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303
Structure-function Relationship of Adenylate Kinase: Glu-101 in AMP Specificity

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Introduction

NMR has been used as an integral part of the analysis of the structure-function relationship of adenylate kinase (AK) from chicken muscle (overexpressed in *Escherichia coli*). The enzyme catalyzes the interconversion between MgATP+AMP and MgADP+ADP. In the earlier part of our studies, various site-specific mutants were constructed. One and two dimensional NMR were used to determine whether the global conformations of the mutants have been perturbed in comparison with the wild-type enzyme. Perturbation of the global conformation, if observed, suggests that the mutated residue is likely to play a structural role. If the global conformation has not been perturbed, then the mutant is subjected to detailed kinetic analysis to determine the functional role of the residue. This approach has allowed us to map out the substrate binding site and to understand the detailed mechanism of catalysis, as summarized in Tsai, *et al.*, 1991 [1].

As a complementary approach, NMR was used to identify the residues in proximity to substrates. The residues were then chosen for site-specific mutagenesis studies to determine their functional roles. To this end, we have performed total assignments of AK complexed to $MgAP_5A$ [2]. AP_5A has five phosphates between two adenosines and is a well established bisubstrate analog inhibitor of AK. Both ^{15}N and $^{15}N/^{13}C$ uniformly labeled enzymes have been used, and various two and three dimensional experiments have been performed. Although AK is a fairly large protein (21.6 kDa) in the NMR standard, we have been able to assign over 90% of the backbone ^{1}H, ^{13}C, and ^{15}N resonances, as well as a substantial amount of side chain resonances. The assignments were then used to determine the secondary structure of AK. Most importantly, the residues in proximity to the adenosine moieties of AP_5A have been identified. Gln-101 is one of the residues identified by NMR to be in proximity to the adenine ring of the AMP moiety. This residue was then further investigated by the first approach for its structural and functional roles. The results are described in this report.

Results and Discussion

The mutant Q101E, in which Gln-101 is replaced by Glu, has been constructed and purified as previously described [3]. As shown in Figure 1, the proton NMR spectrum of Q101E is very similar to that of the wildtype (WT) AK. This was further confirmed by

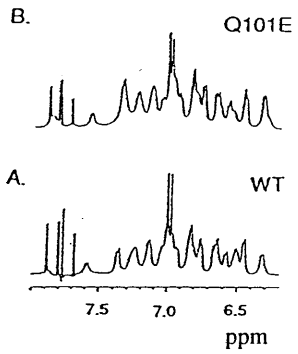

Figure 1. *Partial proton NMR spectra of WT AK (A) and Q101E (B).*

2D NOESY experiments for the enzymes in the free and the $MgAP_5A$ complexed forms (spectra not shown). Thus it can be concluded that the global conformation of the mutant has not been perturbed significantly, and that the functional data of the mutant can be interpreted with confidence.

The binding affinity toward $MgAP_5A$, however, is substantially lower for the mutant. The K_d value obtained from proton NMR titration experiments in Figure 2 is 0.04 mM, which amounts to a binding energy of *ca.* 5 kcal/mol. The corresponding value for WT AK is 11 kcal/mol [4]. Presumably because of the weakened binding affinity, the two adenosine moieties are in fast exchange on the NMR time scale as opposed to slow exchange for wildtype AK and many other mutants [2]. The weakened binding affinity could be caused by a perturbed interaction between the side chain of Glu-101 and the adenosine of the AMP moiety of $MgAP_5A$. This was confirmed by kinetic analysis.

Full kinetic analysis was performed by the procedure described previously [3, 4] and the results are summarized in Table 1. Comparison of the data between Q101E (column 3) and WT (column 2) indicates that k_{cat} decreases to 2% and AMP binding affinity decreases substantially. Furthermore, the specificity toward AMP is relaxed. While the activities of WT AK toward GMP and IMP were too low to be measured quantitatively, the activities of Q101E toward GMP and IMP became significant (columns 4 and 5). The results of NMR and kinetic analyses taken together suggest that the side chain of Gln-101 interacts with the adenine ring of AMP and controls the substrate specificity of AK toward AMP.

Table 1. *Comparison of kinetic data between WT and Q101E.*

Parameters[a]	WT/AMP[b]	Q101E/AMP	Q101E/GMP	Q101/IMP
k_{cat} (s-1)	650	14	1.7	0.094
K_{MgATP} (mM)	0.042	0.17	0.08	0.2
K_{NMP} (mM)	0.098	10.4	8.2	12.6
Ki_{MgATP} (mM)	0.16	0.097	0.094	0.082
Ki_{NMP} (mM)	0.37	5.9	10.9	5.2

[a]K and K_i values are Michaelis and dissociation constants, respectively.
[b]The data for WT are from [6]

Figure 2. *Prot*
mM. The ratio:

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References

1. Tsai, M.-I
2. Byeon, I.-
M.-D., *Bi*
3. Tian, G., S
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4. Yan, H., S
5. Byeon, I.-
6. Tian, G.,
(1990) 42

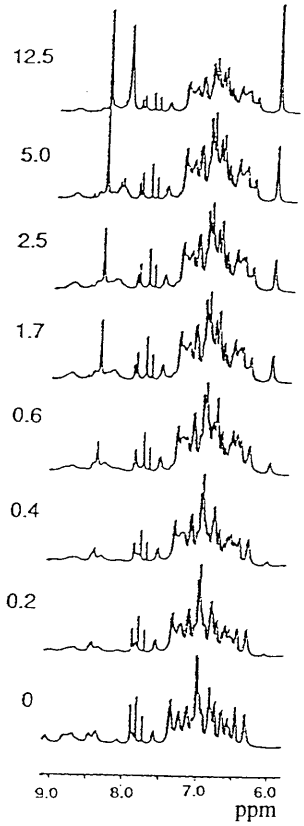

Figure 2. *Proton NMR titration of Q101E with $MgAP_5A$. The starting conc. of free enzyme is 1.8 mM. The ratios of $[MgAP_5A]/[Q101E]$ are shown.*

Acknowledgments

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References

1. Tsai, M.-D. and Yan, H., *Biochemistry*, 30 (1991) 6806.
2. Byeon, I.-J.L., Yan, H., Edison, A.S., Mooberry, E.S., Abildgaard, F., Markley, J.L. and Tsai, M.-D., *Biochemistry*, 32 (1993) 12508.
3. Tian, G., Sanders, C.R., II, Kishi, F., Nakazawa, A. and Tsai, M.-D., *Biochemistry*, 27 (1988) 5544.
4. Yan, H., Shi, Z. and Tsai, M.-D., *Biochemistry*, 29 (1990) 6385.
5. Byeon, I.-J.L., Shi, Z. and Tsai, M.-D., *Biochemistry*, 34 (1995) 3172.
6. Tian, G., Yan, H., Jiang, R.-T., Kishi, F., Nakazawa, A. and Tsai, M.-D., *Biochemistry*, 29 (1990) 4296.