

## The Structural Changes of D-Galactose/D-Glucose-Binding Protein Induced by Calcium Depletion

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The question of how a protein folds into its unique, compact, highly ordered and functionally active form is one of the most intriguing and perplexing questions of structural and cellular biology. Special attention deserves the role of ligands. The convenient subjects of inquiry to elucidate this problem are the periplasmic ligand binding proteins. This work is focused on investigation of D-galactose/D-glucose-binding protein (GGBP) from *E. coli* belonging to this protein family. GGBP molecule can bind not only one molecule of sugar, but has one calcium localized in the loop of the C-terminal domain and coordinated by oxygen atoms from every second residue of this loop and from Glu 205. The aim of this work is to examine the mutual influence of calcium ion and sugar molecule on GGBP structure. Our investigations have been carried out by intrinsic fluorescence of proteins and far-UV CD.

Kinetics of structural changes of GGBP and its complex with D-glucose (GGBP/Glc) induced by EDTA has been studied. Calcium depletion is shown to result in GGBP structural changes recorded by the increase of intrinsic fluorescence intensity up to 35 % in the first 2 min after EDTA addition to protein solutions. Fluorescence intensity of GGBP achieves its equilibrium value in 4–7 min after mixing of protein and EDTA solutions of different concentrations. Calcium removing finally leads to approximately 20 % increase in fluorescence intensity of GGBP and practically does not affect fluorescence intensity of GGBP/Glc complex. These data apparently indicate that 3D structures of GGBP/Glc with and without calcium are the same which allows us to make conclusion about stabilizing action of D-glucose on the protein structure. Far UV CD experiments reveal that GGBP and GGBP/Glc complex with calcium and calcium free possess the same secondary structure. The denaturation kinetics of GGBP and GGBP/Glc calcium free (GGBP-Ca and GGBP-Ca/Glc, respectively) induced by guanidine hydrochloride (GdnHCl) have been measured by monitoring tryptophan fluorescence intensity and parameter  $A$  ( $A=I_{320}/I_{365}$  characterizing position and form of fluorescence spectra). It is shown that the rate of denaturation of GGBP with calcium is less than that of GGBP-Ca. Also, denaturation of GGBP-Ca/Glc takes less time compared to that of GGBP/Glc. The value of parameter  $A$  recorded after proteins incubation in the presence of 1.0 M GdnHCl during 10 minutes illustrates that according to their resistance to GdnHCl denaturing action, proteins are distributed as follows: GGBP-Ca < GGBP < GGBP-Ca/Glc < GGBP/Glc.

The results of our study prove the stabilizing role of calcium in the structure of GGBP obtained earlier by temperature denaturation [1]. Calcium depletion results in 3D structure changes of GGBP. At the same time, the presence of bounded sugar makes GGBP-Ca/Glc more resistant to structure reorganization induced by calcium removal. Kinetic experiments have revealed that GGBP-Ca is less stable than GGBP while D-glucose binding increases GGBP-Ca/Glc resistance against denaturant.

[1] P. Herman, J. Vecer, I. Barvik Jr., V. Scognamiglio, M. Staiano, M. de Champdore, A. Varriale, M. Rossi, S. D'Auria. *Proteins*. 61 (2005) 184-195.

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