Section: Molecular Biophysics

The endonucleolytic reaction catalysed by the EcoRI restriction enzyme is inhibited when the enzyme is in excess over substrate.

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We have studied the cleavage of radioactively labelled double stranded d(GGAATTCC) in single turnover experiments with substrate and enzyme concentration both being in the µM range. As can be seen in fig. 1 the reaction rate increases with enzyme concentration until a 1:1 ratio of substrate and enzyme (dimeric form) is reached, when the enzyme concentration is further increased a sharp decline of the reaction rate is observed.

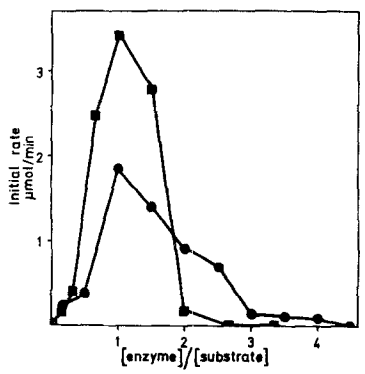

Fig 1: 1.5 µM (●) and 3 µM (■) double stranded d(GGAATTCC) were incubated at 16°C in 0.01 M Tris. HCl pH 7.5, 0.02 M $MgCl_2$, 0.08 M NaCl with EcoRI endonuclease. Samples were withdrawn after 15, 30, 45, 60, 90, 120 sec, the reaction was stopped with EDTA, the samples were analyzed by homochromatography, autoradiography and liquid szintillation counting. Initial reaction rates were determined from a plot of the amount of d(GG) formed vs. time.

We interpret these findings in the following manner:

I. The EcoRI endonuclease shows a half-of-the-sites reactivity either due to a cooperation of two subunits in cleaving the palindromic substrate, or due to a negative cooperativity between two identical catalytic sites.

II. The enzymatic action of the EcoRI endonuclease is inhibited by the unspecific binding of the enzyme to the enzyme-substrate complex, or vice versa the enzyme cannot cleave unspecific enzyme-substrate complexes, possibly due to a distortion of the regular helix geometry induced by the unspecifically bound protein.

Macromolecular substrates show a similar behavior; the cleavage of both pBR322 plasmid DNA (1 EcoRI site) and λ DNA (5 EcoRI sites) is inhibited at high enzyme concentration. The ratio of dimeric EcoRI to DNA base pairs at which inhibition sets in is approx. one enzyme dimer per 6 - 8 base pairs.

Interestingly, the cleavage of the superhelical plasmid DNA is only partially inhibited, since even at the highest enzyme concentrations used one nick is set which yields the relaxed open circular form. Only the cleavage of the second DNA strand is inhibited. We presume that due to the superhelical conformation with two double strands wound around each other the DNA molecule

cannot be as much occupied with enzyme as a relaxed DNA, thus enabling the EcoRI endonuclease to nick the DNA. Once it is relaxed, the reaction is then completely inhibited.

The catalytic mechanism of phenylalanyl-tRNA synthetase of Escherichia coli K10. Conformational change and phenylalanylation of $tRNA^{Phe}$ are concerted

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Catalytic phenylalanylation of $tRNA^{Phe}$ and the reverse, AMP-dependent deacylation of Phe-$tRNA^{Phe}$, have been measured by steady-state and presteady-state kinetic techniques including rapid sampling and fluorescence stopped-flow methods.

(1) Identity of values of rate constants under presteady-state and steady-state conditions for phenylalanylation and AMP-dependent deacylation demonstrates bond making and bond breaking, respectively, to be rate-limiting in catalysis. (2) Values of catalytic rate constants are the same as those of the conformational change of a Phe-site directed enzyme·Phe-$tRNA^{Phe}$ complex. However, the complex at the end of the conformational change is not on the catalytic path. It is concluded that bond making and bond breaking, respectively, are in concert with a similar conformational change of the enzyme. (3) Results are discussed in terms of a model for the phenylalanyl transfer reaction. It involves formation of a tetrahedral adenylate tRNA intermediate which, for geometrical reasons, awaits the conformational change in order to break down into Phe-$tRNA^{Phe}$ and AMP. The model may also offer a unifying basis for mechanisms of discrimination against noncognate amino acids and tRNAs.