# Pre - steady state kinetics of DNA cleavage by the restriction endonuclease Eco RI measured in a pulsed quenched flow apparatus

J. Langowski, C. Urbanke and A. Pingoud

Medizinische Hochschule Hannover, Zentrum Biochemie, Hannover, F.R.G.

Pulsed quenched flow experiments enabled us to follow the kinetics of DNA cleavage by the restriction enzyme Eco RI down to a time range of several milliseconds.
The experiments were done in a microprocessor-controlled pulsed quenched flow apparatus recently constructed by us; analysis of the reaction products was done on agarose gels by photography and scanning of the negatives.
We could establish that with an excess of Eco RI the reaction proceeds in two distinct steps: 1. cleavage of the first strand of DNA under formation of nicked circular DNA from a covalently closed plasmid DNA substrate; 2. cleavage of the second strand under formation of linear DNA.
The intrinsic rate of cleavage at 22° C was faster than 1 $sec^{-1}$ for the first step and in the range of 0.1 $sec^{-1}$ for the second step. Maximum values were reached only at a very high molar excess of enzyme over DNA, which is probably due to the fact that non-specific binding of Eco RI to DNA sequences outside of the recognition site (1,2) leads to non-productive complexes.

1. M. Goppelt et al. , Eur. J. Biochem. 104 (1980) 101-107
2. J. Langowski, A. Pingoud, M. Goppelt, G. Maass, submitted to Nucl. Acids Res.