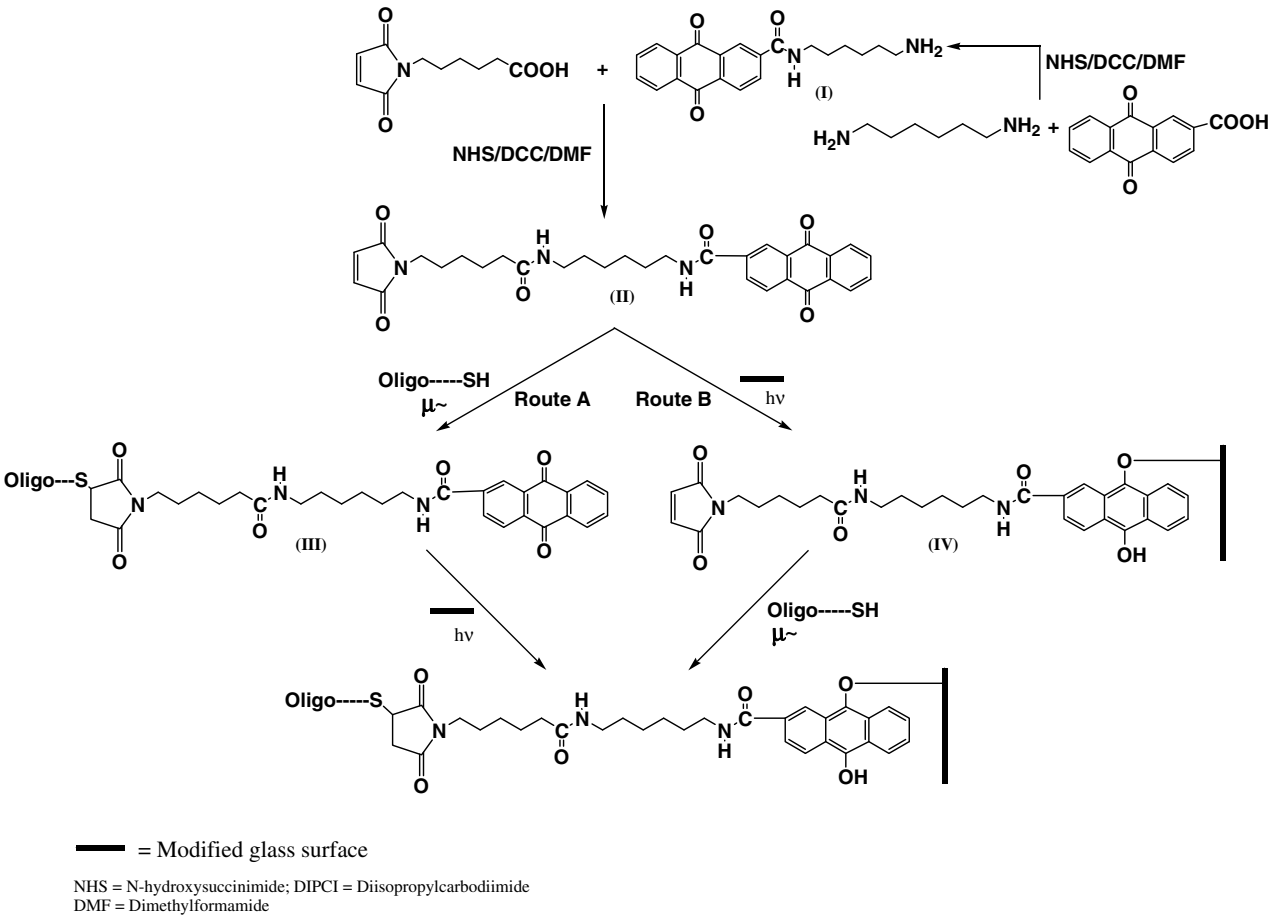

Scheme 1. Synthesis of the reagent, 1-*N*-(maleimidohexanoyl)-6-*N*-(anthraquinon-2-oyl) hexanediamine (MHAHD), and immobilization of oligonucleotides.

synthesis, which allows the generation of high-density oligonucleotide microarrays (up to 10^6 probes). The method is expensive and does not offer flexibility, which limits its widespread implementation. However, the patterning of pre-synthesized oligonucleotides is the preferred method for many research applications requiring low-to-moderate density arrays (up to few hundred probes). This method offers great flexibility and can accommodate different chemistry as well as surfaces of choice. However, for these microarrays to replace conventional diagnostic methods, substrates have to be well characterized with respect to thermal and chemical stability, absolute density of immobilized oligonucleotides, availability of oligonucleotide probes for hybridization and reproducibility of the attachment chemistry.

Primarily due to its transparency, low cost, non-porous nature and resistance to high temperature, glass is a popular material for oligonucleotide microarray technology. Glass possesses surface functionality, which renders it suitable for robust derivatization and oligonucleotide attachment chemistry. It also possesses very low intrinsic fluorescence, which improves the signal-to-noise ratio when high-intensity lasers are used for detection of labeled oligonucleotides. Central to the deposition technologies is the development of efficient chemistry for covalent attachment of oligonucleotides on glass. A number of attachment methods have been published, which vary widely in chemical mechanism, ease of use, probe density and stability. Most of the procedures reported for oligonucleotide immobilization are based on thermochemical reactions, i.e. covalent bond formation between ligands having amino, carboxyl, thiol or aldehyde functions and suitably modified solid supports. These methods often require derivatization of oligonucleotides, glass surface, or both. In addition, the thermochemical reaction may effect biological activity of the ligand and result in multiple-site attachment of individual molecules. Since photoactivatable reagents are topically addressable (i.e. regiospecific immobilization of oligonucleotide probes on a glass microslide in desired orientation), photoimmobilization of biomolecules have become a subject of intense research towards the development of biosensors and biomaterials.

Here, we report the synthesis of a new heterobifunctional reagent, 1-*N*-(maleimidohexanoyl)-6-*N*-(anthraquinon-2-oyl) hexanediamine (MHAHD) **II** (Scheme 1), for the preparation of oligonucleotide microarrays under the influence of light and microwaves. The reagent, MHAHD, carries sulfhydryl-reactive maleimide group on one of the termini, to react with 3′- or 5′-mercaptoalkylated oligonucleotides and an anthraquinone function on the other end, for light dependent covalent attachment to polymer surface. The immobilization of oligonucleotides using the proposed reagent was accomplished via two routes. In route A, an oligonucleotide-anthraquinone conjugate **III** (Scheme 1) was first formed by allowing the reagent, MHAHD, to react with mercaptoalkylated oligonucleotide under microwaves, an alternative energy source [31,32], which was subsequently immobilized on C–H containing polymer surface under UV irradiation (365 nm). In route B, MHAHD was brought in contact with polymer surface under UV light to generate maleimide functions on it **IV** (Scheme 1), which were subsequently coupled with 3′- or 5′-mercaptoalkylated oligonucleotides under microwaves in 15 min. Both the routes worked satisfactorily and the constructed microarrays were analyzed by hybridization assay. The versatility and applicability of