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Synthetic oligonucleotide combinatorial libraries and their applications

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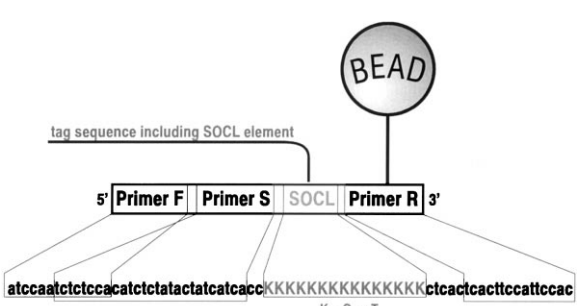
Abstract

The application of synthetic oligonucleotide combinatorial libraries is described in the studies of triplex DNA. A new method of selection of dispersed (beaded) oligonucleotide combinatorial libraries based on the use of streptavidin magnetic beads is presented. A combinatorial chemistry approach is also proposed for studies of polyaminooligonucleotides.

Keywords: Oligonucleotides; Streptavidin; Combinatorial chemistry

1. Introduction

Combinatorial chemistry is an innovative methodology for preparing and studying a large number of related compounds [1]. Different approaches were reported in the literature to prepare and study synthetic compound combinatorial libraries (SCCL). Studies on synthetic oligonucleotide combinatorial libraries (SOCL) were first reported in 1991 and involved the libraries prepared on a surface of appropriately functionalised glass plates [2,3]. SOCLs of integrated type (arrays or chips) are prepared using either mechanical [3] or photolithographic [2] masking of a surface of a future library during each step of a combinatorial synthesis. These SOCLs, so called DNA chips [4–9] are produced mainly to resequence genes and to study gene expression.



Scheme 1. The structure of a tag on a single bead of the [(G/T)15]SOCL.

2. Results and discussion

2.1. Triplex DNA studies

In our laboratory the studies of the properties of oligonucleotides using the dispersed synthetic oligonucleotide combinatorial libraries were initiated several years ago. The dispersed SOCLs (d-SOCLs) are easier to prepare than the integrated ones. Thus, their preparation can be achieved in a laboratory with less sophisticated synthetic equipment and the resulting SOCLs can be used to study the properties of oligonucleotides and their analogues. This is an especially interesting possibility for the study of the properties of oligonucleotides containing modified units and backbones, many of which were proposed as interesting structures for use in antisense and antigene strategy. Moreover, use of SOCLs of unmodified DNA units can be helpful to find DNA targets, other than those expected, within antisense and antigene therapy.

Elements of integrated combinatorial libraries do not require the extra step of reading their structure (sequence). However, the sequencing procedure has to be performed for elements from dispersed SCCLs (d-SCCLs) following a selection procedure. Various approaches were proposed for this purpose [10].

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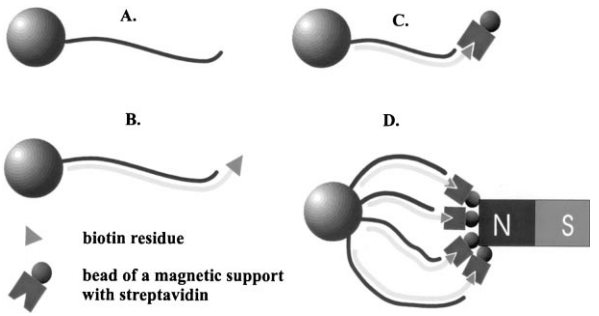


Scheme 2. The selection of elements (beads) of a dispersed combinatorial library with magnetic supports: (A) a library element before 'hybridisation' (selection) and (B) after 'hybridisation' with a biotin-labelled 'complementary' probe; (C) its interaction with a streptavidin coated magnetic support and (D) a mechanical selection of a single or multiple elements of a library at the massive selection step with a permanent magnet.

In the case of the d-SOCLs a single bead is expected to carry up to picomole quantities of oligonucleotide. Therefore, in our experiments we follow the coding approach in which the reading of the structure of a library element is based on the sequencing of a copy of a DNA tag [11,12]. This approach is also convenient because: (i) a sequencing procedure can be repeated, (ii) a library bead can be returned for further selection steps, and (iii) sequencing can be applied for libraries with very small beads (10 μm and smaller) carrying femtomole and even attomole quantities of DNA tag per bead. In our case, the actual randomised oligonucleotide sequence is a part of a tag oligonucleotide. Thus, the tag structure consists of longer oligonucleotide (ca. 70 nucleotides long) and includes a sequence complementary to a PCR 3′-end (reverse) primer, an appropriate tag, a sequence of a sequencing primer and a sequence of PCR 5′-end (forward) primer. A tag sequence was identical to a randomised oligonucleotide of a library and a library contained only complete tag sequences. The model SOCL in which the element of a library was included into the tag sequence is shown in Scheme 1.

We have found that a highly cross-linked polystyrene [13] support with uniform beads of 50 μm diameter and average pore size ca. 1000 Å is well suited for preparation of dispersed SOCLs after appropriate functionalisation [14]. This aminoalkyl support was loaded with 13-*O*-dimethoxytrityl-4,7,10,13-tetraoxatridecanoic acid and used as an universal support to prepare SOCLs [15].

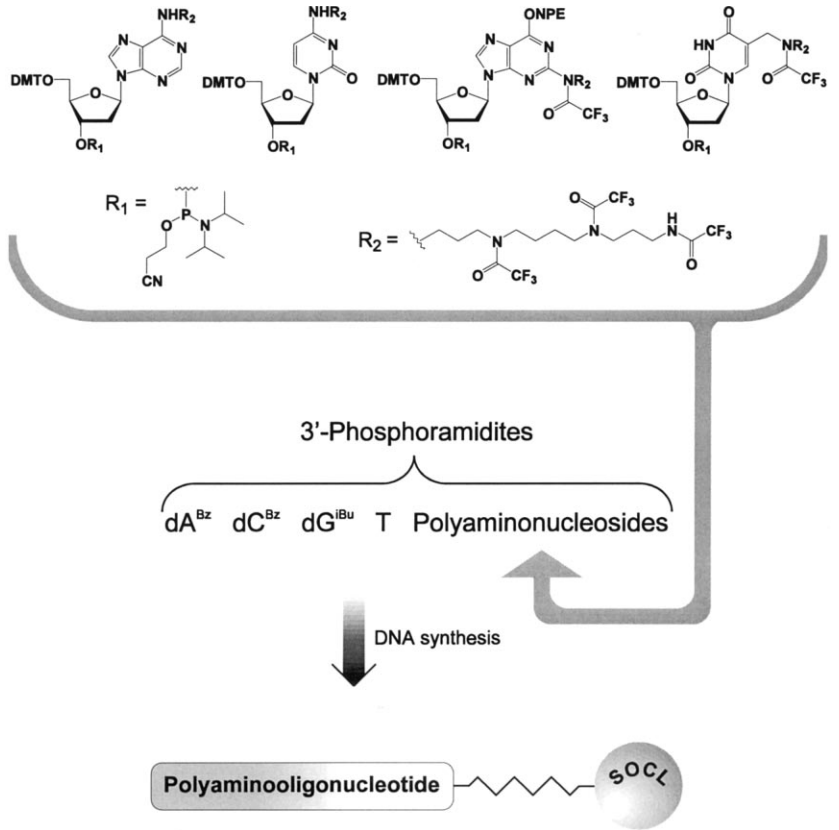
The possibility of using of a SOCL to study triplex DNA formation was chosen for testing the library approach and as a first application. Recently, the application of deoxyguanosine- and deoxythymidine-containing oligonucleotides (GT oligodeoxynucleotides, GT oligo) in the formation of a triplex DNA structure with the promoters of the human epidermal growth factor receptor gene (HER2) was described [16].

Thus, the hairpin oligonucleotide (HER2PIN2) labelled with fluorescein was synthesised – XAG-GAGAAGGAGGAGGTTCCTCCTCCTTCTCCT. It contains, in a duplex region, a sequence of the promoter of human epidermal growth factor receptor gene. The oligonucleotide probe contains at the 5′-end 4-*N*-(8-amino-3,6-dioxaoctyl)-2′-deoxycytidine residue (X) labelled with fluorescein isothiocyanate as described previously [17]. Then the model SOCL with a 15-mer, 5′-ggtggtggttgtggt-3′ was synthesised and found to interact selectively with the probe HER2PIN2 under the conditions reported for triplex DNA formation. In the following experiment, it was established that the probe HER2PIN2 did not hybridise with the model SOCL carrying the tag sequence: 5′-atccaatctctccacatctctatactatcatcaccTATCCTATTTTTACctcactcacttccattccae-3′, showing that the previously used tag could be applied for the studies of triplex formation with the HER2PIN2 probe. The SOCL with a 15-mer G/T randomised region (Scheme 1) was assembled (polystyrene support, ca. 50 000 beads) using the split approach [1,18,19], leading to the SOCL in which each bead should carry an oligonucleotide of unique sequence (one bead — one oligomer). As a result a library containing $2^{15} = 32\,768$ individual oligonucleotide library elements should be produced.

In order to ease a selection of active beads from a SOCL we introduced a new mode of selection, i.e. so-called massive selection. The simultaneous application of rhodamine- and biotin-labelled probes was performed in selection experiments with streptavidin magnetic beads (massive selection) [15]. We report this kind of selection for dispersed combinatorial libraries for the first time (Scheme 2).

The micromanipulation of single beads of SOCLs found during the process of chemoselection is a step that precludes their sequencing. Thus, glass capillaries of 0.01 mm internal diameter were used under the inverted microscope to manipulate the selected library beads.

Then, the [(G/T)15]SOCL was used for the selection of active beads with the probe HER2PIN2 labelled with fluorescein, rhodamine B or biotin. A biotinylated double-stranded PCR product was captured with streptavidin-coated magnetic beads. Then, after an alkaline elution of the nonbiotinylated strand, a magnetic support carrying a single strand copy of the tag sequence of a SOCL was successfully used as a template for DNA sequencing. As a result, several other GT oligonucleotides capable of forming strong complexes with the double-stranded DNA promoter of the *her*2 gene, GGTGGTGGTTGTGGT oligonucleotide, were revealed.



Scheme 3. Polyaminonucleotides were synthesised on the modified polystyrene support using standard 2′-deoxynucleoside 3′-phosphoramidites as well as 3′-phosphoramidites of *N*-trifluoroacetyl-protected polyamino-2′-deoxynucleosides.

2.2. Polyaminooligonucleotides

The ability of natural polyamines to stabilise nucleic acids' tertiary structures and their complexes is well known. In recent years oligonucleotide derivatives bearing various polyamine residues attached at different positions in nucleic acids were synthesised and their properties were investigated. Thus, oligonucleotides derivatised both through 5′- and 2′-hydroxyl functions [20–23], as well as oligonucleotides modified with polyamine moieties attached at different positions of heterocyclic bases [24–28], were obtained. In all cases the stabilising effect of polyamines on complexes of nucleic acids was observed. Recently, we have undertaken systematic studies of polyaminooligonucleotides modified at base moieties [29–32].

Synthesis of 3′-phosphoramidites of polyamino-2′-deoxynucleosides was carried out. The method developed in our laboratory allows the introduction of symmetric polyamines (e.g. putrescine, spermine, etc.) into heterocyclic bases of four major deoxynucleosides. Spermine is the most promising polyamine in relation to DNA. Thus, spermine residue was attached to appropriately modified 5′-*O*-dimethoxytritylated 2′-deoxynucleosides at the N-6 position of dA, the N-4 position of dC, the N-2 position of dG and the C-5 position of T via a methylene group (Scheme 3). Then, amino functions of spermine moieties were protected with trifluoroacetyl groups to eliminate side reactions during a coupling step of automated DNA synthesis. Finally, polyamino 2′-deoxynucleosides were phosphitylated at 3′ position. Such a series of phosphoramidites can be used to obtain different synthetic polyaminooligonucleotide combinatorial libraries (SPOCLs).

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