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Conformational changes induced by Mg^{2+} on the multiple forms of glutamine synthetase from *Bacillus brevis* Bb G1

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ABSTRACT

Conformational changes play an important role in the function of proteins. Glutamine synthetase, an important enzyme of nitrogen metabolism, was purified under sporulating (GSala) and non-sporulating (GSpyr) conditions and the effect of Mg^{2+} on these multiple forms was studied by fluorescence spectroscopy to detect possible conformational changes that occur in the presenceof Mg^{2+}. The substantial changes in the fluorescence emission maximum, fluorescence intensity and lifetime that occur in the presence of different concentrations of Mg^{2+}, indicated major changes in molecular conformations in both forms of this enzyme. The fluorescent changes produced by the effect of Mg^{2+} in GSala was much more prominent than in GSpyr. These observations strongly support the possibility that GSala and GSpyr undergoes a conformational change on binding with Mg^{2+}.

Key words: glutamine synthetase, fluorescence spectroscopy, conformational changes

Introduction

One of the major characteristics of a living system is its ability to respond to stimuli, which can be given due to changes either in environment or chemical milieu within the living system. These responses by the living system are mostly brought about change in the conformation of macromolecules. Interaction of proteins with substrate or other small molecule is structurally complementary and stereo-specific. It often leads to change in the conformation of protein, which has biological implication in catalysis and regulation. During the formation of an enzyme-substrate complex, the conformations of protein and substrate fit each other. The substrate causes changes in the geometry of the enzyme as it fits into the active site. A delicate orientation of catalytic groups is required for enzyme action. The substrate induces this proper orientation by the change in the geometry of the enzyme. Many physicochemical techniques such as optical rotatory dispersion, circular dichroism, electron paramagnetic resonance and nuclear magnetic resonance have been applied to determine the conformational changes in biological systems (Eads & Villafranca, 1987; Varlan & Hillerbrand, 2010a, 2010b). Another technique for studying changes in protein conformation is fluorescence. It gives information on the binding of small molecules, such as substrates, coenzymes and inhibitors. Since fluorescence measurements appear to be more sensitive to molecular environment than many other physical methods, they may even indicate small structural transitions of proteins. The parameters of fluorescence spectroscopy such as fluorescence spectrum, quantum yield and life time are dependent on the molecular structure of the fluorophore. They are, however, also sensitive to the environment in or around the macromolecule carrying the fluorophore. It is this sensitivity of the emission parameters on the environment of the fluorophore, which makes fluorescence spectroscopy a useful tool in the study of conformation and dynamics. A shift in emission maximum, life time or quantum yield gives ample evidence to indicate conformational changes in the protein molecule (Giovanni et al., 2008; Strunk et al., 2008; Shinitzky, 2011; Abraham, 2013). A change in fluorescence intensity indicated by ligand binding to a protein can also result from local conformational changes that alter the interactions of tryptophan residues with their neighboring

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groups (Houston et al., 2003; Varlan & Hillerbrand, 2010a, 2010b).

Glutamine synthetase, an important enzyme of nitrogen metabolism has been suspected to play a regulatory role. Mn^{2+}, Mg^{2+}, Ca^{2+}, Co^{2+} and Zn^{2+} are the divalent cations, which induce specific structural changes in glutamine synthetase. The activation of glutamine synthetase in the biosynthetic reaction by Mn^{2+}, Mg^{2+} or Co^{2+} is intimately linked to the adenylylation state of the enzyme and in this respect these metal ions also could have a regulatory function. The organism under study *Bacillus brevis* Bb G1 is a gram positive, aerobic, spore-forming and rod shaped bacterium. Cells do not form chains. It showed a typical behavior of growth and sporulation. It fails to sporulate only if a single change of carbon source is made from an amino acid (alanine) to its corresponding keto acid (pyruvic acid). In the present investigation, glutamine synthetase was purified under sporulating and non-sporulating conditions and the effect of Mg^{2+} on this multiple forms was studied by fluorescence spectroscopy to detect possible conformational changes that occur in the presence of Mg^{2+}.

Materials and Methods

Most of the chemicals used in this study were purchased from Sigma Chemical Co., USA. All other chemicals used were of analytical grade. Chromatographic columns were procured from Bio Rad Laboratories, California, USA. *Bacillus brevis* Bb G1 was grown in alanine / pyruvate minimal medium. Glutamine synthetase was purified by affinity chromatography using Cibacron Blue as affinity ligand attached to Sepharose 4B. Purification of glutamine synthetase was also done by DE-52 ion exchange chromatography (Tiwari et al., 1989). The protein concentration for the purified enzyme, GSala was 0.089 mg/ml, while the protein concentration for GSpyr was 0.050 mg/ml in 20 mM MES buffer, containing 1 mM $MnCl_2$ at pH 7.0.

The fluorescence emission spectra were recorded by JY3CS spectrofluorometer at room temperature by exciting the sample with a particular excitation wavelength selected by excitation monochromator and recording the intensity distribution of emitted light with wavelengths by scanning with emission monochromator and other accessories for detection and processing of spectrum.

The decay time measurements were made with the help of an Edinburgh model 199 fluorescence time domain spectrofluorometer under single photon counting conditions and data analysis was done with a PDP 11/2 microcomputer by reconvolution method using a least-squares fitting program. The time correlated single photon counting (SPC) perhaps offers the highest sensitivity and accuracy for measuring fast fluorescence decay profiles. The reliability of the instrument was checked by using fluorescence standards, viz, .anthracene in cyclohexane and rose Bengal in ethanol.

To study the effect of Mg^{2+}on GSala/GSpyr, different concentrations of Mg^{2+} were prepared. By adding suitable amounts of Mg^{2+} in 1.5 ml of GSala/GSpyr, the final concentrations of Mg^{2+} were kept as 10, 100 and 150 mM, respectively in the sample. The volume of the sample was always kept 3 ml by the addition of distilled water whenever required. These samples were kept for twenty minutes, then the emission spectra were taken and life times were measured.

Results and Discussion

The fluorescence spectra and the exponential decay curves of GSala, GSala with different concentrations of Mg^{2+}, GSpyr and GSpyr with different concentrations of Mg^{2+} are shown in Figure 1, Figure 2, Figure 3 and Figure 4, respectively.

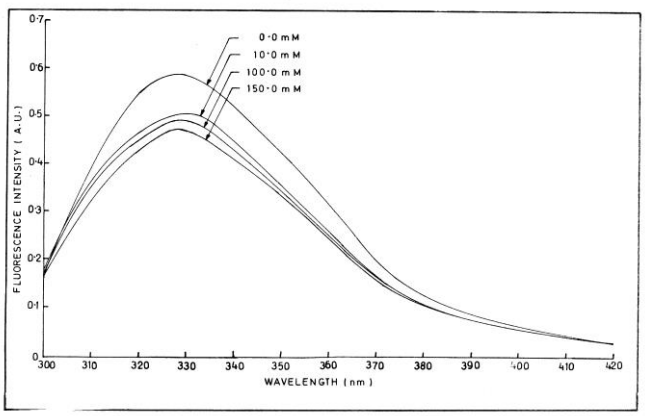

Figure 1. *The fluorescence spectra of GSala with various concentrations of Mg^{2+} at room temperature with excitation wavelength at 284 nm. The protein concentration was 0.089 mg/ml in 20 mM MES buffer, containing 1mM $MnCl_2$ at pH 7.0.*

The fluorescence spectra of proteins containing tryptophan had only one maximum of fluorescence which was characteristic of tryptophan.

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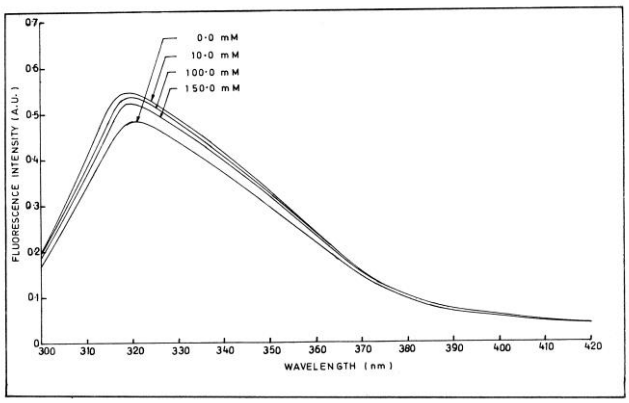

Figure 2. *The fluorescence spectra of GSpyr with various concentrations of Mg^{2+} at room temperature with excitation wavelength at 284 nm. The protein concentration was 0.050 mg/ml in 20 mM MES buffer, containing 1 mM $MnCl_2$ at pH 7.0.*

The shorter shift in the spectrum of fluorescence compared to the free tryptophan indicated that the majority of the fluorescent tryptophan residues in the enzyme are buried inside the protein in a nonpolar hydrophobic microenvironment, supported by the studies done by several workers (Zhang et al., 2007; Chilom et al., 2011; Ge et al., 2011; Ma & Wang, 2011). The two life times indicated that the enzyme contained at least two tryptophan residues that fluoresced in two different environments. This result is consistent with the studies in which it was shown that the life times of tryptophan fluorescence are rather short (2-5 ns) and often multi exponential (Kelkar et al., 2010; Risso et al., 2010; Sarkar et al., 2011). The significant changes between the two life times indicated that one of the tryptophan residues in the enzyme may be relatively exposed whereas the other tryptophan residue appears to be deeply buried inside the enzyme (Ghiron et al., 1988). For GSala, at lower concentrations of Mg^{2+} (10 mM) the emission maximum was shifted to longer wavelength with a decrease in fluorescence intensity, while at higher concentrations of Mg^{2+} (100 and 150 mM) the emission maximum was shifted to shorter wavelengths with a decrease in fluorescence intensity compared to the emission maximum and fluorescence intensity of GSala. The 2 nm red shift would indicate the movement of tryptophan to polar environment, subsequent blue shift would indicate movement towards interior. The life time T1 and fluorescence intensities decreased for all the three concentrations of Mg^{2+}, whereas life time T2 increased for 100 mM Mg^{2+}, decreased for 150 mM Mg^{2+} and no significant change was observed for 10 mM Mg^{2+} in the case of GSala. Distinct transitions were seen in the conformation of GSala due to changes in Mg^{2+} concentrations. For all the three concentrations of Mg^{2+} with GSpyr, the emission maximum was shifted to shorter wavelengths compared to that of GSpyr. The blue shift would indicate the movement of tryptophan towards interior. In the case of GSpyr, fluorescence intensities increased for the three concentrations of Mg^{2+}. The lifetimes T1 and T2 increased for 100 mM Mg^{2+}. Both lifetimes T1 and T2 decreased for 10 mM Mg^{2+}. The lifetime T1 decreased and no significant change was observed for the lifetime T2 for 150 mM Mg^{2+}.

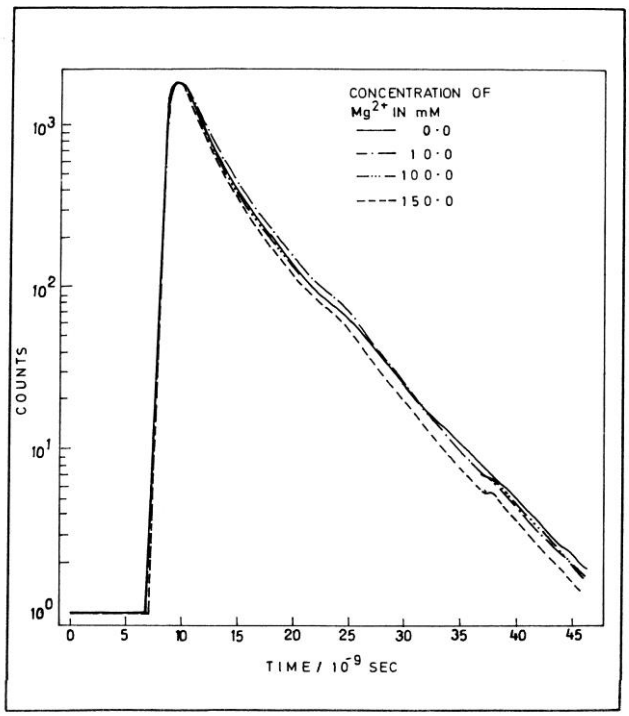

Figure 3. *The decay curves of GSala with different concentrations of Mg2+at room temperature.*

The comparison of fluorescence changes observed in GSala and GSpyr due to different concentrations of Mg^{2+} clearly indicated that GSala was more responsive to Mg^{2+} than GSpyr as former demonstrated larger changes than in GSpyr. It was very interesting to note that GSala did not show continuous gradual changes with increasing concentrations of Mg^{2+}, where as a 2 nm red shift of the emission maximum was seen at lower concentration of Mg^{2+} (10 mM) compared to a distinct blue shift seen at 100 and 150 mM Mg^{2+}. The largest difference was 4 nm blue shift shown by GSala at 150 mM as compared to GSala at 10 mM Mg^{2+}.

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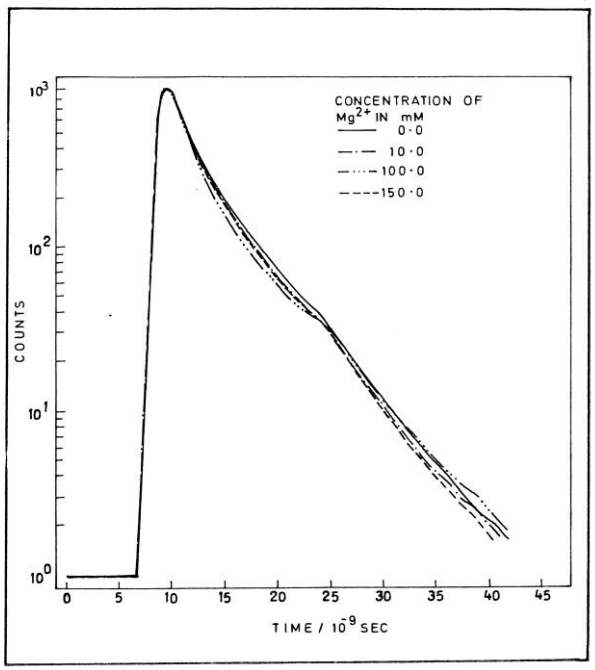

Figure 4. *The decay curves of GSpyr with different concentrations of Mg^{2+} at room temperature.*

In contrast to this, GSpyr emission maxima shifted only by 1 nm and the changes due to varying concentrations of Mg^{2+} were not detected. The fluorescence intensities decreased for all the three concentrations of Mg^{2+} in the case of GSala, whereas in the case of GSpyr, fluorescence intensities increased for all the three concentrations of Mg^{2+}. The life time T1 decreased for all the three concentrations of Mg^{2+}, whereas, life time T2 increased for 100 mM Mg^{2+}, decreased for 150 mM Mg^{2+} and no significant change was observed for 10 mM Mg^{2+} in the case of GSala. In the case of GSpyr, lifetimes T1 and T2 increased for 100 mM Mg^{2+}. Both lifetimes T1 and T2 decreased for 10 mM Mg^{2+}. The lifetime T1 decreased and no significant change was observed for the lifetime T2 for 150 mM Mg^{2+}. These results are consistent with the earlier view that GSala should be more sensitive to Mg^{2+} than GSpyr. Earlier studies in this enzyme showed that different forms of glutamine synthetase differed in their Mg^{2+} sensitivities (Tiwari et al., 1989). These substantial changes in the tryptophan fluorescence parameters such as fluorescence emission maximum, fluorescence intensity and lifetime that occur in the presence of different concentrations of Mg^{2+} indicated major changes in molecular conformations in both forms of this enzyme.

The conclusion is that the effect seen above is due to conformation and not to the interaction of Mg^{2+} with tryptophan, which is supported by the studies done by several workers (MacManus et al., 1984; Maurizi et al., 1987; Sillen et al., 2000; Haghighi et al., 2005). No specific effect of divalent ions like Be^{2+},Mg^{2+},Ca^{2+},Sr^{2+}or Ba^{2+} was seen in the fluorescence of free tryptophan in solution. Because of the absence of a specific effect on tryptophan, any effect on protein fluorescence produced by alkaline earth cations has to be due to conformational changes (Chen, 1976; Sillen et al., 2003; Suh & Savizky, 2011). The addition of Mg^{2+} to relaxed glutamine synthetase produced a decrease in fluorescence intensity with a slight blue shift (Timmons et al., 1974). The binding of Mn^{2+}or Mg^{2+} to each subunit of bovine brain glutamine synthetase produced structural perturbations in the octameric enzyme as evidenced by ultraviolet spectral and tryptophanyl residue fluorescence changes (Maurizi et al., 1987). Upon adding calcium, Trp170 shows a strong fluorescence increase, Trp57 an extensive fluorescence decrease and Trp4 shows no fluorescence change (Sillen et al., 2003). By binding various metal ions to bovine alpha lactalbumin, conformational changes were observed (Noyelle & van Dael, 2002). Heat induced conformational changes were observed in the absence and presence of calcium ions in bovine apo-α-lactalbumin by fluorescence spectroscopic studies. An increase in fluorescence intensity was observed in this protein in the absence of Ca^{2+} (Stanciuc et al., 2011). Conformational changes were observed by adding Ca^{2+} in troponin complex by fluorescence studies (Bains et al., 2011). Increased stability was found by intrinsic fluorescence studies on metal ion binding to human α–lactalbumin (Suh & Savizky, 2011). Conformational change of lig proteins upon Ca^{2+} binding was monitored using tryptophan emission fluorescence and CD spectroscopy (Raman et al., 2010). Ca^{2+} induced conformational changes were observed in prion protein fragment 90-231 by fluorescence studies (Sorrentino et al., 2012). The changes observed in the emission maximum, fluorescence peak intensity and life times in GSala and GSpyr in the presence of different concentrations of Mg^{2+} are summarized in Table1.

Conclusion

Based on these results and discussion, the following conclusions were made. The effect of Mg^{2+} on the multiple forms of glutamine synthetase in *Bacillus brevis* Bb G1 was significantly different from each other and thus resulted in different protein conformations for each form.

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Table 1. *The wavelengths of emission maximum, corresponding fluorescence intensity and lifetimes of GSala, GSpyr, GSala with different concentrations of Mg^{2+} and GSpyr with different concentrations of Mg^{2+}.*

Sample	Emission Maximum (nm)	Fluorescence Intensity	Lifetime T1 (ns)	Lifetime T2 (ns)
GS ala	329	0.589	5.80	1.80
GS pyr	320	0.486	5.08	1.30
GS ala + 10 mM Mg^{2+}	331	0.509	5.30	1.80
GS pyr + 10 mM Mg^{2+}	319	0.549	4.95	1.20
GS ala + 100 mM Mg^{2+}	328	0.494	5.56	1.96
GS pyr + 100 mM Mg^{2+}	319	0.539	5.70	1.40
GS ala + 150 mM Mg^{2+}	327	0.474	5.23	1.52
GS pyr + 150 mM Mg^{2+}	319	0.527	4.83	1.30

The fluorescent changes produced by the effect of Mg^{2+} in GSala was much more prominent than in GSpyr. The effect of different concentrations of Mg^{2+} towards multiple forms of glutamine synthetase in *Bacillus brevis* Bb G1 was considerably different from each other. Thus, characteristically different protein conformations may be obtained at a given concentration of Mg^{2+}. The substantial changes in the tryptophan fluorescence parameters such as fluorescence emission maximum, fluorescence intensity and lifetime that occur in the presence of different concentrations of Mg^{2+} indicated major changes in molecular conformations in both forms of this enzyme. Summarizing, Mg^{2+} induced conformational changes were observed in the multiple forms of glutamine synthetase from *Bacillus brevis* Bb G1 by fluorescence spectroscopic studies.

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