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The effect of pH on the glucose response of the glucose–galactose binding protein L255C labeled with Acrylodan

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ABSTRACT

The glucose–galactose binding protein (GGBP) is used as an optical biosensor in medical and bioprocess applications. This paper investigates the effect of pH on the behavior of GGBP-L255C labeled with Acrylodan for the purpose of finding the optimum conditions for sensing purposes as well as for protein preparation, purification and storage. The Acrylodan–GGBP fluorescence response in absence and presence of glucose was measured under varying buffer and pH conditions. Dissociation constants (K_d) and Gibbs free energies (ΔG) for the protein-glucose binding were calculated. Binding was found to be energetically favored at slightly acidic to neutral conditions, specifically close to the pI of GBP (~5.0). Minimal fluorescence response to glucose was exhibited at pH 3.0 accompanied by a blue shift in the steady state fluorescence spectrum. In contrast, an almost 45% response to glucose was shown at pH 4.5–9.0 with a 13-nm red shift. Frequency domain lifetime measurements and quenching with KI suggest that at highly acidic conditions both the glucose-free and the glucose-bound protein are in a conformation distinct from those observed at higher pH values.

1. Introduction

The glucose–galactose binding protein (GGBP) belongs to a group of soluble proteins that can be found in the periplasmic space of gram negative bacteria, such as *Escherichia coli*. These proteins are responsible for stimulating chemotaxis in low nutrient environments and participate in active transport of small molecules and ions from the periplasm to the cytoplasm. Because of these functions, these proteins have evolved very high sensitivity and selectivity for their substrates [1]. Thus, wild type GGBP has a dissociation constant $K_d < 1$ μM for glucose at pH 7 and slightly higher for galactose, but does not respond to other sugars such as maltose, fructose, etc. In addition, the binding proteins including GGBP undergo substantial conformational changes upon substrate binding, which are then required for docking to a membrane-bound receptor. The process of translocation of the substrate from the periplasm to the cytoplasm requires ATP for energy, thus, constituting the so-called ATP-binding cassette (ABC) transporters. The tertiary structure of GGBP, as is generally the case in the binding proteins, is comprised of two globular domains linked by flexible hinges. In solution, the tertiary structure of GGBP equilibrates between an open structure where the two domains are apart and a closed structure where the hinge bends and the two domains are closer together. The binding of glucose to GGBP favors the closed conformational structure as glucose replaces water within the binding cleft [2–6]. The change from the open ligand-free to the closed ligand-bound structure has been utilized in optical transduction applications by conjugating the protein with a single environment-sensitive dye [7–10]. The protein is thus rendered as a glucose optical biosensor for biomedical and bioprocess applications. It has been used to monitor glucose levels in bioprocesses to ensure healthy cell growth and optimum product yield [10,11]. It has also been used in clinical applications to monitor transcutaneous glucose levels [12] and transdermal glucose [13].

Because GBP is primarily used as a biosensor, understanding its behavior under different pH conditions is very important. As an example, one of the applications we are investigating for the GGBP biosensor is to determine glucose diffusing from the tissues to the surface of the skin [13] in a noninvasive glucose monitoring system. The pH of the skin surface is on average slightly below pH 5.0 [14] and we have to make sure GGBP functions properly at

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this pH. We are also developing the GGBP biosensor in bioprocess and fermentation monitoring. In yeast fermentation in particular, the optimum pH is around 5.0. Thus, we have to show that GGBP labelled with Acrylodan is an appropriate biosensor even under these conditions. Additionally, pH is important for optimum protein preparation, purification and storage conditions.

The experiments reported here include studying the activity of GGBP in various pH and buffers. The GGBP mutant has a cysteine mutation at the 255th position that was then labeled with sulfhydryl reactive Acrylodan dye. Acrylodan is one of a group of polarity sensitive dyes that exhibit changes in their photophysical properties in response to changes in hydrophobicity of the surrounding environment. From previous studies, we have shown that Acrylodan at the 255-position of GGBP gives favorable fluorescence response as glucose is added, an indication that GGBP undergoes conformational changes as it binds to glucose [9,15]. Additionally, Acrylodan is the dye of choice because it absorbs and emits at wavelengths distinct from where biological components autofluoresce [9]. The choice of Acrylodan is also based on previous reports that the fluorescence of Prodan, a dye with the same fluorophore as Acrylodan but without the –SH reactive group, is unaffected by pH [16]. Thus, fluorescence changes observed were the effects of pH on the protein rather than on the dye. This was further confirmed by dissolving free Acrylodan (without the protein) at various pH conditions (Supplemental data 1) and finding no differences in the fluorescence spectra.

To obtain the desired pH, the Acrylodan-labeled GBP is dialyzed in buffer solutions with pH ranging from 3 to 10. Protein behavior was analyzed by observing the steady state fluorescence response, changes in fluorescence lifetime, as well as, KI quenching in the absence and presence of glucose. These fluorescence measurements provide an indirect indication of changes in the protein structure, specifically those associated with interaction of the protein with glucose at this range of pH.

2. Materials and methods

2.1. Materials

D-Glucose, HEPES, acetic acid, Tris–HCl, Bis–Tris, citric acid monohydrate ($C_6H_8O_7{\cdot}H_2O$), boric acid (H_3BO_3), sodium phosphate dibasic (HNa_2O_4P), sodium phosphate monobasic monohydrate ($H_2NaO_4P{\cdot}H_2O$) were purchased from Sigma–Aldrich (St. Louis, MO).

2.2. Biosensor and buffer preparation

The fluorescently labeled L255C GBP biosensor was prepared as described in Ge et al. [7]. Buffers were prepared based on desired concentration and pH. To obtain desired protein (L255C GBP) environment, protein was dialyzed in specified buffers. Specifically, 3 mL of purified GBP was placed in dialysis cassettes (Slide-A-Lyzer, Thermo Scientific) and protein solution was dialyzed in 500 mL of citrate, acetate, HEPES, Bis–Tris, Tris, phosphate or borate buffer for 14 h (overnight) in a cold room. The buffer was changed in the morning and protein solution was dialyzed for 6 additional hours in a cold room. The ionic strength for all the employed buffer solutions was 20 mM. Protein solutions were collected, filtered with vacuum filters (Nalgene 0.2 micron vacuum filters, Fisher Scientific) to ensure buffer sterility, and were stored at room temperature in glass bottles for no more than 24 h.

2.3. Measurements of steady state GBP fluorescence

The activity of the protein was determined by using the steady-state fluorescence spectra with Varian Cary Eclipse fluorescence spectrophotometer. The emission spectra in the range 400–650 nm were recorded by keeping the excitation wavelength constant at 390 nm. A 250 μL GBP sample (3.5 μM) was placed in a quartz cuvette and the fluorescence spectrum was measured. The activity and emission spectrum was then checked by adding 0.1 mM glucose standard to cuvette to obtain final concentration of 0.4 μM. After the reading is taken, this standard was added two additional times for final concentrations of 0.8 μM and 1.2 μM and fluorescence readings were taken for each. The glucose assay was completed by adding 1 mM, 10 mM, and 1 M glucose standard, respectively to the GBP sample in same manner previously described. The final glucose concentrations after addition to GBP solution ranged from 5.2 μM to 16 mM. The fluorescence reading was taken after each glucose addition.

2.4. Determination of GBP fluorescence response of GBP to glucose

As previously mentioned, GBP was labeled with Acrylodan dye at the 255th position. Since this position is opposite the binding site, the fluorescence intensity of the dye decreases with increasing glucose concentration. The percent response, %*R*, of GBP to glucose was calculated using the following equation:

$$\%R = \left\{\frac{(F_0 - F)}{F_0}\right\} \times 100 \quad (1)$$

where F is the fluorescence intensity of glucose-bound GBP and F_0 is the fluorescence intensity of glucose-free GBP.

2.5. Determination of fluorescence lifetime

Lifetime (τ), or decay time, is the average amount of time a fluorophore remains in the excited state following excitation. It is a significant property because it describes the time available for the fluorophore to interact or diffuse in its environment. In frequency-domain fluorometry a sample is excited with intensity-modulated light at a high frequency. As a result of a lag existing between the absorption and emission, emission is delayed in time when compared to the modulation excitation. At each modulation frequency (ω), this delay is described as the phase shift or phase delay (φ_ω). In addition, demodulation of the emission occurring by a factor m_ω of phase angles or modulation at any frequency can be used to calculate the lifetime using the following relationships [17]:

$$\tan\varphi_\omega = \omega\tau \quad (2)$$

and

$$m_\omega = \left(1 + \omega^2\tau^2\right)^{-1/2} \quad (3)$$

To determine fluorescence lifetime of the fluorophore, 250 μL of fluorescent GGBP solution was added to a quartz cuvette and the frequency domain intensity data was measured using a frequency domain fluorimeter (ISS-KOALA, Champagne, IL) with modifications. The excitation source is an LED UV3TZ-405-30405 (Bivar, Inc.) driven by a current source. The modulation voltage was applied through bias T. The excitation light was filtered by 500-, 550-, and 650 FL07 short-wave pass filters (Andover, Salem, NH). The emission light was filtered by a 500 FH90 long-wave pass filter (Andover). Luminescence decay data were analyzed by nonlinear least-squares methods where τ_i and f_i are the decay times and preexponential factors, respectively. These measurements were conducted three times for each of the four selected pH environments (citrate pH 3 and pH 4.5, phosphate pH 7 and borate pH 9), with and without glucose. The average lifetime was calculated using the relation:

$$\tau = \tau_1 f_1 + \tau_2 f_2 \ldots \tau_i f_i \quad (4)$$

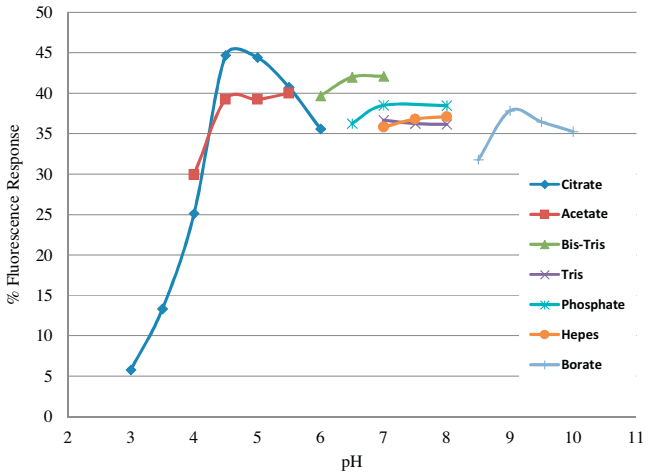

Fig. 1. The percent fluorescence response of acrylodan bound GBP at saturating glucose (500 μM) conditions in different buffers at their working pHs.

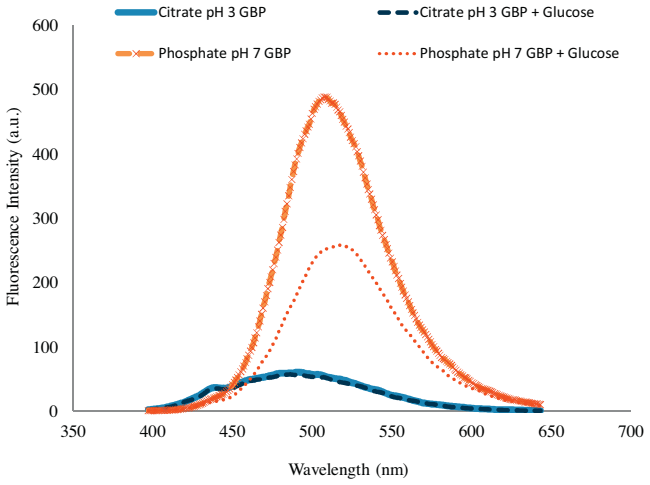

Fig. 2. Steady-state fluorescence spectra of GBP in citrate buffer at pH 3 compared to GBP in phosphate buffer at pH 7.

2.6. Determination of Stern–Volmer quenching constant

Quenching is defined as the decrease in fluorescence intensity. Specifically, collision fluorescence quenching is observed when the excited-state fluorophore is deactivated upon collision with a quencher and can be described by a simplified Stern–Volmer equation [17]:

$$\frac{I_0}{I} = K_{SV}[Q] + 1 \quad (5)$$

where I_0 and I are the fluorescence intensities in the absence and presence of a concentration of quencher, $[Q]$, respectively and K_{SV} is the Stern–Volmer quenching constant. Ultimately, quenching measurements reveal the accessibility of the fluorophore to quenchers in the external aqueous environment.

The quenching experiment was conducted also by adding 250 μL of fluorescent GBP solution to a quartz cuvette. The fluorescence spectrum for glucose-free GBP was measured using the Varian Spectrophotometer. Afterwards, 5 M KI was added to the GBP solution and the fluorescence spectrum was recorded. The quencher, KI, was added 1 μL at a time, up to 10 μL, to ensure a final quencher concentration ranging from 0 to 0.01 M. The fluorescence spectrum was measured after each addition of the quencher. The aforementioned procedure was repeated in the presence of 250 μL GBP–Acrylodan saturated with glucose. Using Eq. (5), the quenching constant was obtained from the slope of a plot of I_0/I versus $[Q]$ data collected from the steady-state fluorescence quenching measurements.

3. Results and discussion

The Acrylodan–GGBP fluorescence response to glucose in varying buffer conditions is shown in Fig. 1. The profiles in the figure represent the glucose induced fluorescence response in seven buffers with pH ranging from 3.0 to 10. At saturating glucose conditions (500 μM), the maximum steady state fluorescence response of GGBP to glucose was calculated using Eq. (1). As shown in the figure, the weakest fluorescence response is seen in the extremely acidic pH 3.0–3.5. Around pH 4 in both acetate and citrate buffers, a gradual increase in the fluorescence response (25 and 30%, respectively) can be observed. The maximum response for GBP, occurring around pH 4.5–5.0, is about 45%. Ultimately, as the pH ranged from pH 4.5 to 10.0, the fluorescence response of GBP plateaus.

In the spectra shown in Fig. 2, this lack of response to glucose at pH 3.0–3.5 can be easily seen where the spectra with and without glucose almost overlap (λ_{max} = 490 nm with and without glucose). In contrast, at pH 7.0, the glucose-bound GBP (λ_{max} = 522 nm) is easily differentiated from the glucose-free GBP (λ_{max} = 509 nm) as shown by a decrease in fluorescence intensity as well as a slight red-shift (~13 nm). This is easily explained with a decrease in hydrophobicity in the Acrylodan environment as the protein assumes the 'closed' glucose-bound conformation. As the protein wraps the glucose within the binding site, the Acrylodan, which is on the opposite side of the binding site at the 255th position, becomes more exposed to the aqueous environment. Note that the same spectral changes associated with glucose binding were observed at pH 4.5–10.0 consistent with the results in Fig. 1. Further in Fig. 2, a blue shift in the Acrylodan emission maximum is observed in pH 3.0 relative to pH 7.0. Additionally, the GGBP at pH 3.0 shows a broader peak and decreased fluorescence intensity than at the higher pH. Taking into account only general solvent effects, a blue shift in the spectrum of a polarity sensitive probe would normally be accompanied by an increase in fluorescence intensity, both attributed to an increase in hydrophobicity in the environment. Thus, the blue shift accompanied by fluorescence observed in our experiments at pH 3.0 is not easily explained by general solvent effects, however, specific solvent effects, such as hydrogen bonding or acid–base reactions in the site where the probe is embedded are highly likely [17]. The strongly acidic environments in pH 3.0–3.5 probably cause proton induced protein conformational changes that affects glucose binding to the protein and/or 'traps' the Acrylodan in a hydrophobic pocket. Nevertheless, the blue shift suggests that only a partial unraveling of the protein occurs at pH 3.0 and some structure remain as evidenced by the hydrophobic pocket where the Acrylodan is embedded. It is also possible that the Acrylodan functional group acts as a seed for the formation of this hydrophobic pocket at acidic conditions.

Values of the normalized percent response for each pH were plotted against concentrations of the unbound glucose to obtain the binding isotherm for GBP–glucose shown in Fig. 3a. The equation describing this isotherm is:

$$\frac{R}{R_0} = \frac{C}{(C + K_d)} \quad (6)$$

where K_d is the dissociation constant for the interaction between GBP and glucose (based on the activity quotient). At the employed low concentration range, the activity is equal to the concentration. R is the normalized response of GBP at any glucose concentration, R_0 is the normalized response at saturating glucose concentration, and

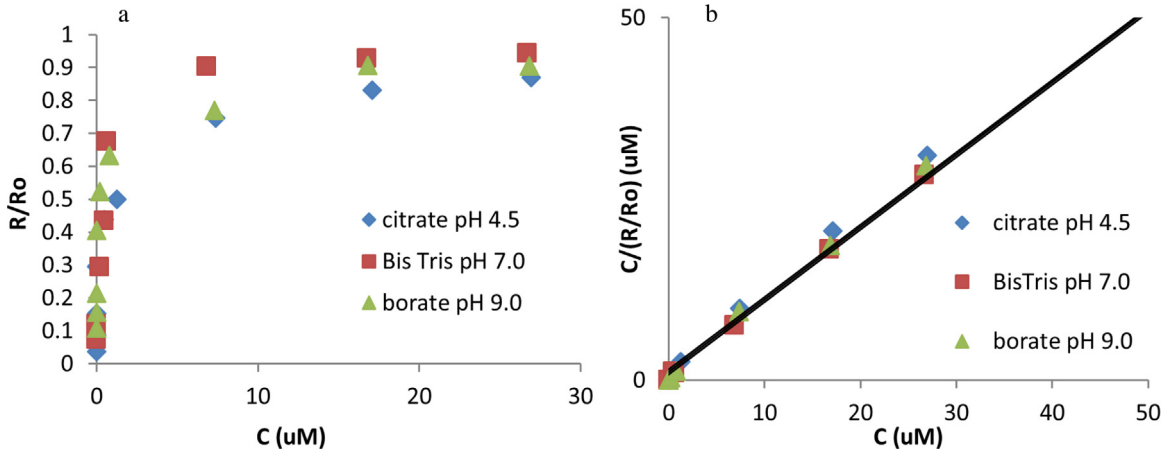

Fig. 3. Binding isotherms (a) and their linear plots (b) for the interaction of GBP with glucose. Measurements were performed at room temperature (20 ± 2 °C) using 20 mM buffer solutions.

C is the concentration of the non-bound glucose (μM), calculated as:

$$C = C_0 - \left(\frac{R}{R_0}\right) C_P \quad (7)$$

where C_0 is the initial free glucose concentration in solution (μM) and C_P is the protein concentration (μM). Rearranging Eq. (7) gives the following linear form:

$$\frac{C}{R/R_0} = K_d + C \quad (8)$$

The isotherm follows a Langmuirian-type behavior where there is a single binding site and hence the isotherm flattens out at saturation conditions. This behavior was also observed by Ge et al., when studying the binding of the dual labeled GBP to glucose at physiological pH and different temperatures [9]. The linearized plot of the isotherm is depicted in Fig. 3b.

Values of the dissociation constants were calculated from the intercepts of the relevant linear plots and were compiled in Table 1. The correlation coefficients (R^2) pertaining to the linear plots were all above 0.900 indicating good fit. As observed from the table, K_d decreased with increasing pH in the acidic buffers (citrate and acetate), as well as the acidic–neutral buffer (Bis–Tris). For extreme acidic conditions between pH 3.0 and 3.5, the R/R_0 value is small, this adds some uncertainty to the calculated K_d. Nevertheless, it may be gleaned that the K_d is relatively higher at these very low pH. Alternatively, under pH conditions above 7.0, K_d increased with pH. The linear plots of $\ln(K_d)$ versus pH as depicted in Fig. 4 manifest these effects. The values of $\ln(K_d)$ can be used to calculate the corresponding changes in Gibbs free energy (ΔG, J/μmol) for the dissociation interaction using the equation:

$$\Delta G = -RT \ln(K_d) \quad (9)$$

where R is the universal gas constant (J/μmol K), T is the temperature in Kelvin, and K_d is the dissociation constant. The term ΔG is instrumental in delineating the energetically favored binding interactions. As is clear from Fig. 4, binding is most energetically favored under slightly acidic to neutral conditions in the pH range of 5.5–7.0 where ΔG of dissociation is positive. Furthermore, the values of $\ln(K_d)$ converge below $x = 0$ in the vicinity of pH 6.0, while they converge at about $x = 0$ in the vicinity of pH 5.0. Knowing that the isoelectric point (pI) of GBP is ~5.0 this suggests that electrostatic interactions play a significant role in glucose binding [18]. Under conditions where the pH is very close to the pI the K_d values are ≤1.0 μM and consequently the ΔG values are ≥0. From the point of view of sensing, GBP is most sensitive (lowest limit of detection) to glucose at the pH range of 5.5–7.0. As also seen in the figure, the profiles of the slightly acidic to neutral buffers (citrate, acetate and Bis–Tris) intersect with the x-axis near pH 5.0. The values of the relevant intercepts provide estimated values for the pI of the protein as given in Table 1. It has to be noted that the rationale for determining the pI from the intercept does not apply for basic medium (above pH 7) since it is not likely that electrostatic interaction would play a significant role in binding the negatively-charged protein (being well above its pI) to the negatively-charged glucose (having a partially negative ring oxygen).

At pH lower than 5.5 or higher than 7.0, i.e., as the pH veers farther from the pI, the ΔG of binding becomes less favorable. For sensing purposes, it is clear that exposure to the low pH 3.0 completely eliminated the glucose sensing property of Acrylodan–GBP, while at pH 4.5 and above, GBP still functions as a glucose biosensor, albeit the sensitivity decreases slightly at the more basic

Table 1
Dissociation constants for the interaction of GBP with glucose in different buffers at their working pHs. Also compiled in Table are the correlation coefficients (R^2) for the plots in Fig. 4 as well as pI values for GBP as estimated from the plots.

Buffer type	pH	K_d	pI estimated	R^2
Citrate	3.0	1.15 ± 0.12	5.15	0.911
	3.5	4.63 ± 0.50		
	4.0	8.92 ± 0.10		
	4.5	2.06 ± 0.13		
	5.0	0.95 ± 0.10		
	5.5	0.48 ± 0.04		
	6.0	0.42 ± 0.04		
Acetate	4.0	3.21 ± 0.16	4.98	0.914
	4.5	1.10 ± 0.11		
	5.0	0.88 ± 0.09		
	5.5	0.54 ± 0.04		
Bis–Tris	6.0	0.80 ± 0.03	5.44	0.918
	6.5	0.56 ± 0.05		
	7.0	0.50 ± 0.05		
HEPES	7.0	0.73 ± 0.06	NA	0.971
	7.5	1.01 ± 0.09		
	8.0	1.84 ± 0.10		
Tris	7.0	0.45 ± 0.04	NA	0.951
	7.5	0.63 ± 0.05		
	8.0	1.37 ± 0.13		
Phosphate	6.5	0.53 ± 0.08	NA	0.912
	7.0	0.55 ± 0.07		
	7.5	1.09 ± 0.12		
	8.0	1.93 ± 0.02		
Borate	8.5	0.91 ± 0.09	NA	0.947
	9.0	1.17 ± 0.13		
	9.5	3.34 ± 0.04		
	10.0	5.12 ± 0.06		

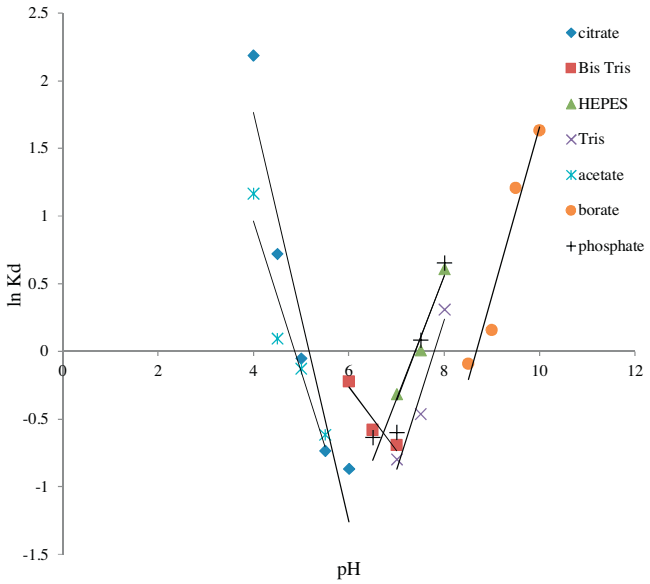

Fig. 4. Plots of lnK_d versus pH for the binding of GBP to glucose in the different employed buffers.

range. Under the highly acidic conditions, the protein appears to be incapable of the expected conformational changes associated with glucose binding. In fact, the fluorescence spectra suggests the Acrylodan is 'trapped' in a hydrophobic pocket distinct from the conformations at pH ≥ 4.5, either for the glucose-free or the glucose-bound.

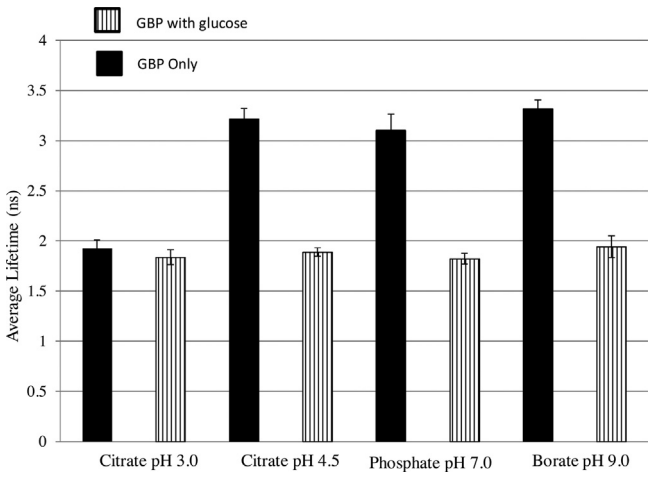

Fig. 6. Frequency domain average lifetime measurements of acrylodan bound GBP in the absence and presence of glucose.

The frequency domain lifetime measurements depicted in Figs. 5 and 6, confirms the minimal response of GBP to glucose at pH 3.0. Biexponential decay fits the frequency domain lifetime measurements at all pH ranges based on nonlinear least squares calculations (Supplemental data 2). For simplicity's sake, the average lifetimes, calculated from Eq. (4), for pH 3.0 with and without glucose are about 2.0 ns, while for pH 4.5–9.0, the lifetimes are 4.3–4.4 ns without glucose and 1.8–3.4 ns with glucose (Fig. 6). From the collected data it can be presumed that at pH 3.0, the GBP (and by consequence, the Acrylodan) assumes a conformation where addition of glucose has no effect, although, we have no way

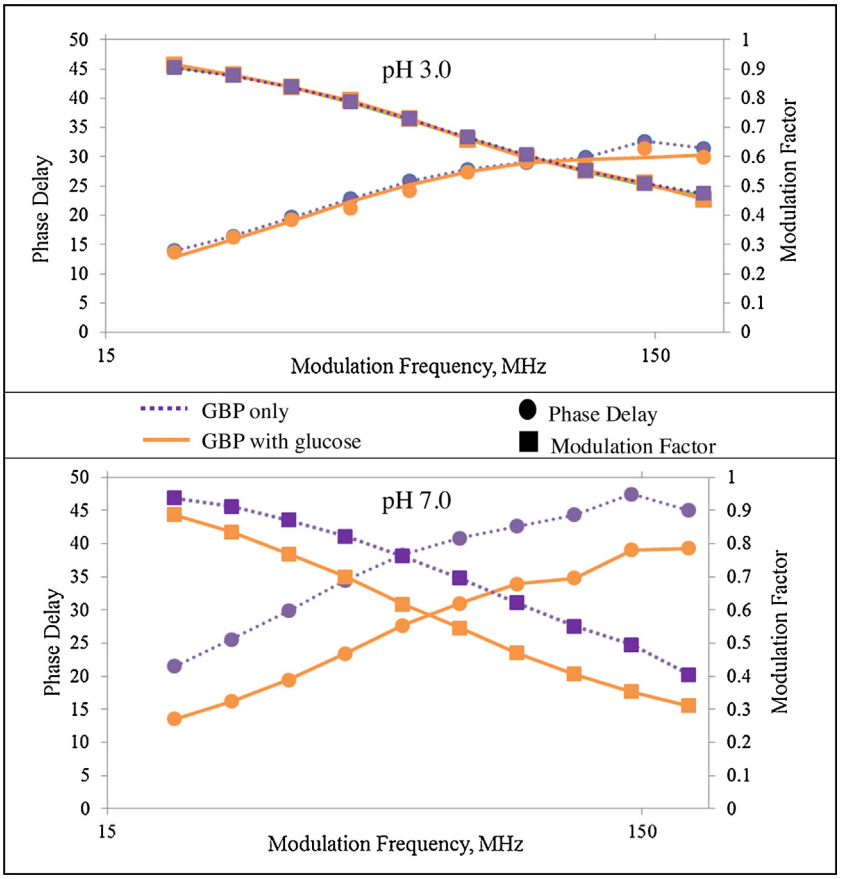

Fig. 5. Frequency domain lifetime measurements of GBP in pH 3 citrate buffer with and without glucose (a), as well as pH7 phosphate buffer with and without glucose (b).

Table 2
Stern–Volmer quenching constant (K_{sv}) of Acr-GBP in the absence and presence of glucose in varying pH.

GBP pH condition	K_{sv} (M^{-1})
pH 3.0	12.35
pH 3.0 + glucose	12.42
pH 4.5	5.24
pH 4.5 + glucose	6.75
pH 7.0	4.77
pH 7.0 + glucose	5.30
pH 9.0	4.00
pH 9.0 + glucose	5.05

of knowing if indeed the glucose fails to attach to the binding site. Interestingly, when both steady-state and time-resolved data are considered, this conformation at pH 3.0 appears to be distinct from that of the glucose-free and glucose-bound conformations at pH 4.5 and higher. A mix of these conformations can be gleaned between pH 3.0 and 4.5 in Fig. 1.

Quenching experiments with KI shows the same K_{sv} at pH 3.0 with and without glucose. At pH 4.5 and higher, the K_{sv} is higher with glucose than without glucose (Table 2). These results are again consistent with the steady state and time- resolved fluorescence results above. The quenching efficiency at pH 3.0 is greater than either the glucose-bound or glucose-free GBP at higher pH. This is to be expected because the negatively-charged I^- readily quenches the Acrylodan embedded in the highly protonated/positively-charged protein. However, no static quenching was observed from the lifetime data (not shown), which indicates the quenching is diffusion-controlled even in these conditions.

4. Conclusion

Previous studies involving electron density maps revealed selective GBP–glucose binding with formation of hydrogen bonds between the side polar groups located in the two globular domains of the protein and both hydroxyl and ring oxygen groups on the glucose [5,6]. As suggested by Borrok et al., in the absence of glucose, the open-form structure of the protein is stabilized by water molecules filling the binding site. In the presence of glucose, hydrogen bonding interactions involving water molecules again play a role in the transition from open to closed states [6]. This concept of the open and closed conformations fit our data at mildly acidic to basic conditions. However, our results indicate that besides the open and closed conformational states, a third one can be induced by highly acidifying the environment of the protein.

The fluorescence data supports this third structure as distinct from the open and the closed structures. This conformational state is incapable of responding to the presence of glucose. Fonin, et al., similarly observed the loss of glucose response at pH 2.8 in the GBP labeled with BADAN in the 152-position [19]. The 152-position is in close proximity to the binding site and is opposite to the 255-position in this paper. A loss of protein structural integrity was concluded by this group because of the red-shift and decrease in intensity of the BADAN fluorescence. In contrast, our fluorescence measurements suggest that there is loss in glucose response but the protein does not undergo a full random unfolding. The blue shift in fluorescence maximum emission supports this. But most notable is the biexponential fit of the decay data. Random unfolding would have resulted in nonexponential decay.

Binding of glucose to GBP was found to be energetically favored at slightly acidic to neutral conditions. That is, pHs close to the isoelectric point of GBP (~5.0). Therefore, a balance between the positive and negative charges on the protein is important for the protein to readily transition from the open to an energetically stabilized closed conformation. On the other hand, as the conditions become more basic, glucose binding becomes less energetically favored but not to a point where it is no longer possible. In fact, once the protein transitions to the closed conformation in the presence of glucose based on our fluorescence data, the structure at the basic pH is almost indistinguishable from the closed conformation at neutral pH. Finally, our findings indicate that slightly acidic to neutral pH range is ideal for use of GBP as a biosensor.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijbiomac.2016.01.077.

References

[1] L. Salins, R. Ware, C. Ensor, S. Daunert, Anal. Biochem. 294 (2001) 19–26.
[2] N.K. Vyas, M.N. Vyas, F.A. Quiocho, Proc. Natl. Acad. Sci. U. S. A. 80 (1983) 1792–1796.
[3] C.L. Careaga, J. Sutherland, J. Sabeti, J.J. Falke, Biochemistry 34 (1995) 3048–3055.
[4] B.H. Shilton, M.M. Flocco, M. Nilsson, S.L. Mowbray, J. Mol. Biol. 264 (1996) 350–363.
[5] D. Cunningham, Julie A. Stenken (Eds.), In Vivo Glucose Sensing, Wiley, New Jersey, 2010, p. 275.
[6] M. Borrok, L. Kiessling, K. Forest, Protein Sci. 16 (6) (2007) 1032–1041.
[7] X. Ge, H. Lam, M. Swati, W. LaCourse, G. Rao, L. Tolosa, J. Diabetes Sci. Technol. 1 (6) (2007) 864–872.
[8] M. Dweik, M. Milanick, S. Grant, Development of a glucose binding protein biosensor, Boston, in: Conference on Smart Biomedical and Physiological Sensor Technology V, vol. 6759, 2007, http://dx.doi.org/10.1117/12.732399.
[9] X. Ge, L. Tolosa, R. Govind, Anal. Chem. 76 (2004) 1403–1410.
[10] X. Ge, L. Tolosa, J. Simpson, G. Rao, Biotech. Bioeng. 84 (2003), 723-721.
[11] X. Ge, G. Rao, L. Tolosa, Biotechnol. Prog. 24 (2008) 691–697.
[12] K. Judge, L. Morrow, A.G. Lastovich, D. Kurisko, S.C. Keith, J. Hartsell, B. Roberts, E. McVey, K. Weidemaier, K. Win, M. Hompesch, Diabetes Technol. Ther. 13 (3) (2011) 309–317.
[13] S. Kanjananimmanont, X. Ge, K. Mupparapu, G. Rao, R. Potts, L. Tolosa, J. Diabetes Sci. Technol. 8 (2) (2014) 291–298.
[14] H. Lambers, S. Piessens, A. Bloem, H. Pronk, P. Finkel, Int. J. Cosmet. Sci. 28 (5) (2006) 359–370.
[15] J.S. Marvin, H.W. Hellinga, J. Am. Chem. Soc. 120 (1998) 7–11.
[16] N. Alizadeh-Pasdar, E. Li-Chan, J. Agric. Food Chem. 48 (2) (2000) 328–334.
[17] J. Lakowicz, Principles of Fluorescence Spectroscopy, 2nd ed., Plenum, New York, 1983, pp. 96, 141–143, 239–243.
[18] M. Stinson, M. Cohen, J. Merrick, J. Bacteriol. 131 (2) (1977) 672–681.
[19] A. Fonin, O. Stepanenko, O. Povarova, C. Volova, E. Philippova, G. Bublikov, I. Kuznetsova, A. Demchenko, K. Turoverov, Peer J. (2014), http://dx.doi.org/10.7717/peerj.275.