

# Inhibition of the Type I Restriction-modification Enzymes *EcoB* and *EcoK* by the Gene *0-3* Protein of Bacteriophage T7

Pradip K. Bandyopadhyay†, F. William Studier

Biology Department  
Brookhaven National Laboratory  
Upton, N.Y. 11973, U.S.A.

Daniel L. Hamilton and Robert Yuan

LBI-Basic Research Program, National Cancer Institute  
Frederick Cancer Research Facility  
P.O. Box B, Frederick, Md 21701, U.S.A.

(Received 15 October 1984)

The gene *0-3* protein of bacteriophage T7 is a potent inhibitor of the restriction-modification enzymes *EcoB* and *EcoK*, both *in vivo* and *in vitro*. We have analyzed the ability of purified *0-3* protein to inhibit different steps in the reactions of *EcoB* and *EcoK* with DNA. Most of our experiments were done with *EcoK*, but selected tests with *EcoB* indicate that the two enzymes are affected by *0-3* protein in the same way. Purified *0-3* protein binds tightly to free enzyme, apparently to one of the small subunits, and prevents it from binding to DNA. If *EcoK* is allowed to form specific recognition complexes with unmodified DNA before *0-3* protein is added, relatively low levels of *0-3* protein prevent the nuclease activity that would otherwise appear upon addition of ATP, but considerably higher levels are needed to prevent formation of filter-binding complexes or ATPase activity. This, together with other results, suggests that the binding site for *0-3* protein is protected in recognition complexes and in the early stages of the ATP-stimulated reactions, but that it becomes accessible again before cleavage of the DNA, perhaps after the translocation step. If added after the nuclease reaction is substantially over, *0-3* protein has little effect on ATPase activity, and indeed, the subunit having the binding site for *0-3* protein apparently dissociates from the enzyme-DNA complex. The methylase activity of *EcoK* on hemi-methylated recognition sites is strongly inhibited by *0-3* protein added at any stage of the reaction.

## 1. Introduction

*EcoB* and *EcoK*, in a complex set of reactions, modify the DNA that is resident in their own cell but degrade unmodified foreign DNA (see review by Yuan, 1980; Endlich & Linn, 1981). The DNA of bacteriophage T7 contains six recognition sites for *EcoB* and four for *EcoK* (Dunn & Studier, 1983). However, when T7 infects *Escherichia coli* B or K-12, its DNA is neither degraded nor methylated by *EcoB* or *EcoK*, because gene *0-3*, the first T7 gene, directs the synthesis of a protein that prevents both

the restriction and modification activities (Studier, 1975). The *0-3* protein is a small protein of 116 amino acids, and it has been purified and shown to inactivate the nuclease and ATPase activities of *EcoB in vitro* (Mark & Studier, 1981; Dunn *et al.*, 1981). The inhibition requires stoichiometric rather than catalytic amounts of *0-3* protein, suggesting that inactivation occurs by binding. Indeed, the *0-3* protein is very acidic, which raises the possibility that it could compete for the DNA-binding site of the restriction enzymes. We have explored further the interaction between purified *0-3* protein and *EcoB* and *EcoK*, and the effects of this interaction on different stages of the complex interaction between these restriction enzymes and DNA.

† Present address: Synergen, 1885 33rd Street, Boulder, Col. 80301, U.S.A.