

Circular Dichroism Studies on C-terminal Zinc Finger Domain of Transcription Factor GATA-2

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The C-terminal zinc finger domain of GATA-2 transcription factor from *Rattus norvegicus* has been expressed and purified to elucidate its secondary structure using circular dichroism spectroscopy. Circular dichroism spectra showed that native GATA-2 C-terminal domain of (Cys)₄ type zinc finger has 12% α -helix, 36% β -sheet and 52% random coil content. The estimated structure was compared with predicted structures by sequence based prediction software SOPMA and found to be similar. Furthermore, the effect of pH on the secondary structure of GATA-2 C-finger was examined. This study provides an insight into the understanding of the structure and function of transcription factor GATA-2.

Key words: circular dichroism; GATA-2; transcription factor; Zn finger

GATA transcription factors comprise a family of six zinc finger proteins (GATA-1 to 6) which regulate cell differentiation and proliferation (Hoene et al., 2001; Ferreira et al., 2005). GATA-2 is an essential transcription factor for the regulation of multiple aspects of hematopoiesis and expressed within the same hematopoietic lineages as GATA-1 and also in several nonhematopoietic cell types (Yamamoto et al., 1990; Arceci et al., 1993; Pandolfi et al., 1995; Dasen et al., 1999; Lilleväli et al., 2006). The most notable features of the GATA proteins are the two adjacent zinc finger domains referred to as the N-terminal zinc finger (N-finger) and the C-terminal zinc finger (C-finger) which have highly conserved amino acid sequences (Lowry and Atchley, 2000; Vonderfecht et al., 2008). In the case of GATA-2, the N-finger and C-finger amino acid residues reside between 295-319 and 349-373 respectively. Members of the family bind to the DNA consensus sequence WGATAR; (W = A/T; R = A/G) by two character-

istic C₄ (Cys-X₂-Cys-X₁₇-Cys-X₂-Cys) zinc finger domains (Orkin, 1992; Ko L et al., 1993). Studies have shown that the C-terminal zinc finger and its adjacent C-terminal basic tail are necessary for GATA to bind to its cognate sequence (Evans et al., 1988; Ko and Engel, 1993; Merika and Orkin, 1993; Omichinski et al., 1993; Visvader et al., 1995; Ferreira et al., 2005). It has been shown that the N-finger of GATA-2 and GATA-3 bind to DNA independently with slightly different sequence preferences while contributing to the stabilization and specificity of DNA binding (Martin and Orkin, 1990; Merika and Orkin, 1993; Pedone et al., 1997; Newton et al., 2001). It has also been shown that zinc finger plays a crucial role in the ability of GATA-1 to induce terminal erythroid differentiation (Wiess et al., 1997). In a recent study a cross talk between GATA-1 and GATA-2 during the proliferation and differentiation stages of erythropoiesis was reported (Huang et al., 2009). It is known that GATA-2 gene is upregulated in the absence of

Abbreviations: CD, circular dichroism; DTT, dithiothreitol; GST, glutathione S-transferase

GATA-1, in other words high levels of GATA-2 are expressed in the proliferating erythroid progenitors. Upon differentiation, GATA-1 expression increases whereas GATA-2 is repressed. In another study by Visvader et al., it was shown that the GATA-2 C-terminal zinc finger can induce megakaryocytic differentiation of an early myeloid cell line (Visvader et al., 1995). Therefore, the structure of C-finger is very crucial in its function for DNA binding and protein-protein interaction. Although the structure and function of GATA-1 have been reported (Omichinski et al., 1993; Bates et al., 2008), there are so far no studies on the structure of GATA-2. GATA-1 C-finger and GATA-2 C-finger of *Rattus norvegicus* have a sequence homology of about 87% (United States National Center for Biotechnology Information Basic Local Alignment Search) but they differ in their functions during erythropoiesis (Huang et al., 2009).

The present study aimed to investigate the secondary structure of GATA-2 C-finger using circular dichroism (CD) spectroscopy. GATA-2 C-finger (amino acid residues 324–387) was sub-cloned into the expression vector pGEX4T-2 (GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom) as a glutathione S-transferase (GST) tag fusion protein. The sub-cloned constructs were transformed into BL21(DE3) *E. coli* for protein expression. The expression of the GATA-2 C-finger was induced by IPTG for 6 h at 37°C. The cells were harvested by centrifugation and resuspended in lysis buffer and stored at –20°C until protein extraction. The cell suspension was thawed and cell lysis was performed by ultrasonication, five times for 30 s. The supernatant after centrifugation at 12,000 rpm was used for the purification. The protein was first purified by affinity chromatography using glutathione sepharose beads and then digested by thrombin protease for 1 h at ambient temperature to remove the GST tag. The protein was further purified through gel chromatography using a column HiLoad 16/60 superdex 30 pg, AKTA prime (GE Healthcare) and stored at 4°C in the presence of 1 mM dithiothreitol (DTT). The protein sample was analyzed by tricine-SDS PAGE and confirmed to be single band.

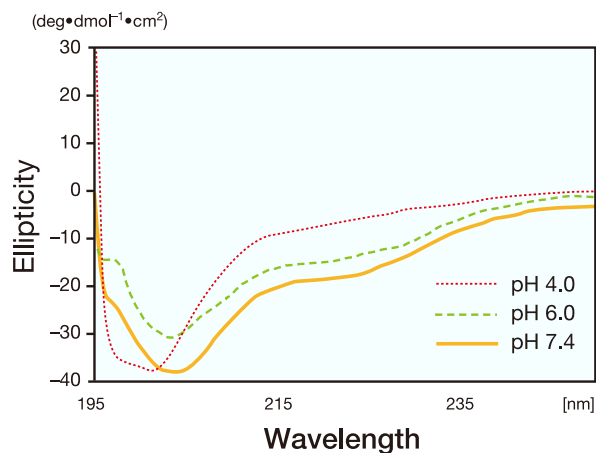


Fig. 1. The effect of pH on the secondary structure of GATA-2 C-finger: CD spectra were recorded for 30 μ M GATA-2 C finger protein in 20 mM Tris-HCl buffer (pH 7.4, solid line), 20 mM phosphate buffer (pH 6.0, dashed line) and 20 mM acetate buffer (pH 4.0, dotted line) containing 150 mM NaCl and 1 mM DTT. The spectra were recorded in 0.1 nm intervals at 50 nm/min at 25°C.

In the present study the CD spectra were recorded from 250 nm to 190 nm using a Jasco J-720 spectrometer (Jasco, Tokyo) purged with nitrogen. The spectra were acquired using a 0.1 cm path-length cuvette at 25°C with a resolution of 0.1 nm and a scanning speed of 50 nm/min. Spectra presented were averages of ten consecutive measurements. CD signals were converted to molar ellipticity (θ) using the equation;

$$\theta = \frac{[\theta]_{\text{obs}} \cdot 10^{-3} \cdot \text{MW}}{C \cdot l \cdot n \cdot 10^{-2}} \text{ deg} \cdot \text{dmol}^{-1} \cdot \text{cm}^2$$

where $[\theta]_{\text{obs}}$ is the observed ellipticity, MW is molecular weight, C is concentration (in mg/mL), l is the path length of the cuvette in centimeters, n is the number of residues, and deg is degrees (Das et al., 2004).

The experiments were carried out separately using five independently purified protein. Our result showed that all the GATA-2 C-fingers display a negative peak near 200 nm and a shoulder around 222 nm, suggesting that the protein is largely in a random coil conformation with some helical content under physiological conditions (20 mM Tris-HCl, 150 mM NaCl, 1 mM DTT, pH 7.4) (Fig. 1). Furthermore, we examined the effect of pH on the

Table 1. Comparison of the secondary structures of GATA-2 C-finger from CD analysis and sequence based prediction software SOPMA

Sample	α -Helix (%)	β -Sheet (%)	Coil (%)	Method
GATA-2 C-finger	12.6 \pm 7.2	36 \pm 5.0	52.1 \pm 6.0	CD analysis*
	12.5	29.7	57.8	SOPMA prediction†

*Dichroweb software package.

†http://npsa-pbil.ibcp.fr/cgi-bin/secpred_sopma.pl

secondary structure of GATA-2 C-finger. The estimated secondary structure of GATA-2 C-finger at pH 6.0 was not significantly different from pH 7.4. We estimated the helical, β -sheet and random coil content by CD spectrum using K2D algorithm from Dichroweb software package (Andrade et al., 1993; Whitmore and Wallace, 2008). We demonstrate in the present study that the secondary structure of GATA-2 C-finger deduced from CD analysis is consistent with that obtained from the sequence based secondary structure prediction software SOPMA presented in Table 1. At low pH the secondary structure of GATA-2 C-finger was altered resulting in a decrease in α -helical content and an increase in β -sheet compared to the native structure. The α -helix content is essential for the zinc finger type protein to carry out certain functions such as DNA binding. The secondary structure estimate showed that GATA-2 C-finger is rich in random coil while it has less β -sheet and α -helical content. This result strongly agrees with other zinc finger domain secondary structures (Lachenmann et al., 2002; Negi et al., 2004; Sakai-Kato et al., 2009). In contrast, the secondary structure of ZIC3 which has a (Cys)₂-(His)₂ type Zinc finger was reported to have a high β -sheet content (Miura et al., 1998). In another study it was reported that histidine residues play an important role in stabilizing the α -helical structure when Zn²⁺ is introduced (Nomura and Sugiura, 2003). However, GATA-2 C-terminal zinc finger is a (Cys)₄ type zinc finger, which has no histidine residues. This might be one of the reasons for the low helical content.

In summary, CD experiments demonstrate that GATA-2 C-finger has 13% α -helix, 36% β -sheet and remaining random coil in its native form. This study provides insight into a better

understanding of the secondary structure and function of (Cys)₄ type zinc finger proteins. Further work needs to be done on the structure of GATA-2 C-finger in relation to the metal binding, thermal stability and DNA and protein interactions.

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Received December 8, 2009; accepted December 28, 2009

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