Conformational stability and domain coupling in d-glucose/d-galactose-binding protein from *Escherichia coli*

Grzegorz PISZCZEK*1, Sabato D’AURIA†, Maria STAiano†, Mosè ROSSI† and Ann GINSBURG*

*Section on Protein Chemistry, Laboratory of Biochemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892-8012, U.S.A., and Institute of Protein Biochemistry, CNR, Via F. Castellino, 111 80131 Naples, Italy

The monomeric D-glucose/D-galactose-binding protein (GGBP) from *Escherichia coli* (Mr 33 000) is a periplasmic protein that serves as a high-affinity receptor for the active transport and chemotaxis towards both sugars. The effect of D-glucose binding on the thermal unfolding of the GGBP protein at pH 7.0 has been measured by differential scanning calorimetry (DSC), far-UV CD and intrinsic tryptophanyl residue fluorescence (Trp fluorescence). All three techniques reveal reversible, thermal transitions and a midpoint temperature (Tm) increase from 50 to 63 °C produced by 10 mM D-glucose. Both in the absence and presence of D-glucose a single asymmetric endotherm for GGBP is observed in DSC, although each endotherm consists of two transitions about 4 °C apart in Tm values. In the absence of D-glucose, the protein unfolding is best described by two non-ideal transitions, suggesting the presence of unfolding intermediates. In the presence of D-glucose protein, unfolding is more cooperative than in the absence of the ligand, and the experimental data are best fitted to a model that assumes two ideal (two-state) sequential transitions. Thus D-glucose binding changes the character of the GGBP protein folding/unfolding by linking the two domains such that protein unfolding becomes a cooperative, two two-state process. A Kd value of 5.6 × 10⁷ M⁻¹ at 63 °C for D-glucose binding is estimated from DSC results. The domain with the lower stability in DSC measurements has been identified as the C-terminal domain of GGBP from thermally induced Trp fluorescence changes.

Key words: circular dichroism (CD), differential scanning calorimetry (DSC), D-glucose/D-galactose-binding protein, intrinsic tryptophanyl residue fluorescence, ligand binding, thermal unfolding.

INTRODUCTION

The understanding of non-covalent interactions that determine specificity and binding is a fundamental aspect of the relationships between structure and function of proteins. Proteins that bind to carbohydrates are present in all living organisms and are involved in a great variety of biological functions. There is an increasing interest in protein–carbohydrate interactions, owing to their essential role in biological recognition and adhesion processes [1,2].

The periplasm of Gram-negative bacteria contains a large family of specific binding proteins that are essential primary receptors in transport, and in some cases, chemotaxis [3,4]. These proteins usually have a monomeric structure that folds in two main domains linked by three strands commonly referred to as the ‘hinge region’. Conformational changes involving the hinge are thought to be necessary for sugars to enter and exit the binding site [5,6]. Differences in the structures of the ligand-bound and ligand-free proteins are essential for their proper recognition by the membrane components [7]. This property of binding proteins makes them good candidates for biological-recognition elements in the development of biosensors [8]. In fact, in the presence of a specific ligand, these proteins undergo a large conformational change to accommodate the ligand inside the binding site [9]. On the basis of this conformational change, sensing systems for maltose [10] and D-glucose [8,11–13] have been developed by using their respective binding proteins.

The thermodynamic parameters associated with the thermal denaturation of, and ligand binding to, the L-arabinose binding protein from *Escherichia coli* have been reported by Fukada et al. [14]. However, at the high ABP (L-arabinose-binding protein) concentrations required in these studies, intermolecular interactions and some irreversible aggregation precluded identification of two unfolding domains.

The D-glucose/D-galactose-binding protein (GGBP) of *E. coli* serves as an initial component for both chemotaxis towards D-galactose and D-glucose and high-affinity active transport of the two sugars. Refined X-ray structures that have been determined by Vyas et al. [15] for GGBP in the absence and in the presence of D-glucose provide a view of the sugar-binding site at the molecular level. The sugar-binding site is located in the cleft between the two lobes of the bilobate protein. Binding specificity and affinity are conferred primarily by polar planar side-chain residues that form an intricate network of co-operative and bidentate hydrogen bonds with the sugar substrate and, secondarily, by aromatic residues that sandwich the pyranose ring. A Kd value of 0.21 μM for D-glucose dissociation from GGBP has been reported by Zukan et al. [16] at pH 8.0 and 4 °C.

Frequent monitoring of the blood D-glucose level can prevent many long-term complications associated with diabetes, a medical condition affecting 16 million people in the United States alone. New non-invasive methods for real time D-glucose-level monitoring include using interstitial fluids [17] and tears [18]. The GGBP protein has a high affinity for D-glucose, and therefore is suitable to measure the low D-glucose concentrations known to be present in those fluids [17,18]. Biosensors for convenient optical measurements of D-glucose concentration are obtained by genetically engineering the protein. GGBP is mutated to introduce a cysteine residue on one of the domains for labelling with a fluorescent probe for intensity [11,13] or lifetime-based...
measurements [8]. Alternatively fluorescent probes are introduced on both domains for fluorescence resonance energy transfer (‘FRET’) measurements [12].

The present study examines the thermal unfolding/folding of GGBP in the absence or presence of D-glucose. The preferential binding of D-glucose to the native folded protein is investigated by DSC (differential scanning calorimetry), far-UV CD and intrinsic Trp fluorescence (intrinsic tryptophan residue fluorescence). All three techniques reveal a substantial transition-temperature increase produced by D-glucose binding. DSC data show two unfolding transitions and a change in the character of the folding/unfolding process in the absence and presence of D-glucose. Moreover, intrinsic Trp fluorescence changes may be ascribed to the C-terminal unfolding, allowing for an identification of thermal transitions. On the basis of these observations, a mechanism of thermal unfolding/folding of GGBP in the absence or presence of D-glucose is postulated.

MATERIALS AND METHODS

Preparation of GGBP solutions

GGBP from E. coli was purified as described by Tolosa et al. [8]. GGBP contains a single structural site for Ca\(^{2+}\), and it was confirmed by atomic-absorption spectroscopy that the purified protein contained one equivalent of Ca\(^{2+}\)/mol of GGBP. Concentrated stock solutions of purified GGBP were dialysed against 10 mM Hepes/NaOH, pH 7.0. The dialysed samples were diluted to an appropriate concentration based upon sensitivity requirements of the technique to be used. All samples were centrifuged and degassed before measurements. The protein concentration was determined by UV absorption using a molar absorption coefficient (ε\(\text{\text{280}}}\) of 37 410 M\(^{-1}\)·cm\(^{-1}\) (M, 33 000), calculated from the protein amino acid composition by the method of Gill and von Hippel [19]. Absorption spectra were measured at 20 °C using a model 8453 Hewlett-Packard spectrophotometer.

CD

CD measurements were performed with a Jasco (Jasco Inc., Easton, MD, U.S.A.) model J-710 spectropolarimeter equipped with a computer controlled Neslab (now Thermo NELLAB, Inc., Newington NH, U.S.A.) model RTE-111 water bath. CD spectra were corrected for the solvent CD signal and normalized for protein concentration using a value of 107.9 for the mean residue molecular mass. The thermal stability of GGBP secondary structure was monitored by heating samples containing 0.17 mg/ml of protein at a rate of 30 °C/h over the range 15–85 °C in a water-jacketed cylindrical cell with a 0.05 cm light path. The mean residue molar ellipticity at 222 nm was recorded at 0.2 °C intervals using a time constant of 8 s. CD data were analysed for two-state transitions using the EXAM thermodynamic analysis program for a two-state model of unfolding [20].

DSC

DSC measurements were performed using a VP-DSC calorimeter [21] from MicroCal, LLC (Northampton, MA, U.S.A.). The VP-DSC instrument was run without feedback with at least 60 min equilibration times prior to, and between, the 30, 60 or 90 °C/h scans. Samples, at concentrations over the range 0.17–0.20 mg/ml, were scanned three times from 15 to 75–85 °C with rapid cooling between scans (compare dotted lines in Figure 1). In all cases thermal unfolding was reversible, with recovery above 90 % for all samples [22]. No visible light-scattering was observed for protein solutions after DSC experiments. DSC data were corrected for instrument baselines (determined by running the dialysis buffer in both reference and sample cells just prior to placing protein in the sample cell) and normalized for scan rate and protein concentration. The excess heat capacity (C\(_p\)) was expressed in kcal·K\(^{-1}\)·mol\(^{-1}\), where 1.000 cal = 4.184 J. Data conversion and analysis were performed with Origin software (OriginLab Corporation, Northampton, MA, U.S.A.) and the EXAM program [20].

Fluorescence

Fluorescence measurements were conducted using an Aminco–Bowman Series 2 spectrofluorimeter. For changes in Trp fluorescence with temperature, the excitation wavelength was 295 nm and emission was measured at the peak wavelength at 340 nm with polarizers under magic-angle conditions. Temperature was controlled by programmable Neslab RTE-111 water bath using water-jacketed fluorescence cuvettes (1 ml, 1 cm pathlength). The sample temperature was monitored by inserting a Teflon®-coated microthermocouple (Omega Inc., Stamford, CT, U.S.A.) into the cell. Progress curves for tryptophan exposure were analysed by the two-state thermodynamic analysis program EXAM [20].

RESULTS AND DISCUSSION

Thermal unfolding of GGBP monitored by DSC

Repetitive DSC profiles for GGBP in the absence or presence of 10 mM D-glucose are shown in Figure 1. DSC scans for a 90 °C/h scan rate give single endotherms, centred at 53 °C and 68 °C respectively in the absence or presence of D-glucose. This result indicates that D-glucose binding causes a large transition-temperature increase. Note that repetitive scans after cooling to
15°C and equilibrating for 60 min give approx. 90% reproducibility under both conditions. The same DSC scans performed at 30 and 60°C/h scan rates also give single unfolding endotherms, with comparable T_m increases produced by D-glucose binding.

DSC data for GGBP were first analysed with the EXAM program, applying an ideal (two-state) unfolding model with a heat-capacity change (ΔC_p) and a variable N value (in mol) for the number of two-state unfolding transitions. The average ΔC_p value for all scans in the absence of D-glucose was 1.7 ± 0.2 kcal·K⁻¹·mol⁻¹, and, in the presence of 10 mM D-glucose, the average ΔC_p was 1.3 ± 0.2 kcal·K⁻¹·mol⁻¹.

For all conditions studied, the number of mol of co-operative units per mol of the protein, obtained by fitting to the two-state model, is close to 2. The same values are obtained when the co-operativity ratio (CR) is calculated:

\[
CR = \frac{\Delta H_{cal}}{\Delta H_{th}}
\]

These results suggest that the domains of the protein unfold as two independent co-operative units with similar thermal stabilities. Assuming this model of unfolding, the broken lines in Figure 1 are constructed from deconvolution of DSC data, taking into account ΔC_p values obtained from the EXAM program.

In order to deconvolute DSC profiles, sigmoidal protein baseline was subtracted from data collected at 30°C/h scan rate, normalized for protein concentration, giving a ΔC_p value of 0 for the baseline shown. Experimental data are shown as open circles, and the continuous line is for a non-two-state unfolding model with two transitions. Upper panel: deviations of experimental data points from the best fits to a two-state model (open symbols) either deconvoluting into two independent (○) or two sequential (□) transitions. Filled squares (■) represent deviations from a non-two-state model with two transitions shown as a continuous line in the lower panel.

Using the MicroCal LLC Origin program [23]. As expected from the results obtained with the EXAM program, analysis with an assumption of a single transition in each case yields a poor fit to the experimental data (Figure 3, upper panel; see below). All data were subsequently analysed with an assumption of two transitions, applying three different unfolding models, namely two independent ideal two-state transitions, two sequential ideal two state transitions and two non-ideal (non-two-state) transitions.

In the absence of D-glucose, experimental data are best fitted to a model for two independent non-ideal transitions (Figure 2, upper panel). Comparison of the data analysis by both the ideal and non-ideal models, and an improvement of the fit for the latter, can be seen in the upper panel of Figure 2, showing residuals obtained for all three analyses. Similar improvements in the fits to a non-ideal model with two transitions were obtained for data acquired for GGBP in the absence of D-glucose at different scan rates. The fact that the unfolding of GGBP in the absence of a ligand is a non two-state process indicates the presence of unfolding intermediates.

Unlike the protein-denaturation transitions in the absence of a ligand, DSC data for GGBP obtained in the presence of 10 mM D-glucose are best fitted to a model for two sequential two-state transitions. In this case, good fits are obtained for two-state models, but there is a significant improvement in the fit when the equation for two two-state sequential transitions is applied instead of that for two two-state independent transitions. A comparison of the fit residuals obtained from analysis with two sequential and two independent transitions (in both cases applying the
Table 1 Thermodynamic parameters for thermal unfolding of GGBP in the absence of D-glucose

Thermodynamic parameters were obtained from deconvolution of DSC data, assuming two transitions and a non-two-state model of unfolding. The standard errors in \( T_m \) are \( \pm 0.2 \) °C and \( \pm 10 \) % in \( \Delta H \) values.

<table>
<thead>
<tr>
<th>Scan rate (°C/h)</th>
<th>( T_m^1 ) (°C)</th>
<th>( \Delta H_m^1 ) (kcal/mol)</th>
<th>( T_m^2 ) (°C)</th>
<th>( \Delta H_m^2 ) (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>49.0</td>
<td>94</td>
<td>53.3</td>
<td>82</td>
</tr>
<tr>
<td>60</td>
<td>51.0</td>
<td>103</td>
<td>55.1</td>
<td>86</td>
</tr>
<tr>
<td>90</td>
<td>51.7</td>
<td>95</td>
<td>55.9</td>
<td>88</td>
</tr>
</tbody>
</table>

* Average values of \( \Delta H \) for individual domains, where \( \Delta H = (\Delta H_m^1 \cdot \Delta H_m^2)^{1/2} \) [23].

Table 2 Thermodynamic parameters for thermal unfolding of GGBP in the presence of 10 mM D-glucose

Thermodynamic parameters were obtained from deconvolution of DSC data, assuming a two-two-state model of unfolding, with two sequential transitions. The standard errors in \( T_m \) are \( \pm 0.2 \) °C and \( \pm 10 \) % in \( \Delta H \) values.

<table>
<thead>
<tr>
<th>Scan rate (°C/h)</th>
<th>( T_m^1 ) (°C)</th>
<th>( \Delta H_m^1 ) (kcal/mol)</th>
<th>( T_m^2 ) (°C)</th>
<th>( \Delta H_m^2 ) (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>62.1</td>
<td>100</td>
<td>65.4</td>
<td>125</td>
</tr>
<tr>
<td>60</td>
<td>64.6</td>
<td>104</td>
<td>68.5</td>
<td>129</td>
</tr>
<tr>
<td>90</td>
<td>69.2</td>
<td>99</td>
<td>69.7</td>
<td>124</td>
</tr>
</tbody>
</table>

two-two-state model of unfolding) is shown on the upper panel of Figure 3. The corresponding reduced \( \chi^2 \) value decrease for sequential versus independent transitions is in the range of 30–50 % for data sets obtained in this study. This result indicates that the thermal unfolding of GGBP in the presence of D-glucose occurs not only with the presence of unfolding intermediates, but also that D-glucose binding imposes an order in which the domains unfold; that is, the stability of one domain is dependent on whether the other domain is folded or unfolded.

The full widths at the half-maximum of GGBP endotherms are approx. 9.5 and 7.5 °C in the absence and presence of 10 mM D-glucose respectively. Constriction of endotherms in the presence of ligand indicates increased co-operativity of the unfolding/folding transition under these conditions. When D-glucose binds to the GGBP, the unfolding of the two domains becomes tightly coupled with transition temperature increases of \( \approx 13 \) °C. The protein remains folded until thermal unfolding of one of the two domains causes ligand dissociation, and unfolding of the second domain follows.

Thermodynamic parameters obtained from the analysis of the GGBP thermal unfolding data in the absence of D-glucose are presented in Table 1. Parameters were acquired by applying a non-ideal model of unfolding for data sets collected at three different scan rates: 30, 60 and 90 °C/h. Table 2 contains corresponding parameters obtained for the GGBP unfolding in the presence of 10 mM D-glucose, using an ideal unfolding model with two sequential transitions. Apparent \( T_m \) values increase with increasing scan rate. This indicates that GGBP unfolding/folding transitions are not at equilibrium during fast scan rates [24]. The \( T_m \) changes with the scan rate are linear with equal slopes, which allows an extrapolation to a 0 °C/h scan rate [25,26]. Extrapolated transitions temperatures for the two GGBP protein domains are 46.9 and 51.2 °C in the absence of D-glucose, and 61.2 and 64.3 °C in presence of 10 mM D-glucose respectively.

Taking into account \( \Delta C_p \), the two unfolding units are approximately the same size with an average heat of unfolding 6.8 cal/g at 63 °C, which is about the same value as that measured for other small globular proteins that undergo two-state unfolding (see the review by Privalov [27]). More recently, Robertson and Murphy [28] have used the expanded database to explore the relationship between the protein stability and structure. These authors suggest some reasons why decomposing the energetics of protein stability in terms of changes in solvent-exposed surface area upon unfolding might be inadequate for accurately predicting thermodynamic parameters.

The fact that transition temperatures of both domains increase to approximately the same extent in the presence of D-glucose suggests that the bound carbohydrate interacts with both domains. This effect occurs through the free energy of binding ligand preferentially to the folded protein [29]. In this case, the affinity constant for the ligand in a two-state unfolding process is related to the increase in \( T_m \) in the presence of ligand. Assuming that the folded GGBP protein has a single site for D-glucose and that D-glucose does not bind to the unfolded protein, eqn (17) of Brandts et al. [30,31] can be applied to calculate the ligand binding constant:

\[
\ln (1 + [L]/K) = (\Delta H_m/R) \cdot (1/T_0 - 1/T) - \Delta C_p/R \ln [T_0/T] + T/\Delta H_m - 1
\]

where \( T_0 \) and \( T \) are endotherm maxima (in K) in the absence and presence of ligand respectively, \([L]\) is the free concentration of ligand, \( R \) is the gas constant, \( \Delta C_p \) is the heat-capacity change for unfolding (assumed to be temperature-independent) and \( \Delta H_m \) is the enthalpy change for unfolding (eqn (1) and yielded a \( K_a \) value of 5.6 × 10⁶ M⁻¹ at 63 °C for D-glucose binding to the GGBP protein.

Thermally induced Trp fluorescence changes

Four of the five GGBP tryptophan residues are located in the C-terminal domain of the protein. The fifth tryptophan residue, at position 284, is located in a C-terminal loop headed toward the N-terminal domain [15]. Measurements of changes in the Trp fluorescence as a function of increasing temperature should therefore predominantly reflect conformational changes in the C-terminal domain of the protein. Thermally induced changes in the tryptophan-residue exposure for GGBP in the absence or presence of 10 mM D-glucose are presented at Figure 4. Progress curves of intrinsic Trp fluorescence as a function of temperature are shown as open symbols, and continuous lines represent the fit of each data set to a two-state model. The experimental data obtained in the presence of D-glucose are fitted well to a two-state unfolding model. A small deviation of experimental data points in the region near to the midpoint of the transition in the absence of D-glucose can be attributed to the presence of unfolding intermediates. The analysis for two-state unfolding reactions yield \( T_m \) values of 48.6 and 61.3 °C, and \( \Delta H \) values of 105 and 102 kcal·mol⁻¹ respectively in the absence and presence of 10 mM D-glucose. These values are in good agreement with \( T_m \) and \( \Delta H \) values obtained from the deconvolution of the DSC data under the same conditions with the same 30 °C/h scan rate (compare Table 1 with Table 2). Similar correlation can be observed in Figure 6, where the main peak of the first derivative of Trp fluorescence intensity (bottom panel) has the same position as the peak at the lower temperature of two transitions obtained by deconvolution of the
D-Glucose/D-galactose-binding protein: domain coupling

Figure 4 Thermally induced changes in tryptophan residue exposure

Open circles represent normalized Trp fluorescence changes of GGBP upon heating at a scan rate of 30 °C/h in the absence or presence of 10 mM D-glucose. The excitation and observation wavelengths were 295 and 342 nm respectively. The fit of each data set to a two-state model of unfolding is shown as a solid line with Tm values of 48.6 and 61.3 °C in the absence or presence of D-glucose respectively.

DSC data (top panel). This indicates that the unfolding of the C-terminal domain of GGBP occurs with a lower Tm value than that of the N-terminal domain.

CD measurements of GGBP thermal unfolding

Far-UV CD spectra of the GGBP at 15 °C in the absence or presence of 10 mM D-glucose are essentially superimposable, indicating that the effect of ligand binding on the overall secondary structure of the protein is negligible (Figure 5, upper panel). An estimate of 40% α-helical content in GGBP has been calculated from the far-UV CD spectra using the algorithm of Perczel et al. [32]. This estimate of α-helices in GGBP is in very good agreement with the structural data [15].

Temperature-induced CD changes of GGBP monitored at 222 nm in the absence or presence of 10 mM D-glucose reveal a Tm increase produced by ligand binding (Figure 5, lower panel). CD progress curves, analysed by the two-state unfolding model, yield Tm values of 50.8 and 64.5 °C and ΔH values of 86 and 99 kcal·mol⁻¹ respectively in the absence or presence of 10 mM D-glucose. These Tm values approximately correlate with the positions of the endotherm maxima obtained in the DSC experiment performed under the same conditions and at the same 30 °C/h scan rate. The correlation in the position of the midpoints, and in the width of the transition, can be seen in Figure 6, which shows comparisons of a DSC endotherm (upper panel) with the first derivative of a CD progress curve (middle panel). It is noteworthy that thermally induced secondary-structural changes do not reveal two distinct unfolding domains as do DSC measurements. This could be because ellipticity changes at 222 nm primarily reflect variations in helical content, whereas DSC measurements detect changes in tertiary structures and exposure of the protein hydrophobic residues upon unfolding.

Post-transitional baselines of CD progress curves obtained for GGBP in the absence or presence of 10 mM D-glucose overlap, confirming that, in both cases, the protein unfolds to the same extent. However, the CD pretransitional baseline measured in the absence of D-glucose is steeper than that obtained with 10 mM D-glucose. This indicates the presence of thermal fluctuations in the native conformation of GGBP [33] that are consistent with the non-ideal unfolding observed in DSC experiments. The pretransitional baseline for thermally induced intrinsic Trp fluorescence changes is also steeper in the absence than in the presence of 10 mM D-glucose (Figure 4).

Conclusions

When D-glucose binds to the ligand-binding site located in the cleft between the two GGBP domains of the native folded protein, the relative positions of domains change, which involves some of the amino acids in a network of hydrogen bonds [34]. These interactions result in a large Tm increase of ≈13 °C that can be observed by calorimetric (DSC) and optical (CD and Trp fluorescence) methods.

Heat-capacity change is proportional to the change in polar and apolar solvent accessible surface areas associated with unfolding (see Murphy and Freire [35]). For GGBP unfolding, ΔCp is 1.7 and 1.3 ± 0.2 kcal·K⁻¹·mol⁻¹ in the absence and presence of

© 2004 Biochemical Society
Figure 6 Thermal unfolding of GGBP in the absence of α-glucose

Upper panel: normalized DSC scan (after pre- and post-transitional baseline subtraction) obtained in the range from 20 to 75 °C. The fit of the experimental data points (open circles) to a non-two-state model assuming two unfolding transitions is shown by the broken line. Individual deconvoluted components of this fit are shown by the continuous lines. Vertical dotted lines are drawn at the $T_m$ value of each component and the continuous line at the midpoint of the DSC endotherm. Middle panel: first derivative of CD data from Figure 5. Bottom panel: first derivative of Trp fluorescence (TrpFl) data from Figure 4. CD and fluorescence data were smoothed by adjacent averaging over the range of 1.5 °C prior to differentiation. All data collected at a 30°C/Ch scan rate.

D-glucose respectively. For comparison, $\Delta C_p$ values for ABP unfolding in the absence and presence of L-arabinose have been reported to be 3.16 and 2.74 ± 0.07 kcal·K$^{-1}$·mol$^{-1}$ [14]. The fact that the $\Delta C_p$ values for unfolding the ABP protein are approximately twice those for GGBP unfolding in the absence and presence of ligand suggests that more apolar surfaces are exposed in ABP than in GGBP, since the hydration of apolar groups has a positive contribution to $\Delta C_p$ values.

An affinity constant of $5.6 \times 10^8$ M$^{-1}$ at 63 °C for D-glucose binding to the GGBP protein has been estimated from DSC data. This value is in good agreement with that previously reported from equilibrium dialysis measurements at pH 8.0 and 4 °C [16]. This indicates that there is little temperature-dependence of D-glucose binding to GGBP.

Knowledge of the details of structural properties as well as the conformational stability of the protein is needed when developing biotechnological applications. Stability of the protein has been found in the present study to be controlled by C-terminal domain unfolding. Mutations in the C-terminal domain may affect the stability of the whole protein and biosensor developers employing such a strategy should be aware of this possibility.

This project was realized in the frame of CRdC-ATIBB POR UE (Centro Regionale di Competenza–Applicazioni Tecnologico-Industriali di Biomolecole e Biosistemi–Programmi Operativi Regionali dell’Unione Europea–Campania Misura 3.16 activities (grants to S.D.A. and M.R.) This work was also supported by an F.I.R.B. (Fondo per gli investimenti in ricerca di base) grant (to S.D.A. and M.R.).

REFERENCES

D-Glucose/D-galactose-binding protein: domain coupling


Received 11 February 2004/18 March 2004; accepted 22 March 2004
Published as BJ Immediate Publication 22 March 2004, DOI 10.1042/BJ20040232