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Engineering periplasmic ligand binding proteins as glucose nanosensors

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Abstract

Diabetes affects over 100 million people worldwide. Better methods for monitoring blood glucose levels are needed for improving disease management. Several labs have previously made glucose nanosensors by modifying members of the periplasmic ligand binding protein superfamily. This minireview summarizes recent developments in constructing new versions of these proteins that are responsive within the physiological range of blood glucose levels, employ new reporter groups, and/or are more robust. These experiments are important steps in the development of novel proteins that have the characteristics needed for an implantable glucose nanosensor for diabetes management: specificity for glucose, rapid response, sensitivity within the physiological range of glucose concentrations, reproducibility, and robustness.

Keywords: *biosensor*; *glucose*; *diabetes*; *nanosensor*; *periplasmic ligand binding protein*; *protein design*

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Diabetes is a current major health problem that affects over 100 million people worldwide. Disease management for many patients requires frequent measurement of blood glucose concentrations, which usually requires pricking of a finger to draw a blood sample. There is great interest in developing new methods of monitoring glucose levels. Of particular interest would be an implantable device for continuous monitoring. New methods would require specificity for glucose, rapid response, sensitivity to glucose concentrations within a physiological range (1.7–33 mM) (1), reproducibility, and robustness.

Protein-based biosensors for detecting specific small molecules have previously been derived from several members of the periplasmic ligand binding protein family (PLBP) (2). The PLBP proteins contain two large globular domains with a connecting flexible hinge region (3). Upon ligand binding, the protein undergoes a large conformational change that brings the two lobes together with the ligand binding pocket located at the interface of the two domains (Fig. 1). A method of detecting the conformational change is attached to the protein, for example, a fluorescent tag that undergoes a change in fluorescence when the protein switches between the ligand bound and unbound conformations. The two conformations of the PLBP serve as an on/off switch that can be used to detect bound ligand in a reagentless manner; no substrate is used up. Several labs have used this general method to produce variants of the *Escherichia coli* glucose/galactose-binding protein (GBP) to serve as biosensors for glucose (4–13). Recent developments described below include (1) mutant forms of the GBP that have binding constants for glucose that are within the physiological range of blood glucose levels and increased specificity for glucose over other sugars, (2) the use of new reporter groups, and (3) the use of a thermostable GBP from *Thermotoga maritima* that is more robust than the *E. coli* GBP.

One disadvantage of using the *E. coli* GBP as a glucose sensor has been its high-binding affinity for glucose, 0.2 μM (14). Modified forms of the protein are needed that can respond to physiological levels of glucose without being saturated. Recently, three labs used site-directed mutagenesis guided by the GBP X-ray crystal

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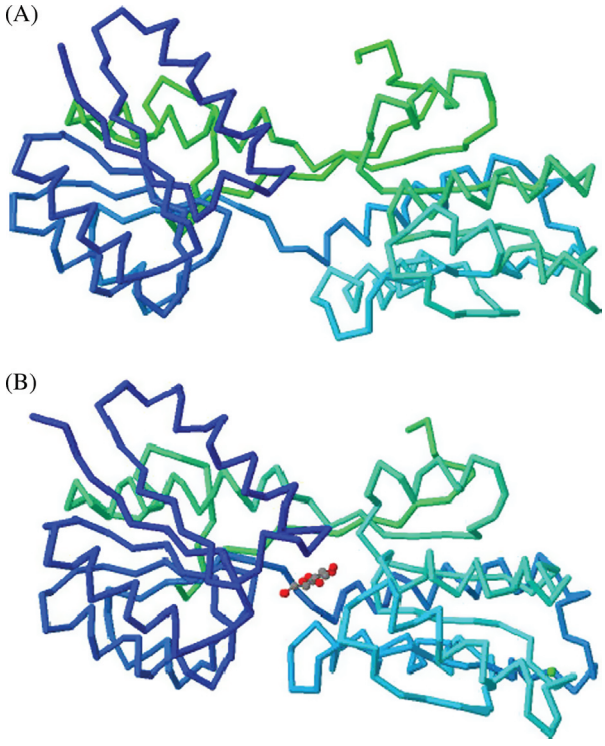

Fig. 1. The glucose/galactose binding protein undergoes a large conformational change that brings two lobes together when glucose is bound. (A) An alpha carbon trace of the glucose/galactose binding protein in its open form without glucose (PDB ID 2FWO). (B) Glucose/galactose binding protein in its closed form with glucose (ball-and-stick model) bound in the ligand binding pocket (PDB ID 2HPH).

structures to develop mutant forms of the *E. coli* GBP with changes in the ligand binding pocket that resulted in a new binding affinity that is within the physiological range for blood glucose monitoring (Fig. 2) (15–17). A fourth lab used structure-guided random mutagenesis of a selected region in the ligand binding site and also successfully created a mutant protein that has a binding constant for glucose within the physiological range (18).

Another recent development is several GBP-based sensors with new reporter groups. Der and Dattelbaum used their mutant GBP protein described above to develop a glucose biosensor that uses molecular exciton luminescence as a reporter (16). Two identical rhodamine derivative tags were covalently bound to cysteines that were introduced through site-directed mutagenesis at opposite ends of the glucose-binding cleft (Fig. 3A). The tags are far apart when the protein has not bound glucose. Upon glucose binding, the GBP conformational change brings the two tags close enough so that they form dimers with altered excitation and fluorescence.

Two labs have developed GBP derivatives with bioluminescent reporters to obtain glucose-responsive bioluminescence. The Sode lab constructed a hybrid protein with their modified GBP described above and split

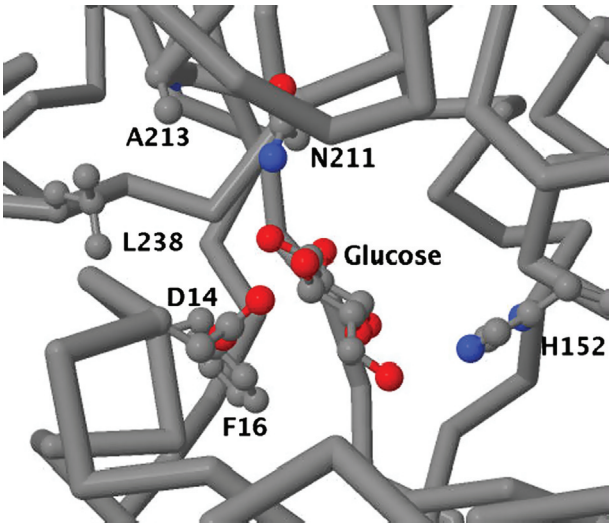

Fig. 2. Several labs have identified amino acid substitutions in or near the glucose binding site of the glucose/galactose binding protein that adjust the binding affinity to within the range needed for monitoring blood glucose levels. The protein backbone is shown as an alpha carbon trace in grey. Glucose and some of the amino acids that were substituted, singly or in groups, to alter the binding affinity are shown as ball-and-stick models. Mutations that brought the binding affinity into the physiological range include D14E/F16A (15), R96C/D168C/N211A/A213R (16), H152C/A213R/L238S (17), and A213R (18).

luciferase (19). Two halves of the luciferase protein that are not individually luminescent are fused to the two lobes of the GBP (Fig. 3B). When the chimeric protein binds glucose, it changes conformation and brings the

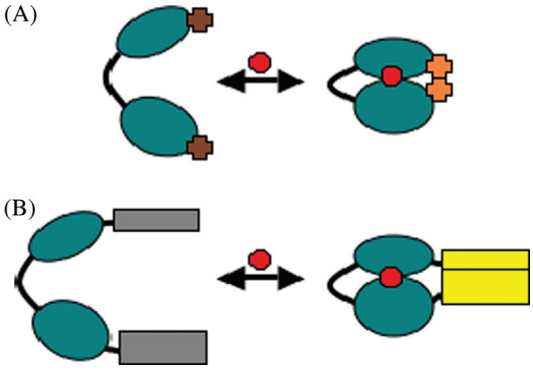

Fig. 3. Fluorescent or bioluminescent probes attached to the GBP in appropriate places can undergo changes in fluorescence or bioluminescence when the GBP changes conformation. (A) Two identical fluorescent molecules placed in appropriate locations are brought together and change fluorescent properties when the GBP switches from the open conformation to the closed glucose bound conformation. (B) The large conformational change in the GBP can also bring together two domains of a bioluminescent protein, such as split luciferase or aequorin, so that the reporter protein becomes luminescent when glucose binds to the GBP.

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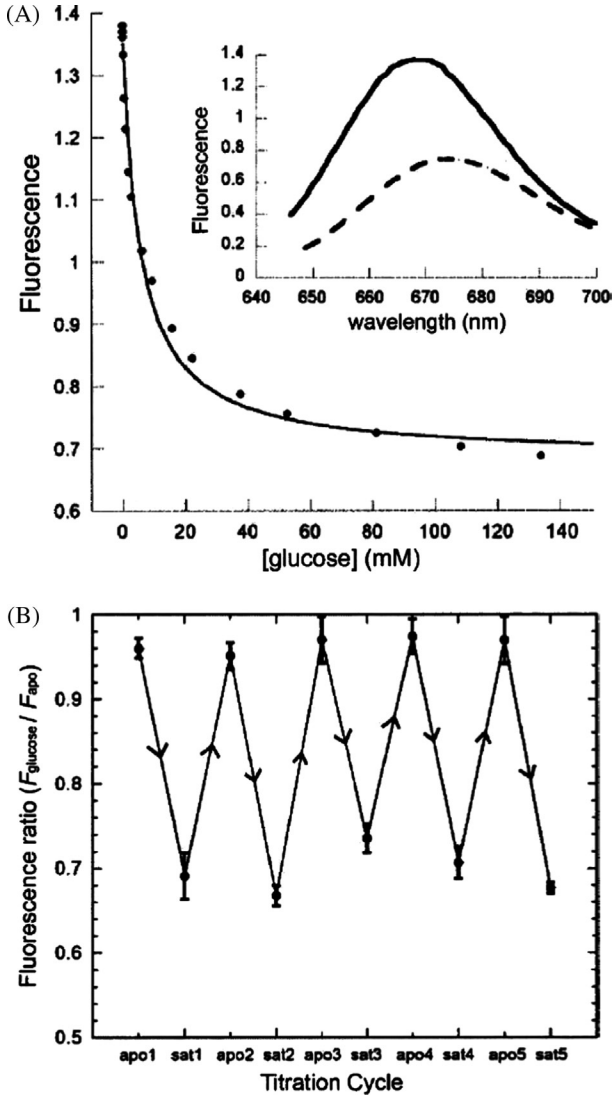

Fig. 4. A thermostable glucose binding protein (tmGBP) was used to develop a more stable glucose biosensor. (A) Glucose binding causes a change in fluorescence of the protein/dye conjugate (Y13C-Cy5). Inset: Emission spectra are shown in the absence (solid line) and presence of 140 mM glucose. (B) When immobilized on microtiter plates, the tmBGP-Y13C protein can undergo multiple cycles of glucose binding and washing and still maintain its ability to respond to glucose. Data for the unbound and glucose bound states for five cycles are connected by arrows. (Reproduced from Tian et al., 2007, with permission)

two luciferase domains close enough together so that they can reassemble into a bioluminescent protein.

Similarly, the Daunert lab created a hybrid glucose-responsive bioluminescent protein by using aequorin (AEQ) as a reporter (20). They identified a large interdomain loop in the X-ray crystal structure of AEQ where the glucose binding protein could be inserted, separating the AEQ into two domains that are not bioluminescent. Glucose binding to the GBP brings the two segments of AEQ together, the coelenterazine cofactor is converted to coelenteriamide, and bioluminescense emission is increased.

In addition to improving the range of sensitivity and adding novel modes of detection of glucose binding to the GBP, other important requirements for use as a biosensor are that the protein is robust enough to be used repeatedly and it maintains function while immobilized on a surface. The Hellinga lab identified and solved the crystal structure of a GBP from the thermophile *Thermotoga maritima*, tmGBP, which does not unfold below 100°C (21). Based on the crystal structure of tmGBP, they introduced a fluorescent reporter group covalently bound to cysteines introduced at key positions where conformational changes due to glucose binding might affect the fluorescence of the tag. They successfully demonstrated that changes in fluorescence emission of the reporter group are indeed coupled to glucose binding (Fig. 4A). The thermostable GBP is more stable to storage and can be immobilized on a surface through biotin coupled to exposed lysines without losing sensitivity (Fig. 4B). It senses glucose within the range of 1–30 mM glucose, which includes the range of physiological glucose levels.

The development of these new GBP-based glucose nanosensors are important steps toward using modified proteins to serve as specific, reusable, sensitive, and robust sensors for the measurement of glucose levels. They are potentially important steps in the direction of an implantable continuous glucose monitor that would aid in the management of diabetes and improve patients' ability to avoid complications due to hypoglycemia or hyperglycemia.

Conflict of interest and funding

There is no conflict of interest in the present study for the author.

References

1. Sacks DB, Bruns DE, Goldstein DE, Maclaren NK, McDonald JM, Parrott M. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Clin Chem 2002; 48: 436–72.
2. Dwyer MA, Hellinga HW. Periplasmic binding proteins: a versatile superfamily for protein engineering. Curr Opin Struct Biol 2004; 14: 495–504.
3. Quiocho FA, Ledvina PS. Atomic structure and specificity of bacterial periplasmic receptors for active transport and chemotaxis: variation of common themes. Mol Microbiol 1996; 20: 17–25.
4. Deuschle K, Okumoto S, Fehr M, Looger LL, Kozhukh L, Frommer WB. Construction and optimization of a family of genetically encoded metabolite sensors by semirational protein engineering. Protein Sci 2005; 14: 2304–14.
5. Thomas KJ, Sherman DB, Amiss TJ, Andaluz SA, Pitner JB. A long-wavelength fluorescent glucose biosensor based on

bioconjugates of galactose/glucose binding protein and Nile Red derivatives. Diabetes Technol Ther 2006; 8: 261–8.
6. Tolosa L, Gryczynski I, Eichhorn LR, Dattelbaum JD, Castellano FN, Rao G, et al. Glucose sensor for low-cost lifetime-based sensing using a genetically engineered protein. Anal Biochem 1999; 267: 114–20.
7. Moschou EA, Sharma BV, Deo SK, Daunert S. Fluorescence glucose detection: advances toward the ideal in vivo biosensor. J Fluoresc 2004; 14: 535–47.
8. Salins LL, Ware RA, Ensor CM, Daunert S. A novel reagentless sensing system for measuring glucose based on the galactose/glucose-binding protein. Anal Biochem 2001; 294: 19–26.
9. Fehr M, Lalonde S, Lager I, Wolff MW, Frommer WB. In vivo imaging of the dynamics of glucose uptake in the cytosol of COS-7 cells by fluorescent nanosensors. J Biol Chem 2003; 278: 19127–33.
10. de Lorimier RM, Smith JJ, Dwyer MA, Looger LL, Sali KM, Paavola CD, et al. Construction of a fluorescent biosensor family. Protein Sci 2002; 11: 2655–75.
11. Ye K, Schultz JS. Genetic engineering of an allosterically based glucose indicator protein for continuous glucose monitoring by fluorescence resonance energy transfer. Anal Chem 2003; 75: 3451–9.
12. Ge X, Tolosa L, Rao G. Dual-labeled glucose binding protein for ratiometric measurements of glucose. Anal Chem 2004; 76: 1403–10.
13. Khan F, Gnudi L, Pickup JC. Fluorescence-based sensing of glucose using engineered glucose/galactose-binding protein: a comparison of fluorescence resonance energy transfer and environmentally sensitive dye labelling strategies. Biochem Biophys Res Commun 2008; 365: 102–6.
14. Vyas NK, Vyas MN, Quiocho FA. Sugar and signal-transducer binding sites of the *Escherichia coli* galactose chemoreceptor protein. Science 1988; 242: 1290–5.
15. Sakaguchi-Mikami A, Taneoka A, Yamoto R, Ferri S, Sode K. Engineering of ligand specificity of periplasmic binding protein for glucose sensing. Biotechnol Lett 2008; 30: 1453–60.
16. Der BS, Dattelbaum JD. Construction of a reagentless glucose biosensor using molecular exciton luminescence. Anal Biochem 2008; 375: 132–40.
17. Khan F, Saxl TE, Pickup JC. Fluorescence intensity- and lifetime-based glucose sensing using an engineered high-Kd mutant of glucose/galactose-binding protein. Anal Biochem 2010; 399: 39–43.
18. Amiss TJ, Sherman DB, Nycz CM, Andaluz SA, Pitner JB. Engineering and rapid selection of a low-affinity glucose/galactose-binding protein for a glucose biosensor. Protein Sci 2007; 16: 2350–9.
19. Taneoka A, Sakaguchi-Mikami A, Yamazaki T, Tsugawa W, Sode K. The construction of a glucose-sensing luciferase. Biosens Bioelectron 2009; 25: 76–81.
20. Teasley Hamorsky K, Ensor CM, Wei Y, Daunert S. A bioluminescent molecular switch for glucose. Angew Chem Int Ed Engl 2008; 47: 3718–21.
21. Tian Y, Cuneo MJ, Changela A, Höcker B, Beese LS, Hellinga HW. Structure-based design of robust glucose biosensors using a *Thermotoga maritima* periplasmic glucose-binding protein. Protein Sci 2007; 16: 2240–50.

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