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J. Am. Chem. Soc., 1996, 118(47), 11701-11714, DOI:10.1021/ja961788h

Table 1. Quantification of the Ligation Product Resulting from Treatment of the Purified Topoisomerase I - DNA Complex with Acceptor Oligonucleotides **I** - **IV**, as a Function of pH

	pH	% ligation[a]
I	7.5	100
	8.0	96.7
	8.5	85.8
	9.0	91.5
II	7.5	32.8
	8.0	33.7
	8.5	31.2
	9.0	32.6
III	7.5	3.8
	8.0	3.1
	8.5	2.5
	9.0	3.1
IV	7.5	2.6
	8.0	4.0
	8.5	8.4
	9.0	12.0

[a]The calculated values were normalized to the ligation value obtained for **I**.

Legends to Figures

Figure 1. Autoradiogram of a 20% denaturing polyacrylamide gel demonstrating cleavage of the 5' ^{32}P-end labeled partial duplex and subsequent ligation of acceptor oligonucleotides **I** and **II**: lane 1, partial duplex + **I** in the absence of topoisomerase I; lane 2, partial duplex + **I** in the presence of topoisomerase I; lane 3, partial duplex + **II** in the absence of topoisomerase I; lane 4, partial duplex + **II** in the presence of topoisomerase I. The heterogeneity of the cleavage bands reflects incomplete digestion by proteinase K.[8]

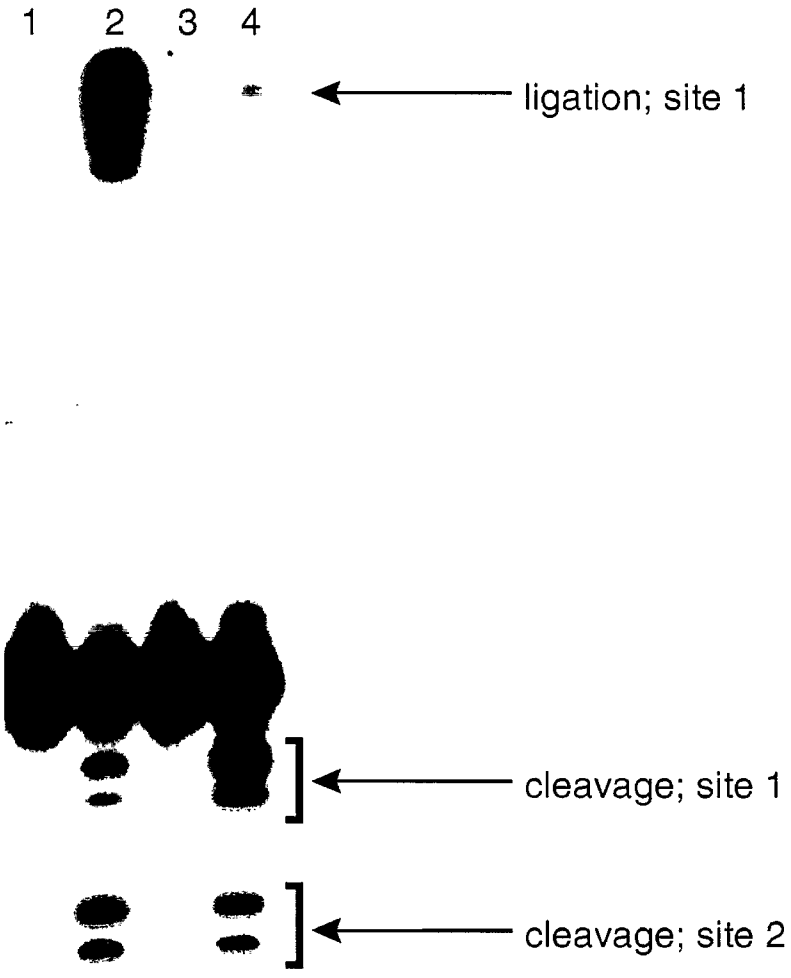
Figure 2. Autoradiogram illustrating DNA sequence analysis of the product resulting from ligation of the acceptor strand to the truncated DNA - enzyme binary complex.

Figure 3. Phosphorimager analysis of the gel illustrated as Figure 5 in the text.

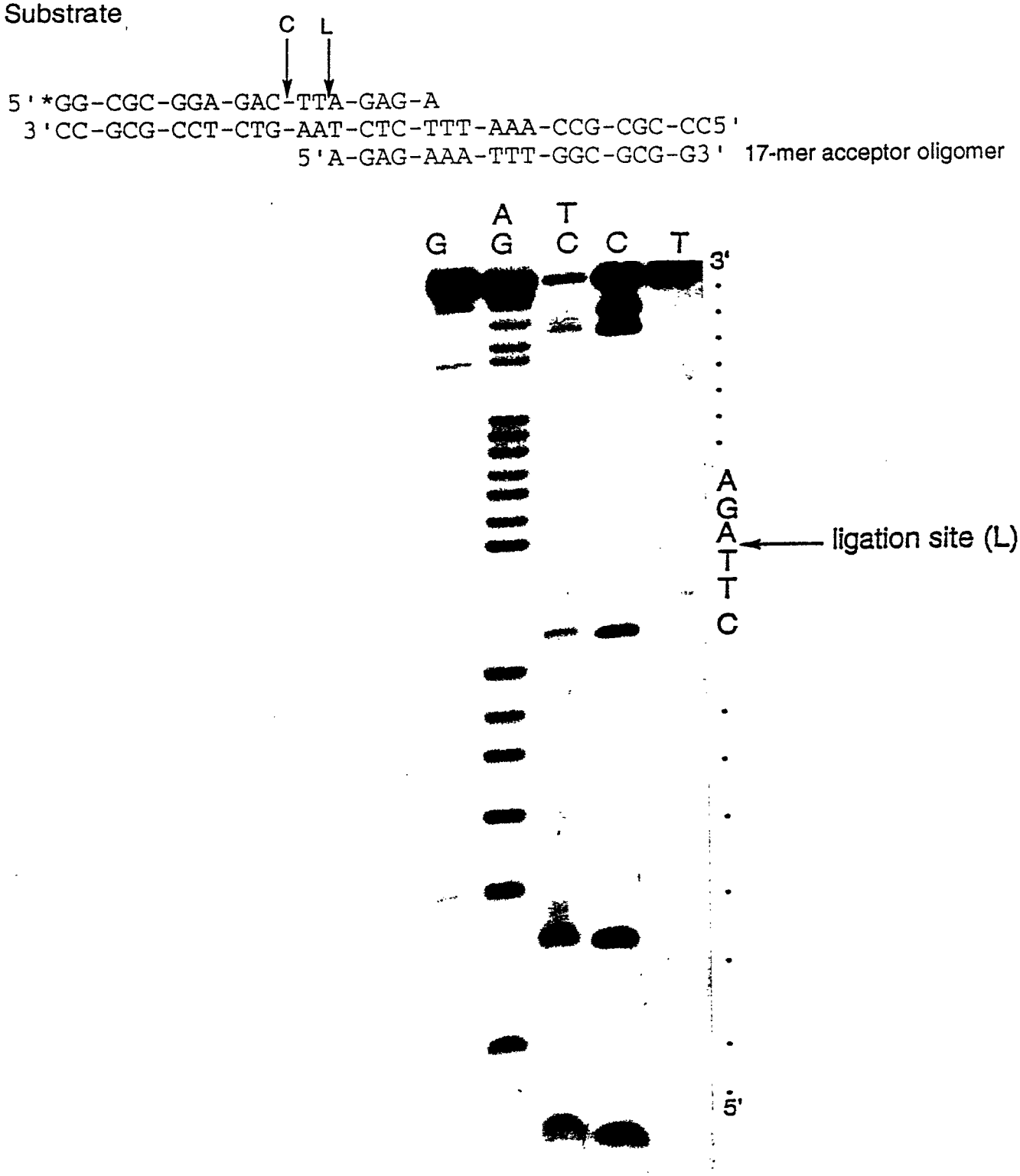
Figure 4. Phosphorimager analysis of a 20% denaturing polyacrylamide gel illustrating the ligation of **I** and **III** as a function of Mg^{2+} and Mn^{2+} utilizing the 5'-^{32}P end labeled topoisomerase I - DNA complex: lane 1, topoisomerase I - DNA complex + **III** in the presence of Mn^{2+}; lane 2, topoisomerase I - DNA complex + **III** in the presence of Mg^{2+}; lane 3, topoisomerase I - DNA complex + **I** in the presence of Mn^{2+}; lane 4, topoisomerase I - DNA complex + **I** in the presence of Mg^{2+}.

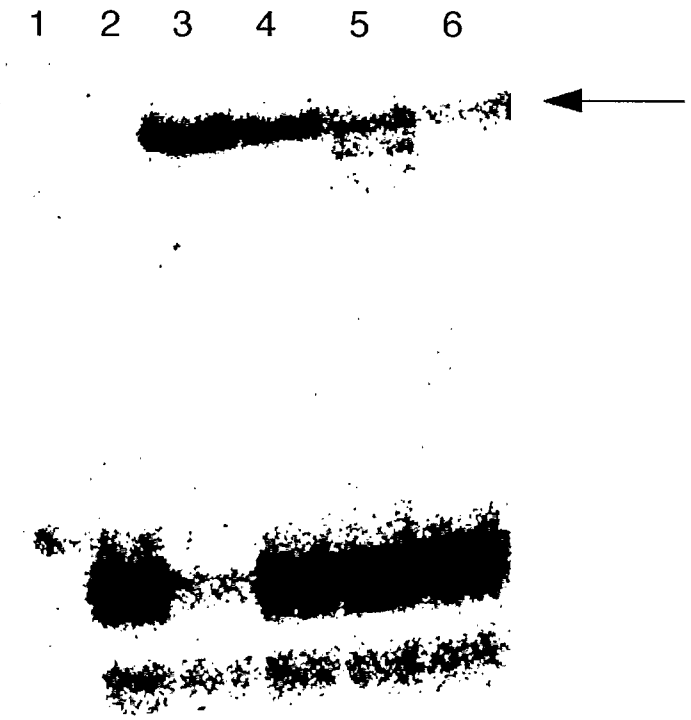
Figure 5. Phosphorimager quantification of the ligation of **IV** as a function of pH.

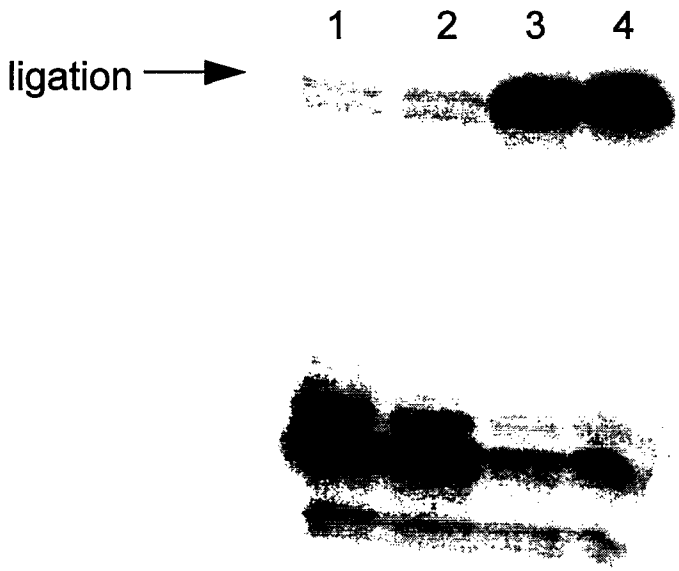
Figure 6. Autoradiogram of a 20% denaturing polyacrylamide gel illustrating exonuclease III digestion of the enzymatic ligation products derived from acceptors **I** and **II**, in comparison with the digestion products of authentic synthetic standards. Lane 1, G; lane 2, A + G; lane 3, exonuclease III treatment of the product resulting from ligation of oligonucleotide **I** with the topoisomerase I - DNA covalent complex; lane 4, exonuclease III treatment of the product resulting from ligation of oligonucleotide **II** with the topoisomerase I - DNA covalent complex; lane 5, exonuclease III treatment of the authentic synthetic standard containing nucleoside **2a** at site 1; lane 6, exonuclease III treatment of the authentic synthetic standard containing normal 3' → 5' phosphodiester bonds at all positions. The additional site of stalling in the enzymatic product lane (lane 4) was also observed with the synthetic product in other experiments.

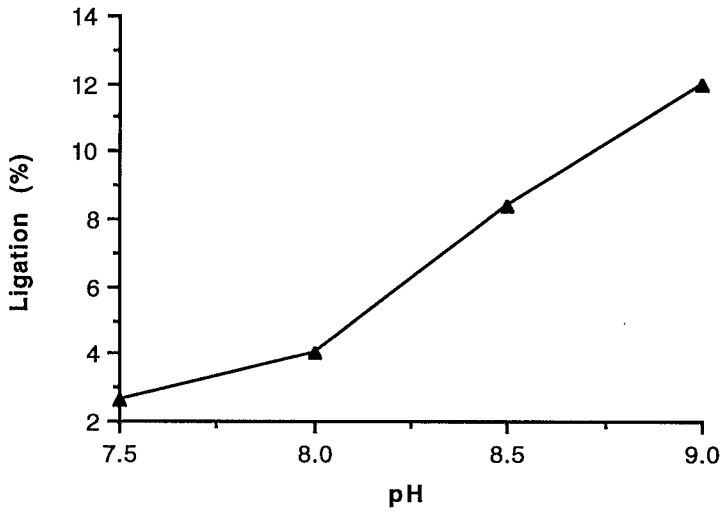

1
2
3
4
ligation; site 1
cleavage; site 1
cleavage; site 2

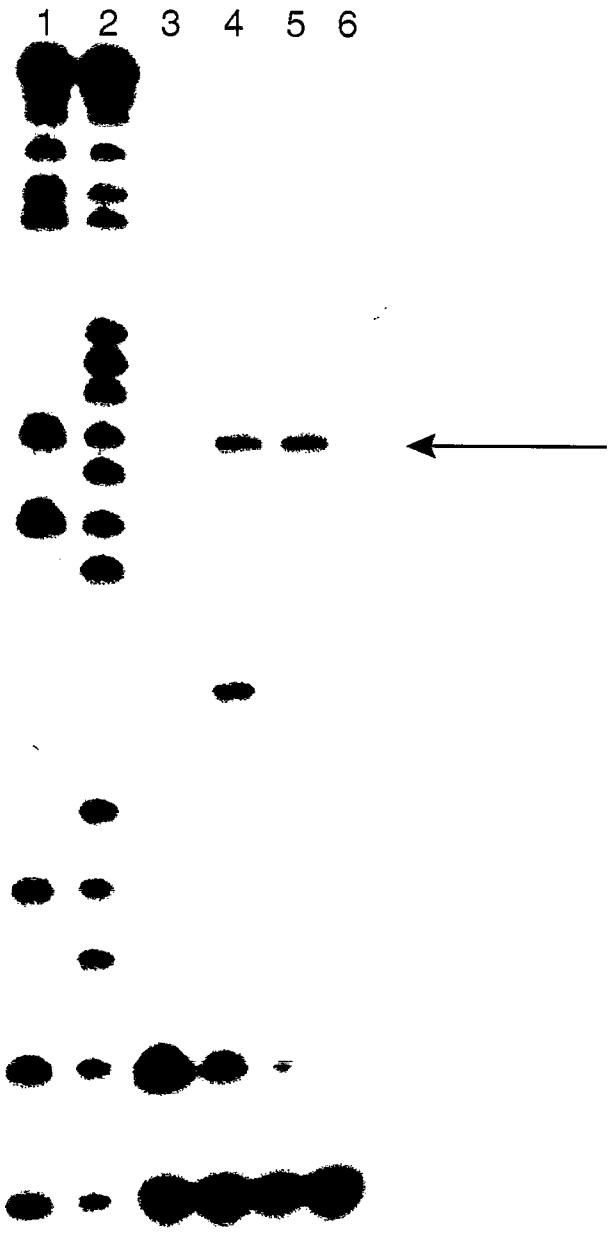
Substrate


C
L
5'*GG-CGC-GGA-GAC-TTA-GAG-A
3'CC-GCG-CCT-CTG-AAT-CTC-TTT-AAA-CCG-CGC-CC5'
5'A-GAG-AAA-TTT-GGC-GCG-G3'
17-mer acceptor oligomer
A T
G G C C T
3'
A
G
A
T
T
C
ligation site (L)
5'


1 2 3 4 5 6


1
2
3
4
ligation


14
12
10
8
6
4
2
Ligation (%)
7.5
8.0
8.5
9.0
pH


1 2 3 4 5 6