Mechanism of Adenylate Kinase. 20. Probing the Importance of the Aromaticity in Tyrosine-95 and the Ring Size in Proline-17 with Unnatural Amino Acids
Zhong Zhao,1 Xiaobing Liu,1 Zhengtao Shi,1 Lora Danley,1 Baohua Huang,1 Ru-Tai Jiang,1 and Ming-Daw Tsai1,2,3

Departments of Chemistry and Biochemistry and Ohio State Biochemistry Program
The Ohio State University, Columbus, Ohio 43210

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We report an application of the unnatural amino acid mutagenesis developed by Schultz1 to probe the importance of the aromaticity of tyrosine-95 and the ring size of proline-17 in the function of adenylate kinase (AK, from chicken muscle, overexpressed in Escherichia coli).2 AK catalyzes the reaction MgATP + AMP ↔ MgADP + ADP. On the basis of structural analyses by X-ray3 and NMR,4 Tyr-95 is located in proximity to the adenosine moiety of AMP (within the range for amino-aromatic interaction, a weakly polar interaction5) and is also likely to be involved in amino-aromatic interactions6 with Phe-12 and Phe-105 (distances between centroids are 6.1 and 5.9 Å, respectively).6 A stereo view of the structure of E. coli AK complexed with AMP and AMPNP (adenosine 5'-(β,γ-imido)triphosphate) is shown in Figure 1.7 The aromaticity of residue 95 is absolutely conserved; while it is Tyr in muscle AK, it is Phe in yeast and E. coli AK. Replacement of the Tyr-95 of muscle AK with leucine led to no detectable changes,8 while replacement with nonaromatic residues in both muscle and E. coli AK led to decreases in activity. Since natural nonaromatic amino acids are very different from Tyr or Phe in the side chain dimension, we used 2,5-dihydroxyphenylalanine (DihPhe, Figure 2) to probe the importance of aromaticity in Tyr-95. DihPhe can provide ε-electrons without aromaticity, and its ring is close to planar.7

Proline-17 (Figure 1) is absolutely conserved in the phospho binding loop (P-loop, GPGXGXGK) in the AK family.8

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* Address correspondence to: Department of Chemistry, The Ohio State University, 100 West 18th Ave., Columbus, OH 43210-1173.
1 Department of Chemistry.
2 Ohio State Biochemistry Program.
3 Department of Biochemistry.
4 University of California at Berkeley.

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Figure 1. Stereoview of the structure of E. coli AK complexed with AMP and AMPNP. Pro-9 and Phe-86 correspond to Pro-17 and Tyr-95, respectively, in muscle AK.

Figure 2. Structures of amino acids substituted for Tyr-95 and Pro-17: Tyr (tyrosine); DihPhe (2,5-dihydroxyphenylalanine); Pro (proline); Dhp (3,4-dehydropipolic acid); Pip (pipolic acid); HPPip (homopipolic acid); Azep (azetidine 2-carboxylic acid); McGly (N-methyl glycine). Substitutions of Pro-17 with natural amino acids caused perturbations in substrate binding parameters. However, natural amino acids cannot probe the importance of ring size, which is the key feature of proline. We used four proline analogs with different ring sizes (Figure 2), pipolic acid (Pip), homopipolic acid (HPPip), 3,4-dehydropipolic acid (Dhp), and azetidine 2-carboxylic acid (Azep), to probe the importance of the ring size of Pro-17 in the function of AK.

The unnatural amino acids were purchased or synthesized according to known procedures. The suppressor tRNA aminocylated with unnatural amino acids was prepared according to the procedures of Schultz,1 to facilitate the purification of the in vitro synthesized AK, a six-histidine tag15 was attached to the C-terminus of AK by modifying the gene of AK. The AK with the six-His tag (AKH) was first expressed in E. coli, purified, and shown to behave essentially the same as wild type (WT) AK. The AKH gene was then cloned into a high-copy-number expression vector PUK constructed in our lab.12 In vitro protein syntheses were carried out with the coupled transcription/translation system of E. coli developed by Zabay13a with some modifications by Collins,13b Pratt,13c and Schultz.1 In vitro expression of the wild type AKH gene under the control of a tac promoter in the PUK vector afforded ca. 5

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10. All amino acids used were L configuration. Pip, Azep, Dhp, and McGly were commercially available. DihPhe was synthesized from Phe by Birch reduction as in ref 7b. HPPip was initially provided by D. Seebach and J. Podlech at the Swiss Federal Institute of Technology in Zurich and then synthesized according to Seebach, D.; Dzidulewicz, E.; Berhendt, L.; Cantoreggi, S.; Fitzi, R. Liebig's Ann. Chem. 1989, 1215.