

SHORT PAPER

Tripeptide, Arg-Gly-Asp, inhibits the transfection of protein-linked DNA of bacteriophage M2

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ABSTRACT

The effect of tripeptide, Arg-Gly-Asp (RGD), on the transfection activity of *Bacillus* phage M2 DNA was examined. The transfection activity decreased when M2 DNA was preincubated with RGD before it was added to competent cells.

1. INTRODUCTION

Protease-sensitive transfection has been described in several *Bacillus* phages, Ø29 (Hirokawa, 1972), Ø15, M2 (Hirokawa *et al.*, 1977), and GA-1 (Arwert and Venema, 1974), all of whose genome is a linear DNA covalently linked with a terminal protein at both 5' ends. Thermolabile transfection has been observed with the temperature-sensitive mutants of the gene encoding terminal protein in both Ø29 (Yanofsky *et al.*, 1976) and M2 (Kobayashi, 1987). These indicate that an intact terminal protein is essential for the transfection of those DNAs.

Recent reports on fibronectin show that the tripeptide, arginine-glycine-aspartic acid (RGD), is the key structure for the adhesion of several proteins (Pierschbacher and Ruoslahti, 1984). When synthetic peptide containing RGD is added to a cell suspension, the adhesion between cells is prevented. Unexpectedly, we have found the RGD sequence in the terminal protein of both M2 (our unpublished result; Leavitt and Ito, 1987) and Ø29 (Yoshikawa and Ito, 1982). In this paper, we describe a new evidence of the tripeptide, RGD, preventing the transfection of protease-sensitive bacteriophage DNAs, such as M2 and Ø29.

2. MATERIALS AND METHODS

Phages and bacterial strains

Bacteriophage M2H6 (Hirokawa *et al.*, 1982) and SP50 (Spatz and Trautner, 1970) were from our stock. *Bacillus subtilis* 222 (Hirokawa, 1972; Trautner, *et al.* 1974) was used for preparation of competent cells (Spatz and Trautner, 1970).

Bacillus subtilis SR22 was used for the indicator.

Media and chemical

A tryptone-yeast extract (TY) medium and LTT plates were used for transfection assay (Hirokawa, 1972). A synthetic tripeptide RGD was provided by Dr. K. Miura, University of Tokyo.

Transfection

The extraction of phage DNA was performed as described by Hirokawa (1972), except that the titer of a phage suspension was adjusted to 10^{11} plaque forming units/ml. The DNA preparation ($0.1 \mu\text{g}/0.1 \text{ ml}$) was mixed with an equal volume of RGD solution (0.1 ml) and incubated at 37°C for the indicated periods of time. The subsequent transfection was performed as described by Hirokawa (1972).

3. RESULTS AND DISCUSSION

The effect of tripeptide, RGD, on the transfection of bacteriophage M2 DNA was examined. A preincubation of the DNA with RGD was essential for the

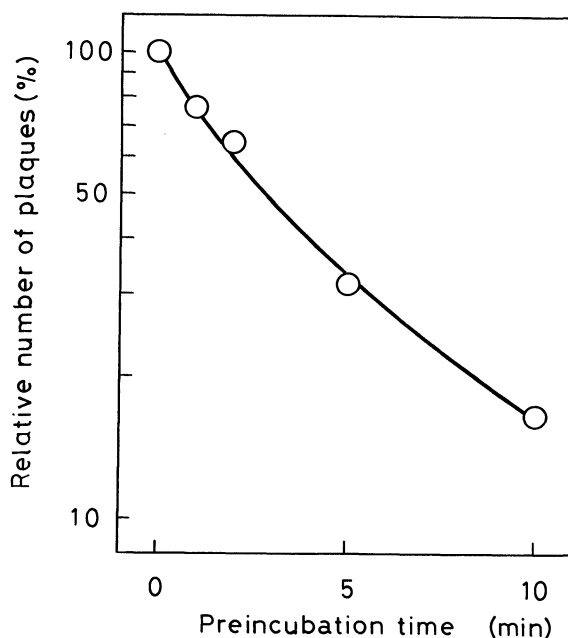


Fig. 1. Inhibitory effect of RGD on the transfection of M2 DNA. The M2 DNA ($0.1 \mu\text{g}/0.1 \text{ ml}$) was incubated with RGD ($0.3 \text{ mg}/0.1 \text{ ml}$) at 37°C for the indicated periods of time. Transfection was performed as described by Hirokawa (1972). The relative number of plaques indicates the percentage to non-treated DNA. The actual numbers of plaque in transfections, without the preincubation and in the absence of RGD, were 543 and 556, respectively.

inhibitory effect on transfection activity (Fig. 1). No difference in the number of transfective centers was obtained when transfection was done in the presence of RGD without preincubation, or in its absence. Competent cells, whether pretreated with RGD or not, exhibited the same response to transfection (data not shown). These results suggest that RGD has an inhibitory effect on transfective DNA, but not on competent cells. To examine whether RGD has an inhibitory effect on TP only or not, the transfection of SP50 DNA which has no terminal protein was examined in the presence of RGD. By pretreatment with a high concentration of RGD, 5 mg/ml, the transfection of SP50 DNA was not affected at all. At the same concentration, the transfection of M2 DNA decreased to 5% (Fig. 2). Transfection of Ø29 DNA resulted in the same response to RGD as M2

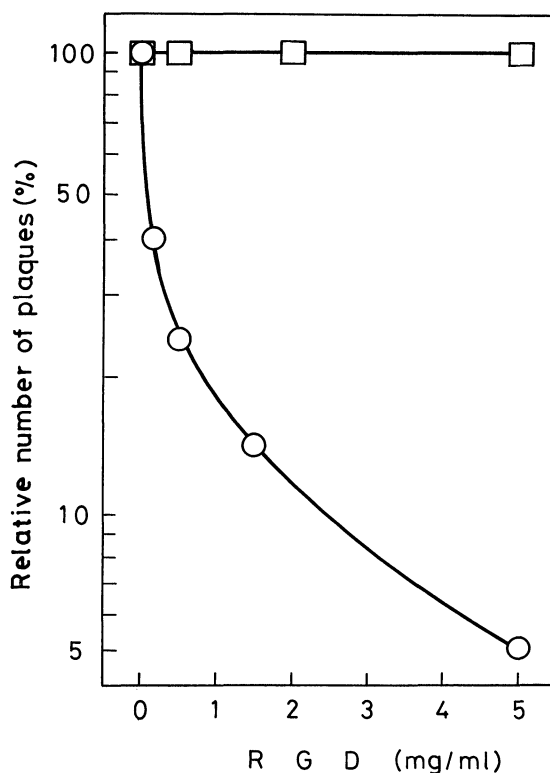


Fig. 2. Dose effect of RGD on the transfection of M2 DNA. The M2 DNA (0.1 μ g/0.1 ml) was incubated with RGD (0.1 ml) in the indicated concentrations at 37°C for 10 min. Transfection was performed as described by Hirokawa (1972). The transfection activity of SP50 DNA was determined by the same manner as M2 DNA except for the DNA concentration (1.0 μ g/0.1 ml) to adjust the number of molecules in the transfection assay. Because, the molecular weight of SP50 DNA is ten times larger than that of M2 DNA. The circle indicates M2 DNA. The square indicates SP50 DNA. The actual numbers of plaques at the 100% value for non-treated M2 DNA and SP50 were 211 and 1214, respectively.

DNA (data not shown).

Transfective DNAs of M2 (Hirokawa, 1988) and Ø29 (Hirokawa, 1969; Ortín *et al.*, 1971) are easily converted to circular and multimeric forms from monomeric molecules. This conversion is due to the adhesion between the terminal proteins (Ortín *et al.*, 1971; Hirokawa, 1988). The terminal proteins would have a recognition site for each other. When the transfective M2 DNA, which is a terminal protein-DNA complex, is preincubated with RGD, the terminal protein could be modified by binding of RGD. Thus, the modified terminal protein of DNA would lose its ability for molecular recognition. Also it is possible that the modified transfective DNA is incapable of entering competent cells. Further studies are needed to determine the cause. The inhibitory effect of synthetic tripeptide RGD on the transfection opens a valuable approach to understand the multiplication of bacteriophage M2.

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