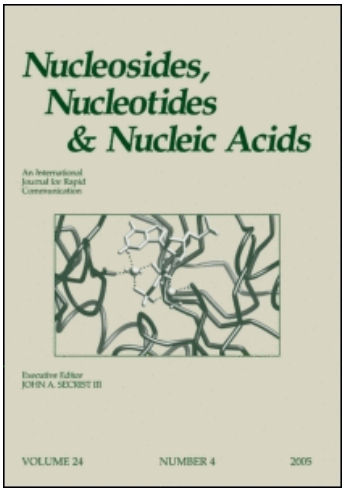

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Site-Directed Mutagenesis of Stahphylococcal Nuclease: Role of Tyrosine Residues in Substrate Binding and Catalysis

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SITE-DIRECTED MUTAGENESIS OF STAHPHYLOCOCCAL NUCLEASE: ROLE OF TYROSINE RESIDUES IN SUBSTRATE BINDING AND CATALYSIS

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Abstract: Oligonucleotide directed mutagenesis was used to change specific aminoacid residues in the active site of Staphylococcal nuclease. From the determined kinetic parameters we have calculated the interaction energy of the Tyr side chain with the substrate nitrophenyl-pTp.

Staphylococcal nuclease, an extracellular 5'-phosphodiesterase from the Foggi strain of Staphylococcus aureus, catalyzes the hydrolysis of singlestranded DNA and RNA to yield 3'-nucleotides as products. Although the nuclease has extensively been studied by techniques of chemistry, physical biochemistry and x-ray crystallography[4], questions still remain about the function of crucial residues in the active site. Using oligonucleotide directed site specific mutagenesis[1], we replaced specific Tyr residues (position 85, 113 and 115) with Phe residues so as to eliminate the hydroxy group and maintain the aromatic moiety.

It was postulated that the hydroxy group of Tyr 85 makes a hydrogen bond with the 3'-phosphate group of the substrate[2] and that the phenolic group of this Tyr must be protonated[3] for the enzyme to be active. Furthermore, possible direct involvement of Tyr 113 in the catalytic mechanism has been suggested[2,4]. From x-ray crystallographic data it was suggested that Tyr 115 is inside the catalytic pocket when no substrate is bound, but moves out of this region when substrate binds[2,5]. To test the role of the several Tyr residues in substrate binding and catalysis we made the following alterations: (Tyr 85 to Phe), (Tyr 113 to Phe), (Tyr 115 to Phe) and (Tyr 113, Tyr 115 to Phe,Phe) by means of site-directed mutagenesis of the nuclease gene[6]. For the expression of the desired mutated nuc genes in E. coli we used the alkaline phosphatase expression system of D. Shortle (unpublished) having the advantage that the expressed enzyme can easily be isolated from the periplasmic space of E.coli in its active form. After purification of the crude enzymes by ionexchange chromatography, they were shown by SDS-PAGE to be pure and to have the expected molecular weight.

Kinetic measurements were done as described by Taniuchi[7] to give the K_M and K_{cat} values for the synthetic substrates nitrophenyl-pTp and nitrophenyl-pT[7,8]. A comparison of kinetic parameters in hydrolysis of nitrophenyl-pTp and the corresponding 3'-hydroxy-substrate nitrophenyl-pT gives a 40 times lower relative specificity[9] for the Phe 85 mutant than for the wild type enzyme, reflecting the interaction of Tyr 85 hydroxy group with the 3'-phosphate group of the nucleotide substrate.

From the k_{cat}/K_M values we calculated the interaction energy of the Tyr side chain hydroxy group with the substrate nitrophenyl-pTp. Replacement of Tyr 85 by Phe weakens the binding energy by 2 kcal/mol which agrees with the finding of Fersht et al[9] that deletion of an uncharged

sidechain group on the enzyme that forms a hydrogen bond with a charged group on the substrate (3'-phosphate) weakens binding energy substantially (up to 3.5 kcal/mol). Substitution of Tyr 113 by Phe results in a loss in energy of 0.8 kcal/mol, which again agrees with Fersht that deletion of a sidechain group that forms a hydrogen bond with an uncharged group on the substrate weakens binding energy by 0.5 - 1.1 kcal/mol. On the contrary, replacement of Tyr 115 by Phe results in a negligible change of binding energy (0.1 kcal/mol).

We could also confirm that Tyr 115 is not involved directly in catalysis regarding the synthetic substrates. Interestingly, substitution of Tyr 115 by Phe led to an increased k_{cat} value (30 p.c. relative to the wilde type). Additionally, in the case of the double mutant (Phe 113, Phe 115) a similar increase in k_{cat} (35 p.c. relative to the single mutant Phe 113) could be observed.

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REFERENCES

1. M. J. Zoller and M. Smith; DNA 3, 479 (1984)

2. P. W. Tucker, E. E. Hazen and F. A. Cotton; Mol. Cell. Biochem. 23, 67 (1979)

3. P. Cuatracasas, S. Fuchs and C. B. Anfinsen; J. Biol. Chem. 243,4787 (1968)

4. F. A. Cotton, E. E. Hazen and M. J. Legg;
Proc. Natl. Acad. Sci. USA 76, 2551 (1979)

5. A. Arnone, C. J. Bier, F. A. Cotton, E. E. Hazen;
D. C. Richardson and J. S. Richardson;
Proc. Natl. Acad. Sci. USA 64, 420 (1969)

6. D. Shortle; Gene 22, 181 (1983)

7. D. S. Parker, A. Davis and H. Taniuchi;
J. Biol. Chem. 256, 4557 (1981)

8. P. Cuatrecasas, M. Wilchek and C. B. Anfinsen;
Biochem. 8, 2277 (1969)

9. A. R. Fersht, J.-P. Shi, J. Knill-Jones, D. M. Lowe,
A. J. Wilkinson, D. M. Blow, P. Brick, P. Carter,
M. M. Y. Waye and G. Winter; Nature 314, 235 (1985)