Crystalline and Solution State Comparison of the Structure of Arginine Kinase by Residual Dipolar Couplings

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Introduction

Arginine kinase (AK) is an invertebrate homologue of mammalian creatine kinase (CK), and both of them belong to the phosphagen kinase family. The main function of AK is catalyzing reversible phosphoryl transfer between arginine and ATP:

$$\text{Arginine phosphate} + \text{MgADP}^- + \text{H}^+ \leftrightarrow \text{Arginine} + \text{MgATP}^{2-}$$

Through the catalysis, the ATP concentration is buffered in response to the cell's energy requirement.

A series of crystal structures of apo AK, transition state AK and AK mutants have been solved [1-2], but the dynamic processes of AK in solution are still not clear. The goal of our research is to interpret the dynamics of AK in solution through NMR and improve our understanding of its enzyme catalysis mechanism.

Experimental

All NMR experiments were acquired at 298 K at 800 MHz field strength. Details of sample preparation have been described elsewhere [3]. After data collection of isotropic samples, the AK samples were weakly aligned in 3% PEG and 10 mg/ml Pf1 Phage. Residual dipolar couplings (RDCs) were determined from the differences of multiplet splittings of the ^{15}N resonances measured in the anisotropic and isotropic samples using HSQC and TROSY-type spectra. All spectra were processed and analyzed using NMRPipe and Sparky.

Results and Discussion

RDCs measured in 3% PEG range from about -25 to 24 Hz, and about 18 to 30 Hz in 10 mg/ml Pf1 Phage. The correlations between experimental RDCs and the predicted RDCs based on crystal structures were determined both for full AK and for individual sub-domains of AK (Table 1). Low Q values reflect good agreement between the experimental RDC data and a structural model. The relatively high Q values of full AK indicate distinct differences between the structure in the crystalline environment and in solution. These results are largely consistent with differences observed between experimental chemical shifts and ones predicted of the X-ray structure in the same regions where deviations of residual dipolar couplings are found.

Table 1. Q values for the whole AK and subdomains of AK

MEDIA		**PEG**		**PHAGE**	
PDB		1M80	1M15	1M80	1M15
Full AK		0.45	0.49	0.52	0.46
Sub-domain of AK	Domain 1	0.36	0.34	0.32	0.33
	Domain 2	0.66	0.70	0.66	0.72
	Domain 3	0.83	0.66	0.76	0.45
	Domain 4	0.24	0.24	0.33	0.27
	Domain 1+4 only	0.32	0.36	0.33	0.32

Conclusions

Similar regions in AK exhibit discrepancies both for RDCs and chemical shifts, which indicate structural dynamic differences in these regions between crystalline and solution environments.

Acknowledgements

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References

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