Abstract

Both patient baselining and monitoring of compliance in insulin-dependent therapy is accomplished clinically by measurement of levels of glycated hemoglobin (HbA1c). BioTex researchers are developing DNA aptamers (as alternatives to antibodies) to HbA1c and other protein analytes as additional indicators of blood glucose exposure in Type 1 and Type 2 diabetes. To select aptamers against these targets, various proteins were glycated non-enzymatically, and the products were separated by affinity chromatography. DNA aptamers were then selected against the glycated protein targets, and aptamers clones were then characterized by surface plasmon resonance (SPR) binding studies. Equilibrium and kinetic analyses were in agreement showing affinity in nanomolar range. Additional studies on specific aptamer binding to the glycated versus non-glycated versions of the same protein were also performed. These studies demonstrate the utility of the novel aptamers as the basis for quantitative assays for HbA1c as well as additional protein analytes with complementary serum lifetimes.

Introduction

Diabetes affects over 100 million people worldwide and nearly 18 million in the United States [1]. In the U.S. this disorder along with its associated complications is ranked as the seventh leading cause of death [2]. One of the mechanisms underlying the morbidity of diabetes is the abnormal formation of sugar adducts on proteins (glycation). In contrast to glycosylation, glycation is not mediated by enzymes; the formation of Schiff bases is proportional to the ambient glucose concentration followed by slower formation of Amadori products. Glycated hemoglobin, specifically so-called HbA1c, is now routinely used clinically as an index of glucose concentration over the prior 6-8 weeks. We are presently developing aptamer affinity reagents to additional glycated protein analytes with different serum half-lives as a means to provide a more complete retrospective clinical picture of historical blood glucose concentrations. In contrast to instrumentation-intensive chromatography and mass spectrometric assays, properly selected aptamer reagents provide an opportunity to develop simple, rapid tests for such analytes.

Aptamers are single-stranded DNA or RNA oligonucleotides selected to have unique three dimensional folding structure for binding to a variety of targets such as proteins, peptides, and even small molecules with affinity and specificity rivaling that of antibodies. They are typically selected in vitro by a process commonly referred to as “SELEX” [3,4] as depicted in Figure 1. Using a proprietary variant of this process, we are developing aptamers to multiple targets simultaneously.

Methods

Boronate affinity chromatography. Proteins were glycated in 1M glucose for 18 hrs at 37°C. Glycated and unglycated proteins were then separated by boronate sepharose affinity column chromatography (Figure 2). The outlet of the column was monitored by a standard UV-Vis detector (ISCO “UA”) with analog signal digitized by a USB analog-to-digital converter (Dataq Instruments).

Aptamer selection. A proprietary, patent pending method of multiplexed aptamer selection is frequently employed at BioTex with commercial services available through affiliate, “ic9 Biotechnologies”. Briefly, both glycated and non-glycated targets were immobilized on a solid phase surface. Following rounds of selection and enrichment against a randomized DNA library, a proprietary method of deciphering which DNA molecules are bound to which protein species is employed. Following DNA sequencing, all subsequent binding studies (see below) were performed on cloned DNA materials.

SPR binding studies. Biomolecular interaction studies were done using a surface plasmon resonance (SPR) based instrument, SensiQ (ICX Nomadics, Oklahoma City, OK). SPR signals of the molecules on the surface of the sensor were studied and the kinetic and equilibrium analyses were performed done in order to get the affinity constant Kd, which aided in validating the aptamers generated. Aptamers having strong affinity towards target shows lesser Kd value, in the range of 1-50 nM.

Results

A typical chromatogram for preparing purified, glycated protein target by boronate affinity target for subsequent aptamer selection is presented in Figure 2 below. Representative characterization of aptamers to both glycated IgM mu chain as well as glycated hemoglobin are presented in Figures 3a and 3b. Figure 4 demonstrates the intended specificity of a single aptamer clone for the glycated version of hemoglobin according to the ELASA method described above. Finally, Figure 5 depicts an analysis of aptamer dissociation from gly-hemoglobin and a number of other gly- and non-gly targets including non-gly-hemoglobin. As can be seen, after 5 minutes of washing, less than 5% of the original aptamer material remains bound to non-target proteins.

Discussion

The results here completely demonstrate the feasibility of novel, aptamer-based clinical diagnostic and research assays for quantifying glycated protein targets. While many groups have developed aptamers to recombinant proteins, very few have demonstrated the rapid ability to develop novel aptamers for discriminating between such small chemical differences between otherwise identical targets. These methods should then be relevant to development of additional aptamers specific to other post-translational modifications and unnatural adducts of other disease states.

References


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