NANOTECHNOLOGY

Use of Oligonucleotides Conjugated to Gold Nanoparticles and Streptavidin for Amplification of Optical Biosensor Signal during Detection of Telomeric Repeats

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Hybridization of telomeric repeats with a complementary oligonucleotide probe was studied by the surface plasmon resonance method. Conjugation of the probe with streptavidin and gold nanoparticles was shown to amplify the signal at similar concentrations of this probe (by 60 and 300 times, respectively). Nanoparticles can be used for biosensor signal amplification in studying the telomerase activity of malignant cells.

Key Words: *biosensor; nanoparticles; detection; telomeric repeats*

The measurement of telomerase activity (TA) is of particular importance for experimental and clinical oncology. TA is closely related to malignant transformation, serves as a potential target for the development of new anticancer drugs, and can be used as a marker of tumor diseases [1]. The methods for evaluation of TA are based on *in vitro* elongation of the telomerase substrate (TS) with telomerase. The elongated substrate is amplified (polymerase chain reaction) or directly detected [2]. Studying the elongation of TS with telomerase forms the basis for the detection of TA by optical biosensors [6,7]. Elongated TS can be detected by the direct (change in biosensor signal) [6] or indirect method (hybridization of elongated TS with the probes complementary to telomeric repeats) [7]. The possibility of TA detection with a biosensor holds much promise for the development of high-efficacy automated methods for quantitative analysis of TA. Successful development of these methods depends on detection sensitivity, since the relative amount of elongated TS is usually small. One of the approaches to increase the detection sensitivity of optical biosensors suggests the use of nanoparticles. This approach can be used in optical biosensors that are based on the effect of surface plasmon resonance (SPR). Published data show that the detected signal is directly proportional to the weight of the substance, which binds to an effective area of the biosensor [3].

This work was designed to evaluate the possibility of signal amplification in an optical biosensor by using the oligonucleotide probes conjugated to streptavidin or gold nanoparticles.

MATERIALS AND METHODS

The study was performed on a Biacore-3000 optical biosensor (Biacore AB). The detection with a biosensor is based on the SPR effect, which allows estimat-

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ing the amount of any substance bound to the effective area at each moment of time. Microfluidic system of the biosensor operates to deliver a specified microvolume of various fluids through a 4-channel chip. The signal (resonance units, RU) reflects the interaction of biomolecules in the solution with biomolecules that are immobilized on the chip surface. The signal is independently detected by each channel and appears in the form of sensograms, which reflects changes of this signal in time. The study was conducted with SA optical chips (Biacore AB). Streptavidin was immobilized on the effective area of these chips. Sensograms were processes by means of BIAevaluation 4.1. software (Biacore AB). The measurements were performed in buffer R with 10 mM HEPES (pH 7.4), 150 mM NaCl, 10 mM $MgCl_2$, and 10 mM EGTA at 37°C. The reagents were obtained from Sigma-Aldrich (ACS [American Chemical Society] quality or better).

Oligonucleotides were synthesized on an ASM-800 DNA synthesizer (Biosset) using phosphoramidites, ethylthiotetrazole, and biotin-CPG (Glen Research). Other reagents were obtained from Acros Organics. Oligonucleotides had the following designations and sequences:

AO, 5'-*GATGAAAGGATAAGGATGGAAAG*GGAC-3'-Biotin;

TR0, 5'-*CTTTCCATCCTTATCCTTTCATC*CATCATT CCGCCGTCGAGCAGAG;

TR4, 5'-*CTTTCCATCCTTATCCTTTCATC*CATCATT CCGCCGTCGAGCAGAG TTAGGGT**T**AGGGT**T**AGGGT**T**AGGGTTAG-3';

and

CX, 5'-CCCT**T**ACCCT**T**ACCCT**T**ACCCTAAAA AAAA-3'-Biotin.

The underlined nucleotides correspond to a region of 4 telomeric repeats (TR4) and partially complementary region CX. Noncomplementary nucleotides are shown in bold type. The presence of noncomplementary nucleotides decreases the affinity of CX for TR4, which facilitates the removal of bound probes and conjugates during regeneration of biochips with 0.5% solution of sodium dodecyl sulfate. Biotin (3'-terminal) was used for immobilization of AO on the effective area of SA chips and binding of CX probes to streptavidin (ST:CX conjugates) and gold nanoparticles (GP-CX conjugates). TR0 and TR4 were immobilized on the chip surface due to hybridization with AO. Complementary regions of oligonucleotides are shown in italic type. Oligonucleotide solutions were heated at 96°C for 5 min and rapidly cooled on ice.

Experiments were performed with recombinant streptavidin (Sigma-Aldrich) and streptavidin-coated nanoparticles of gold (nominal diameter 10 nm, Sigma-Aldrich). ST:CX conjugates were obtaining by mixing streptavidin and biotinylated probe at equimolar concentrations in buffer R. The concentrations of oligonucleotides and streptavidin were measured spectrophotometrically. One optical unit at 260 nm corresponds to 33 μg oligonucleotide. Molar extinction coefficient of streptavidin at 280 nm was 1.9×10^6 $M^{-1}cm^{-1}$ [4]. To obtain GP:CX conjugates, the commercial product of nanoparticles was 10-fold diluted with buffer R (optical density A_{520}=0.25) and mixed with an equal volume of CX (1 μM solution) in the same buffer under thorough agitation. These mixtures were incubated at room temperature for 1 h to obtain the complexes. In experiments with GP:CX conjugates, nanoparticles were pelleted by repeated centrifugation (2 times) at 15,000*g* for 30 min. The pellet was resuspended in a working buffer. The concentration of particles was evaluated from molar extinction coefficient of 1.5×10^8 $M^{-1}cm^{-1}$ [5]. The probe/particle ratio in GP:CX conjugates corresponds to two oligonucleotides per one nanoparticle.

Optical density of oligonucleotide solutions, streptavidin, and preparations of nanoparticles was measured on a ND-1000 spectrophotometer (NanoDrop Technologies).

RESULTS

Figure 1 shows typical sensograms reflecting the interaction of probe CX and its conjugates with immobilized oligonucleotides. These sensograms were presented by a signal from the channel with immobilized TR4 after deduction of a signal from the channel with TR0. Rapid increase in the signal under various conditions reflects binding of CX, ST:CX, and GP:CX to the chip surface due to hybridization with the complementary region TR4. Due to slow kinetics of bind-

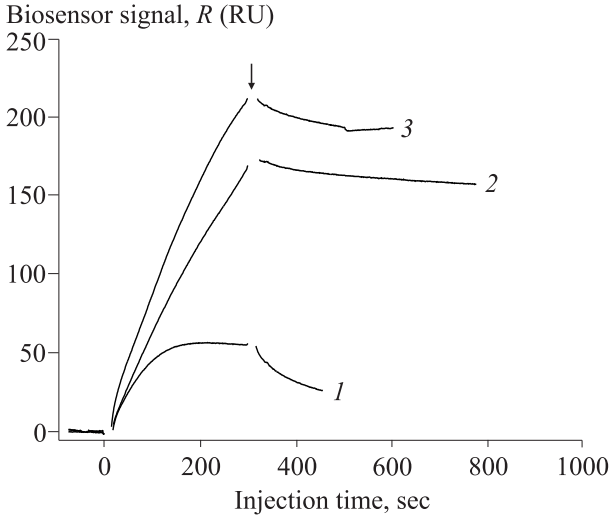

Fig. 1. Typical sensograms. CX, 20 nM (*1*); ST:CX, 10 nM (*2*); GP:CX, 0.13 nM (*3*). Zero point: start of injection of probes or conjugates. Arrow: start of injection of buffer B.

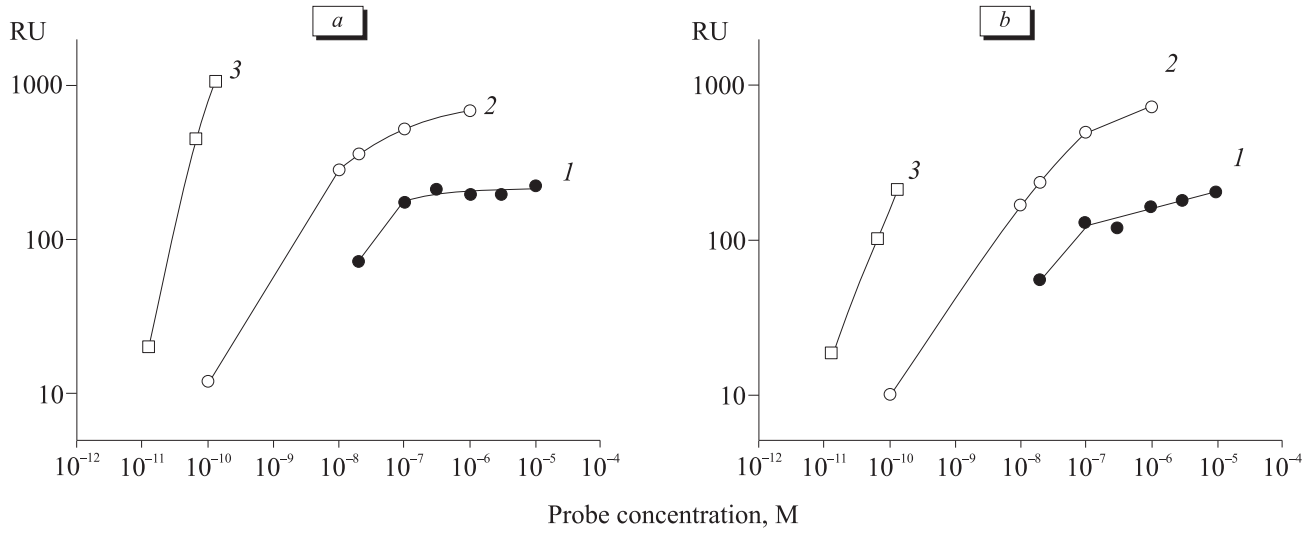

Fig. 2. Dependence of the equilibrium (*a*) and nonequilibrium biosensor signal (*b*) on probe concentration. CX (*1*), ST:CX (*2*), GP:CX (*3*).

ing for conjugates, all measurements were performed after 5-min injection of a sample. Further injection of buffer R was accompanied by the dissociation of CX complexes and conjugates with immobilized TR4 (Fig. 1).

Biosensor signal reflects the formation and dissociation of complexes between immobilized and dissolved biomolecules. Therefore, this signal does not depend on the concentration of dissolved biomolecules at a constant density of immobilization. Comparative analysis should be performed at the same or similar concentrations of a probe to evaluate the degree of signal amplification after conjugation of this probe with protein and nanoparticles. Figure 2 shows the dependence of an equilibrium (R_{eq}) and nonequilibrium biosensor signal (R) on probe concentration. The concentration of conjugates was shown to correspond to the concentration of streptavidin-bound (ST:CX) or nanoparticle-bound probes (GP:CX). The nonequilibrium signal corresponds to a signal recorded over 5-min injection of solutions of the probe or conjugates. The equilibrium signal was evaluated by extrapolation of a biosensor signal with BIAevaluation 4.1 software (Biacore AB). The association and dissociation rate constants are determined from sensograms and used for signal extrapolation. The signal of CX over 5-min injection was practically equal to the equilibrium signal.

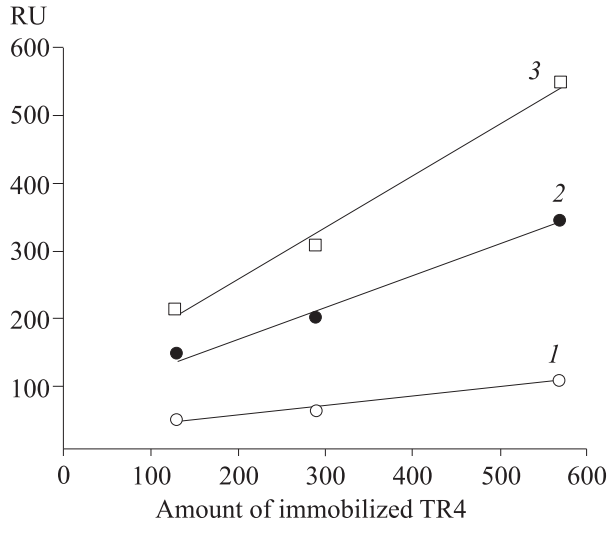

Fig. 3. Dependence of biosensor signal (injection time 5 min) on the amount of TR4 immobilized on the chip surface. CX, 1 μM (*1*); GP:CX, 0.32 nM (*2*); ST:CX, 1 μM (*3*).

The extrapolated equilibrium signal of GP:CX was 60-fold greater than that of ST-CX. Moreover, the extrapolated equilibrium signal of ST-CX was 5 times greater than that of CX. The differences were observed at similar concentrations of conjugated and nonconjugated probes. The use of GP:CX at concentrations comparable to those of CX will be probably accompanied by a 300-fold signal amplification. This amplification will be observed at high concentration of gold nanoparticles. It will increase the cost of TA evaluation. The equilibrium signal of GP:CX can be achieved after a longer time of nanoparticle injection. The study of sensograms shows that the association rate constants for GP:CX and TR4 are 2.5-fold lower than those for CX and TR4 (1.4×10^5 $M^{-1}sec^{-1}$ and 3.5×10^5 $M^{-1}sec^{-1}$, respectively). The signal recorded over 5-min injection of GP-CX is much greater than that of ST:CX and CX (by 16 and 70 times, respectively). Figure 3 shows that the signal of conjugates and probes depends linearly on the density of immobilized TR4. This approach allows us to valuate the relative amount of elongated TS from the signal intensity.

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