Volume 14 Number 19 1986 Nucleic Acids Research

Regulation of inter- and intramolecular ligation with T4 DNA ligase in the presence of polyethylene glycol

Ken'ichiro Hayashi, Masako Nakazawa, Yukuo Ishizaki, Nobutsugu Hiraoka and Akira Obayashi

Takara Shuzo Co., Ltd., Central Research Laboratories, 3-4-1 Seta, Otsu-shi, Shiga 520-21, Japan

Received 16 June 1986; Revised and Accepted 16 September 1986

ABSTRACT

Polyethlene glycol (PEG) stimulates ligation with T4 DNA ligase. In 10% (w/v) PEG 6000 solutions, only intermolecular ligation is enhanced by monovalent cations, while both inter- and intramolecular ligation occur without their presence. Similar stimulation was also caused by divalent cations or polyamines in the PEG 6000 solutions. Such properties of the ligase could be applied to control the extent of inter- and intramolecular ligation. Ligation with cations or polyamines in 10% PEG 6000 solutions was effective for intermolecular ligation. Ligation without cations or polyamines in 6.0% to 10% PEG 6000 solutions was effective for intramolecular ligation.

INTRODUCTION

The relative amounts of the products by inter- and intramolecular ligation is calculated with two parameters, *i* and *j*; *i* is the total concentration of DNA ends, and *j* is the effective concentration of one end of a DNA molecule in relation to the other end (1). The optimum condition to form linear oligomers is $j/i < 1$; the concentration of the DNA must be high. However, only intermolecular ligation is markedly stimulated by monovalent cations in polyethlene glycol (PEG) solutions even when the concentration of the DNA is low; this is, when the ratio of j/i is 2.4. Such stimulation occurs in 10% (w/v) PEG 6000 solutions with T4 DNA ligase (2) and in 10% or 15% PEG 6000 solutions with *Escherichia coli* DNA ligase (3). This stimulation arises from the increased affinity of the enzyme for the DNA substrate, because the first reaction of DNA ligase, formation of the enzyme-AMP complex, is not stimulated by the cations in PEG 6000 solutions. We think that the neutralization

of the negative charges of the DNA phosphate is important in increasing the concentration of DNA termini around the enzyme in PEG solutions, and also that intermolecular ligation is stimulated by any cation in PEG solutions.

Here, we investigated the effects of divalent cations such as Mg^{2+} and Ca^{2+} and those of polyamines such as spermidine (3+) and spermine (4+) on ligation with T4 DNA ligase in 10% PEG 6000 solutions, and found that divalent cations or polyamines also enhanced intermolecular ligation similar to monovalent cations. We also applied these properties of the ligase to gene cloning.

MATERIALS AND METHODS

Enzymes and DNA

T4 DNA ligase was purified from *E. coli* 1100 lysogenic for NM989(λ T4*lig*) (4, 5). The definition of 1 unit of this ligase is given elsewhere (2). Alkaline phosphatase from *E. coli* C75 was purified by the procedure of Nakata *et al.* (6). The restriction endonucleases *Eco*RI, *Ava*I, *Pvu*II, *Sca*I, *Hin*cII, and *Bgl*II were also purified in our laboratory.

pBR 322 DNA was isolated from transformed *E. coli* C600 (7). λ phage DNA was prepared from *E. coli* K-12 W3350 (λ cI857 S7) by the procedure of Thomas and Davis (8), and pUC 18 DNA was isolated from transformed *E. coli* K-12 JM109 (9).

E. coli chromosome DNA prepared as described by Berns and Thomas (10) was completely digested by *Eco*RI and 3000- to 6000-bp DNA fragments were purified by agarose gel electrophoresis and extraction with phenol/chloroform.

*Eco*RI-digested λ gt11 DNA (11) treated with alkaline phosphatase was purchased from Promega Biotec.

Cohesive-ended or blunt-ended DNA fragment containing tetracycline-resistant gene (Tet^r gene) was isolated from pBR 322 DNA as follows: pBR 322 DNA was digested by either *Eco*RI and *Ava*I or *Pvu*II and *Sca*I, and then cohesive-ended DNA fragment containing Tet^r gene (1428 bp) or blunt-ended DNA fragment containing Tet^r gene (2583 bp) was purified by agarose gel electrophoresis and extraction with phenol/chloroform.

*Eco*RI- and *Ava*I-digested pUC 18 DNA (2670 bp) treated with

alkaline phosphatase and HincII-digested pUC 18 DNA (2686 bp) treated with alkaline phosphatase were used as the plasmid vectors.

Ligation

Ligation was done in a mixture of 66 mM tris-HCl (pH 7.6), 6.6 mM $MgCl_2$, 10 mM dithiothreitol, 0.1 mM ATP, the DNA substrates, and indicated percentages (w/v) of PEG 6000.

Examination of the effects of divalent cations or polyamines on ligation in 10% PEG 6000 solutions. The ligation activity was assayed at 16 to 37°C for 30 min with 250 ng of linearized pBR 322 DNA in 20 µl of the mixture containing different amounts of either cations or polyamines, and 10% PEG 6000. The ratio of j/i was 2.4.

Insertion of E. coli chromosome DNA (3000 to 6000 bp) into the EcoRI arm of λ gt11 DNA. Ligation was done at 26°C in 15 µl of the mixture containing 250 ng of dephosphorylated λ gt11 DNA, 18.75 ng of EcoRI-digested E. coli chromosome DNA, indicated amounts of monovalent cations, divalent cations or polyamines, and 10% PEG 6000. The ligated DNA (4 µl) was used for in vitro packaging.

Self-circularization. 250 ng of ScaI-digested pBR 322 DNA was ligated at 16°C in 45 to 75 µl of the mixture containing different percentages of PEG 6000. The ratio of j/i was 5.5 to 9.1.

Insertion of DNA fragment containing Tet^r gene into pUC 18 DNA. Ligation of the DNA fragment containing Tet^r gene and the dephosphorylated pUC 18 DNA was done at 16°C in 45 to 75 µl of the mixture containing different percentages of PEG 6000. The ligated DNA was used for transformation.

Insertion of BglII linker DNA into the PvuII site of pBR 322 DNA. The self-complementary phosphorylated BglII linker DNA, d(pC-A-G-A-T-C-T-G) was ligated to the PvuII site of pBR 322 DNA at 10°C in 45 to 75 µl of the mixture containing 10% PEG 6000. The ligated DNA was digested by PvuII, and then the DNA was used for transformation.

Agarose gel electrophoresis

Details of the procedure are described elsewhere (2).

In vitro packaging

In vitro packaging was done using a λ DNA in vitro packaging kit (Amersham International plc.). E. coli Y1090 (r-) (12) was used as the indicator bacterium. After infection of the phage into the indicator strain, cells were plated on medium containing the chromogenic indicator 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal). The plate was incubated at 37°C for 12-15 hr. The in vitro packaging kit used in this experiment could give 1.0 x 10^8 plaques/μg of concatemeric λ DNA (cI857 S7).

Transformation

The ligated DNA (up to 50 ng) was transformed into E. coli HB101 competent cells prepared with $CaCl_2$ and $RbCl_2$ (13) with some modifications and the cells were plated on an LB-plate (10 g of Bacto-tryptone, 5 g of yeast extract, 5 g of NaCl, 1 g of glucose, and 15 g of agar per liter, pH 7.5) containing 50 μg/ml ampicillin and 5 μg/ml tetracycline. The plate was incubated at 37°C for 12-15 hr. The competence of the cells used in this experiment was 1.0 x 10^7 transformants/μg of pBR 322 DNA RFI.

RESULTS

Stimulation of intermolecular ligation in 10% PEG 6000 solutions

Effects of divalent cations or polyamines on ligation. We investigated the influence of divalent cations such as Mg^{2+} and Ca^{2+} and those of polyamines such as spermidine (3+) and spermine (4+) on ligation with T4 DNA ligase in 10% PEG 6000 solutions and compared the results to those of monovalent cations. Na^+ or K^+ both promote intermolecular ligation in 10% PEG 6000 solutions; ligation is maximum at 150-200 mM Na^+ or 200-250 mM K^+ at 16°C. The minimum amount of the enzyme needed to ligate cohesive- or blunt-ended DNA substrate almost completely with monovalent cations at 16°C for 30 min was 0.35 units or 7.0 units, respectively (2). Similar stimulation was also caused by divalent cations or polyamines. Figure 1 shows the effects of divalent cations or polyamines on blunt-end ligation with PvuII-digested pBR 322 DNA at 16°C. The concentrations of divalent cations or polyamines at which the extent of ligation was maximum were as follows: 15-25 mM for

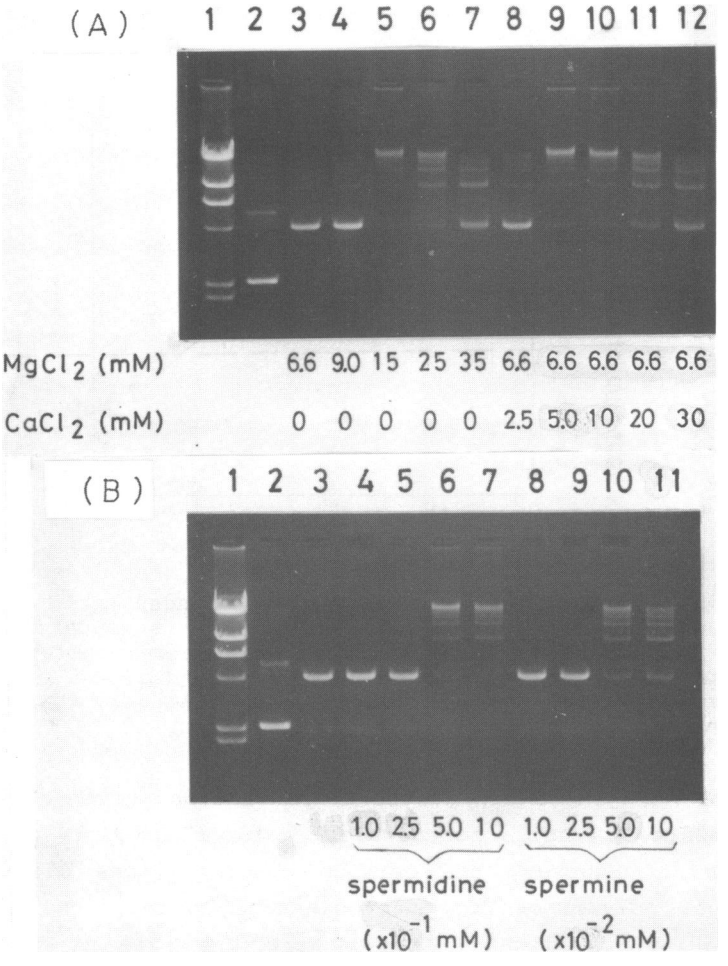

Figure 1. Effects of divalent cations(A) and polyamines(B) on blunt-end ligation in 10% PEG 6000 solutions. Assay mixtires were as in Methods, and contained PvuII-digested pBR 322 DNA and only 10% PEG 6000. Divalent cations or polyamines were present at the concentrations indicated. The amount of T4 DNA ligase added to mixtures was as follows: 1.4 units for (A) and 0.35 units for (B). Incubation was at 16°C for 30 min. HindIII-digested λ DNA, Lane 1; pBR 322 DNA RFI, Lane2; control with 6.6 mM of Mg^{2+}, Lane 3.

Mg^{2+}, 5-10 mM for Ca^{2+}, 0.5-1.0 mM for spermidine, and 0.05-0.1 mM for spermine. All of these concentrations except for Mg^{2+}, were in the presence of 6.6 mM of Mg^{2+}. The minimum amount of the enzyme necessary to bring the extent of blunt-end ligation

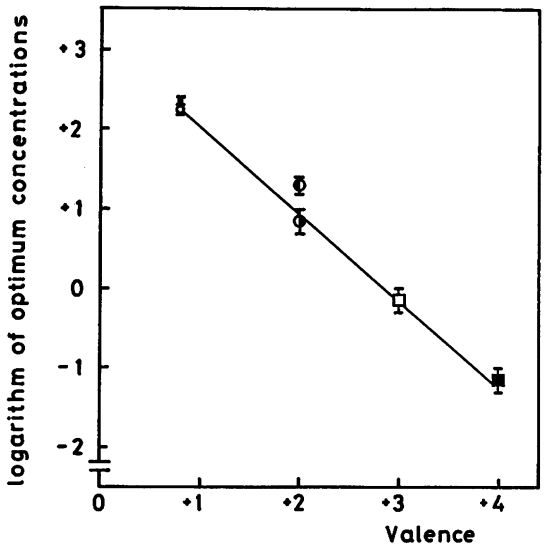

Figure 2. Correlation between optimum concentrations of the cations or polyamines at which ligation was stimulated in 10% PEG 6000 solutions and their valence. Concentrations except for Mg^{2+} were in the presence of 6.6 mM Mg^{2+}. (o) Na^{+}, (•) K^{+}, (◐) Mg^{2+}, (◑) Ca^{2+}, (□) spermidine(3+), and (■) spermine(4+).

to 90% or more in 30 min at the concentrations given above were as follows: 1.4 units for divalent cations and 0.35 units for polyamines. However, the optimum concentrations of divalent cations or polyamines for intermolecular cohesive-end ligation with EcoRI-digested pBR 322 DNA were the same as those for intermolecular blunt-end ligation, the minimum amount of the enzyme needed to ligate the DNA substrate almost completely in 30 min was 0.35 units in each case (data not shown). Temperature had an influence on divalent cations or polyamines induced ligation efficiency in th same way as when done with monovalent cations. As the temperature was raised from 16 to 26 or 37 °C, the extent of intermolecular ligation caused by the cations or polyamines increased, and the concentrations of the cations or polyamines that effected such ligation could be at low concentrations (data not shown). We also found that there was correlation between the optimum concentrations of monovalent cations, divalent cations, or polyamines and their valence;

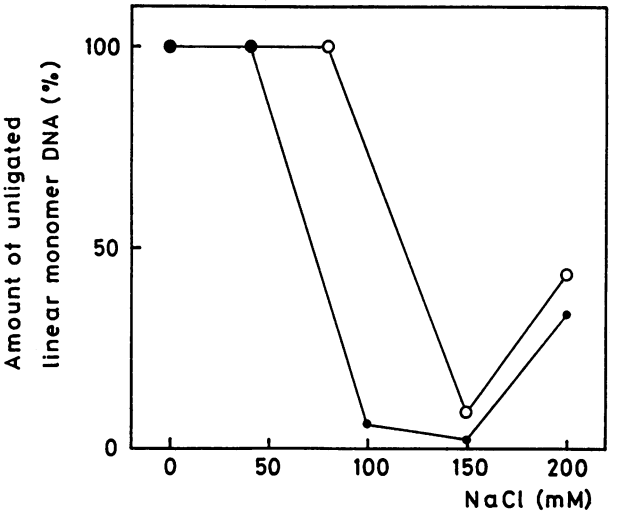

Figure 3. Effects of Na^+ on blunt-end ligation with T4 DNA ligase in 10% PEG 6000 with or without spermidine. Assay mixtures were as in Methods, and contained PvuII-digested pBR 322 DNA and 10% PEG 6000. NaCl was present at the concentrations indicated, and T4 DNA ligase (7.0 U) was added. Incubation was at 16°C for 30 min. The amount of unligated linear monomer DNA was measured using a densitometer. (○) spermidine-free, (●) 0.1 mM spermidine.

their concentrations decreased logarithmically as thier valence increased (Fig.2).

Ligation in the presence of both polyamine and monovalent cation. The effects of Na^+ on blunt-end ligation were examined in a mixture containing 0.1 mM or 1.0 mM spermidine and 10% PEG 6000. Figure 3 shows the effects of Na^+ at 16°C when 0.1 mM of spermidine was either present or absent. Although intermolecular ligation was not stimulated at 100 mM Na^+ without spermidine, stimulation occured at 100 mM Na^+ when 0.1 mM of spermidine was present. In the presence of 1.0 mM of spermidine, the extent of ligation decreased as the concentration of Na^+ increased, and ligation was completely inhibited at 150 mM Na^+ using 0.35 units of the enzyme (data not shown).

Effects of ligation with cations or polyamines in 10% PEG 6000 solutions on in vitro packaging

When a phage vector is used, the ligation products must be

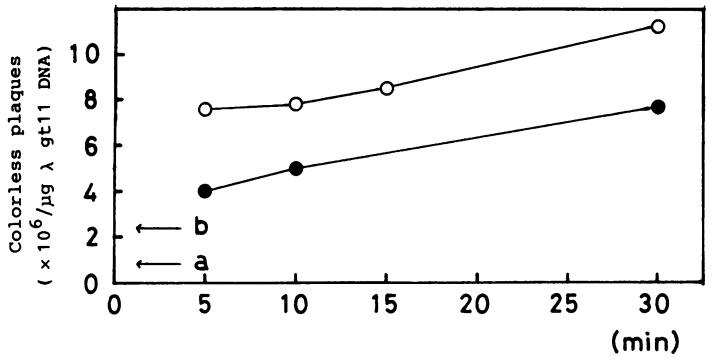

Figure 4. Effects of ligation with 10% PEG 6000 and 150 mM Na^+ on cloning of EcoRI-digested E. coli chromosome DNA (3000 to 6000 bp) into the EcoRI arm of λ gt11 DNA. The ligation was done as in Methods, with 150 mM NaCl. Incubation was at 26°C for 5 to 30 min. The amount of the ligase added to the mixtures is given here; (●) 0.35 units and (○) 7.0 units. The arrows are for the plaque-forming efficiency with 7.0 units (a) and 350 units (b) of the ligase in the usual ligation buffer without PEG 6000 and Na^+ at 16°C for 15 hr.

linear oligomers, because the packaging efficiency with circular monomer is very low (14). We expected the ligation with 10% PEG 6000 and cations or polyamines to be very effective for in vitro packaging. We first examined the ligation in 10% PEG 6000 solution containing 150 mM Na^+ for cloning of EcoRI-digested E. coli chromosome DNA with λ gt11 DNA. Only a 5 min of ligation with 0.35 units of the ligase at 26°C gave more plaque-forming efficiency than the usual ligation with 350 units of the ligase for 15 hr (Fig. 4). Similar effects were obtained with K^+, spermidine, and spermine, but divalent cations were not effective (Table 1).

Effects of ligation with PEG 6000 on self-circularization

Both inter- and intramolecular ligation occur in 10% PEG 6000 solutions when cations or polyamines are absent, and the ligation is inhibited as the temperature is raised from 16 to 37°C (2). We examined the effects of PEG 6000 on ligation with different amounts of T4 DNA ligase. Figure 5 shows the results by agarose gel electrophoresis. There was no difference in the ligation in the different PEG 6000 solutions when 70 units of the ligase was added; both inter- and intramolecular ligation

Table 1. Efficiency of cloning of EcoRI-digested E. coli chromosome DNA into the EcoRI arm of λ gt11 DNA by ligation with 10% PEG 6000 and cations or polyamines.

Cations or polyamines	Colorless plaques/μg of λ gt11 DNA
150 mM Na^+	4.0×10^6
200 mM K^+	3.1×10^6
20 mM Mg^{2+}	0.2×10^6
7.5 mM Ca^{2+}	0.3×10^6
0.75 mM spermidine	5.6×10^6
0.075 mM spermine	5.1×10^6

Ligation was done as in Methods, with 0.35 units of the ligase at 26 °C for 5 min.
Except for the case with Mg^{2+}, 6.6 mM Mg^{2+} was present.

occurred in all solutions (Fig. 5, Lanes 2, 4, 6, and 8). However, the extent of ligation after 60 min in PEG 6000 solutions with 70 units of the ligase was higher than after 14 hr in the usual ligation solution without PEG but with 350 units of the ligase (Fig. 5, Lane 10). In 10% PEG 6000 solutions, intermolecular ligation was enhanced and intramolecular ligation was inhibited when 350 units of the ligase was added (Fig. 5, Lane 9). The transformation of these ligation products into E. coli HB101 also indicated that intermolecular ligation was stimulated when an excess of the ligase was added even in a 10% PEG 6000 solution without cations or polyamines; transformation efficiency with 350 units of the ligase in 10% PEG 6000 solution was lower than with the same amount of the ligase in 6.0% to 9.2% PEG 6000 solutions or with 70 units of the ligase in 6.0% to 10% PEG 6000 solutions (Fig. 6). We next examined the effects of time length of ligation on transformation in 6.0% to 9.2% PEG 6000 solutions. The incubation time was 10, 30, or 60 min with 70 or 350 units of the ligase. The number of the transformants obtained by 30 min of incubation with 70 units of the ligase was one-half that obtained by 60 min of incubation with the same amount of the ligase. There was no difference

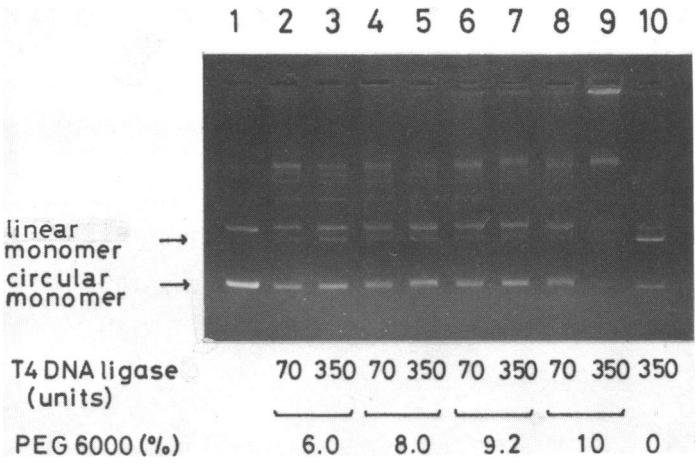

Figure 5. Effects of the amount of T4 DNA ligase on ligation of ScaI-digested pBR 322 DNA in PEG 6000 solutions. Assay mixtures were as in Methods, and contained the indicated percentages of PEG 6000. The amount of the ligase added to the mixtures is given here. Incubation was at 16°C for 60 min except for ligation in the usual buffer without PEG 6000. pBR 322 DNA RFI, Lane 1; ligation with 350 units of the ligase for 14 hr in the usual buffer without PEG 6000, Lane 10.

between 10 and 60 min of incubation when 350 units of the ligase was added (data not shown).

Effects of ligation with PEG 6000 on the insertion of a DNA fragment into plasmid DNA

We examined the effects of ligation in PEG 6000 solutions on the insertion of cohesive-ended DNA fragment containing Tet^r gene into pUC 18 DNA. The ligation between cohesive-ended DNA fragment containing Tet^r gene (1428 bp) and cohesive-ended and dephosphorylated pUC 18 DNA (2670 bp) was measured in PEG 6000 solutions at 16°C for 30 min with 7.0, 70, or 350 units of the ligase. Figure 7 shows the transformation efficiency when the ligation products in the reaction mixtures containing 140 ng of the pUC 18 DNA and 80 ng of the DNA fragment containing Tet^r gene were transformed into E. coli HB101. There were more transformants by ligation in 6.0% to 10% PEG 6000 solutions than by the usual ligation with 350 units of the ligase for 4 hr, and less by ligation in 9.2% and 10% PEG 6000 solutions with 350 units of the ligase than by ligation in 6.0% to 10% PEG 6000

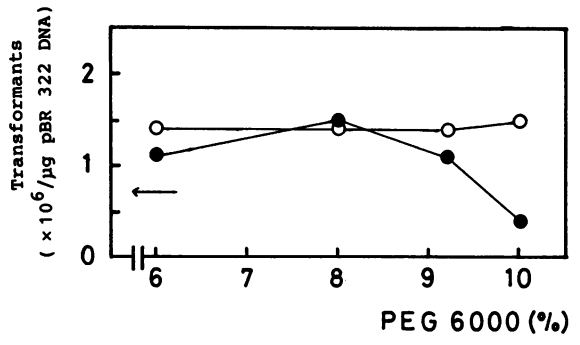

Figure 6. Transformation efficiency with ligation products of ScaI-digested pBR 322 DNA in PEG 6000 solutions. (○) 70 units of the ligase and (●) 350 units of the ligase. The arrow is for the efficiency of transformation with ligation products using 350 units of the ligase at 16°C for 14 hr in the usual ligation buffer without PEG 6000.

solutions with 70 units of the ligase or in 6.0% to 8.3% PEG 6000 solutions with 350 units of the ligase (Fig. 7). These results showed that stimulation of intermolecular ligation

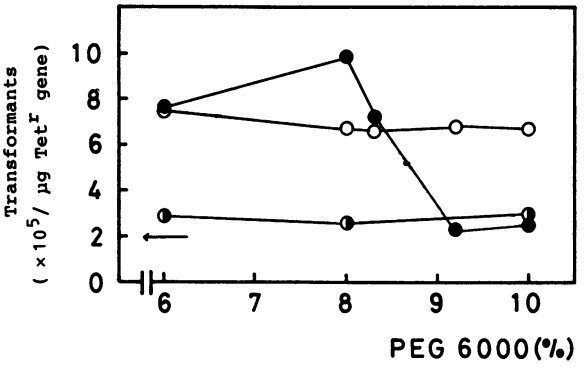

Figure 7. Effects of ligation with PEG 6000 on cloning of cohesive-ended DNA fragment containing Tet^r gene into pUC 18 DNA. The ligation of the DNA fragment containing Tet^r gene and the pUC 18 DNA was measured in the mixtures as in Methods; they contained 80 ng of the DNA fragment containing Tet^r gene, 140 ng of the pUC 18 DNA, and the indicated percentages of PEG 6000. The amount of the ligase added to the mixtures is given here; (◑) 7.0 units, (○) 70 units, and (●) 350 units. The arrow is for the efficiency of transformation with ligation products using 350 units of the ligase at 16°C for 4 hr in the usual ligation buffer without PEG 6000.

Table 2. Efficiency of cloning of blunt-ended DNA fragment containing Ter^r gene into the HincII site of pUC 18 DNA by ligation with PEG 6000 compared to results by the usual ligation.

pUC 18 (ng) :	Tet^r gene (ng)	Transformants/μg of Tet^r gene 10% PEG 6000(a)	PEG 6000 none(b)
70 :	50	1.1×10^5	0.2×10^5
140 :	50	1.2×10^5	0.5×10^5
350 :	50	1.6×10^5	0.5×10^5
700 :	50	0.2×10^5	----NT
350 :	0	0	----NT

a: Ligation was done in 10% PEG 6000 solution at 16°C for 30 min with 70 units of the ligase.
b: Ligation was done in the usual ligation buffer without PEG 6000 at 16°C for 16 hr with 350 units of the ligase.
NT: not tested.

depended on the amount of the ligase in PEG solution. Ligation with PEG 6000 was also effective for insertion of the blunt-ended DNA fragment into the vector DNA. We ligated blunt-ended DNA fragment containing Tet^r gene (2583 bp) and also blunt-ended and dephosphorylated pUC 18 DNA (2686 bp) with 70 units of the ligase in 10% PEG 6000 solutions. Table 2 shows results compared to those of the usual ligation without PEG 6000

Table 3. Efficiency of insertion of BglII linker DNA into the PvuII site of pBR 322 DNA by ligation with PEG 6000 compared to results by the usual ligation.

pBR 322 (ng) :	BglII linker (ng)	Transformants/μg of pBR 322 DNA 10% PEG 6000(a)	PEG 6000 none(b)
125 :	120	1.2×10^4	0.3×10^4
250 :	120	1.5×10^4	0.6×10^4

a: Ligation was done in 10% PEG 6000 solution at 10°C for 30 min with 70 units of the ligase.
b: Ligation was done in the usual ligation buffer without PEG 6000 at 10°C for 16 hr with 350 units of the ligase.

when the ratio between the pUC 18 DNA and the DNA fragment containing Tet^r gene was varied.

Ligation with PEG 6000 could also be applied to the insertion of linker DNA. We constructed a BglII site on pBR 322 DNA, which has no cutting site for BglII, using BglII linker DNA. Ligation of the BglII linker DNA and PvuII-digested pBR 322 DNA was examined in 10% PEG 6000 solutions. The ligation in 10% PEG 6000 solution gave higher transformation efficiency than the usual ligation when linker DNA was an insert (Table 3). The plasmid DNA isolated from the transformants were shown to be digested by BglII.

DISCUSSION

In 10% PEG 6000 solutions, divalent cations or polyamines also enhanced intermolecular ligation similar to monovalent cations. Such stimulation with cations or polyamines is brought about by an increase in the affinity of the enzyme for the DNA substrate. That only intermolecular ligation was enhanced even in 10% PEG 6000 solution without cations or polyamines except for the minimum amount of Mg^{2+} when an excess of the ligase was added indicated that the presence of cations or polyamines increased the concentration of the enzyme around the DNA substrate. However, it is unclear by what mechanism the affinity is increased by cations or polyamines in PEG solutions. The volume-excluding effect of PEG (15) and DNA condensation by polyamines (16-18) or high polymers (19, 20) seem to be closely related to this stimulation. Changes in the higher structure of DNA can occur when the negative charges of DNA phosphate are neutralized by polyamines. We think that neutralization of the negative charges of the DNA phosphate by cations or polyamines is important in increasing the affinity of the enzyme for the DNA substrate in 10% PEG 6000 solutions. The following results strongly support this idea: there was correlation between the concentrations of cations or polyamines at which intermolecular ligation was promoted and their valence, and the optimum concentration of Na^+ for such ligation extended downward when 0.1 mM of spermidine was present in 10% PEG 6000 solutions. We think the probability of the DNA terminus of one molecule

approaching that of another would be increased by neutralization of the DNA phosphate with cations, because such neutralization compensates for the elsctrostatic repulsion of the negatively charged DNA. Moreover, divalent cations or polyamines may fix the distance between the DNA terminus of one molecule and that of another by binding to 5'-phosphate terminus of one molecule and 3'-hydroxyl terminus of a neighboring molecule.

The properties of T4 DNA ligase in 10% PEG 6000 solution were very useful in making recombinant DNA, because inter- and intramolecular ligation could be easily regulated by the concentration of cations or polyamines, and because the time required to complete ligation was very short. Ligation in 10% PEG 6000, particularly with cations or polyamines, stimulated intermolecular ligation when the concentration of DNA was very low, so *in vitro* packaging was effective even when the concentration of the phage vector DNA was low. However, ligation in 10% PEG 6000 solution with divalent cations was less effective than with monovalent cations or polyamines for cloning the gene into the phage vector by *in vitro* packaging. This result seems to arise from inhibition of *in vitro* packaging of the ligated DNA.

ACKNOWLEDGEMENTS

We thank other members of our laboratory for supporting this work.

REFERENCES

1. Dugaiczyk, A., Boyer, H.W., and Goodman, H.M. (1975) J. Mol. Biol. 96, 174-184.
2. Hayashi, K., Nakazawa, M., Ishizaki, Y., and Obayashi, A. (1985) Nucl. Acids Res. 13, 3261-3271.
3. Hayashi, K., Nakazawa, M., Ishizaki, Y., Hiraoka, N., and Obayashi, A. (1985) Nucl. Acids Res. 13, 7979-7992.
4. Wilson, G.G., and Murray, N.E. (1979) J. Mol. Biol. 132, 471-491.
5. Murray, N.E., Bruce. S.A., and Murray, K. (1979) J. Mol. Biol. 132, 493-505.
6. Nakata, A., Yamaguchi, M., Izutani, K., and Amemura, M. (1982) J. Bacteriol. 134, 287-294.
7. Guerry, P., LeBlanc, D., and Falkow, S. (1973) J. Bacteriol. 116, 1064-1066.
8. Thomas, M., and Davis, R.W. (1975) J. Mol. Biol. 91, 315-328.
9. Yanisch-Perron, C., Vieira, J., and Messing, J. (1985)

Gene 33, 103-119.
10. Berns, K.I., and Thomas, C.A.Jr. (1965) J. Mol. Biol 11, 476-490.
11. Young, R.A., and Davis, R.W. (1983) Proc. Natl. Acad. Sci. USA 80, 1194-1198.
12. Young, R.A., and Davis, R.W. (1983) Science 222, 778-782.
13. Kushner, S.R. (1978) Genetic Engineering, Boyer, H.B., and Nicosia, S. Eds., pp 17-23, Elsevier, Amsterdam
14. Feiss, M., Fisher, R.A., Clayton, M.A., and Enger, C. (1977) Virology 77, 281- 293.
15. Minton, A.P. (1981) Biopolymers 20, 2093-2120.
16. Gosule, L.C., and Schellman, J.A. (1978) Nature 259, 333-335.
17. Gosule, L.C., and Schellman, J.A. (1978) J. Mol. Biol. 121, 331-326.
18. Chattoraj, D.K., Gosule, L.C., and Schellman, J.A. (1978) J. Mol. Biol. 121, 327-337.
19. Lerman, L.S. (1971) Proc. Natl. Acad. Sci. USA 68, 1886-1890.
20. Lerman, L.S. (1973) Cold Spring Harbor Symp. Quant. Biol. 38, 59-73.