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Solid Supported Chemical 5'-Phosphorylation of Oligodeoxy-Ribonucleotides that can be Monitored by Trityl Cation Release Application to Gene Synthesis

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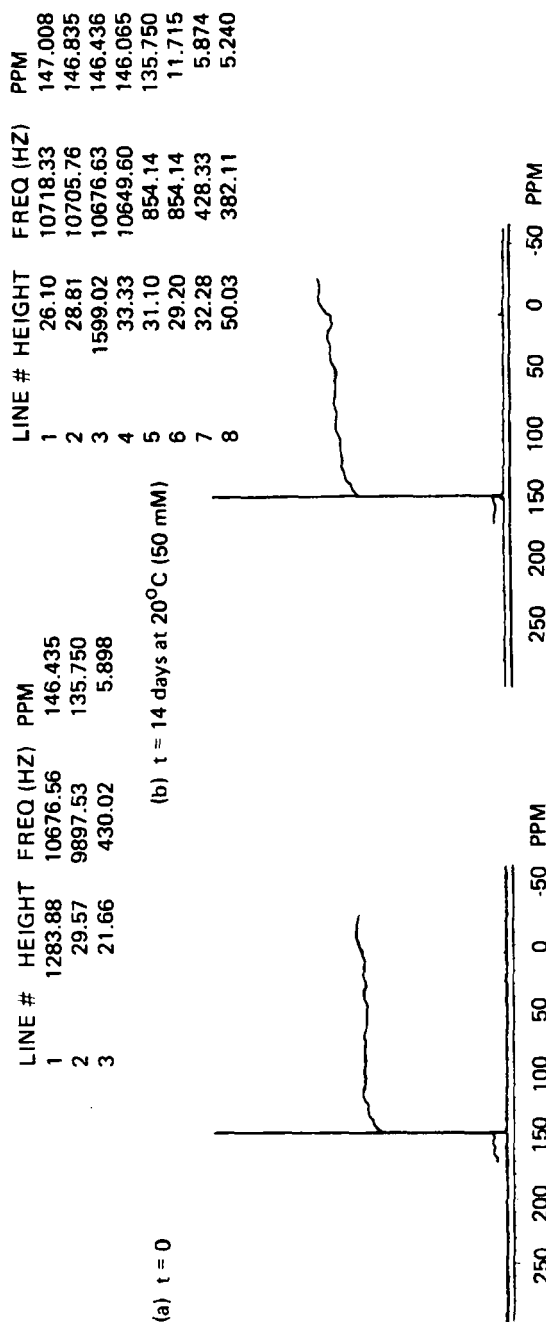
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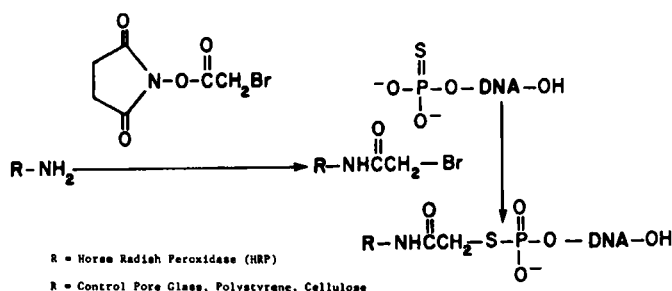
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ABSTRACT: The gene for human lysozyme was assembled from oligonucleotides that were chemically phosphorylated with a novel phosphorylation reagent.

In order to circumvent the use of T4 polynucleotide kinase and ATP for the 5'-phosphorylation of synthetic oligodeoxyribonucleotides after deprotection and purification, we and others (1,2) have developed chemical phosphorylation reagents that can be used on automatic DNA synthesis instruments. However there is no convenient way to determine the coupling efficiency of these compounds.

We now report a phosphorylation procedure that can be monitored by the release of the orange dimethoxytrityl cation after acid treatment (3). The phosphite-derived compound (2-cyanoethoxy)-2-(2'-O-4,4'-dimethoxytrityloxyethyl sulfonyl)ethoxy-N,N-diisopropylaminophosphine was designed

FIGURE 1. ^{31}P -NMR of phosphorylating compound.



SCHEME I.

and synthesized. The phosphitylating compound is very stable as determined by ^{31}P -NMR of a 50 mM solution in dry acetonitrile after a period of 14 days at 20 C (Figure 1). The reagent is coupled to solid-supported fragments and oxidized under the same conditions as nucleoside phosphoramidites. The resultant 5'-phosphate oligomers are then fully deprotected in standard ammonium hydroxide solution at elevated temperature.

Reverse-phase HPLC, silica gel TLC and ^{31}P -NMR analyses of chemically phosphorylated thymidine were compared with a variety of phosphorus derivatives of thymidine. All evidence suggested that the nucleoside was converted to the 5'-phosphate form quantitatively with the reagent. By substituting sulfur for iodine in the oxidation step (4) the 5'-phosphorothioate was obtained in high yield.

The 5'-phosphorothioate oligonucleotides can be used with maleimido- and bromoacetyl-containing reagents to

LeuAlaArgThrLeuLysArgLeuGlyMetAspGlyTyrArgGlyIleSerLeuAlaAsn
 HL-1(47) p
 2 CTAGCTAGAACTTTGAAGAGATTGGGTATGGACGGTTACAGAGGTATCTCCTTGGCTAAC
 GATCTTGAAACTTCTCTAACCATACCTGCCAATGTCTCCATAGAGGAACCGATTG
 HL-9*(19) p HL-10*(48)
 TrpMetCysLeuAlaLysTrpGluSerGlyTyrAsnThrArgAlaThrAsnTyrAsnAla
 HL-2(48) p HL-3(48)
 62 TGGATGTGTTTGGCCAAGTGGGAATCTGGTTACAACACCAGAGCTACCAACTACAACGCT
 ACCTACACAAACCGGTTACCCCTTAGACCAATGTTGTGGTCTCGATGGTTGATGTTGCGA
 p HL-11*(48) p¹
 GlyAspArgSerThrAspTyrGlyIlePheGlnIleAsnSerArgTyrTrpCysAsnAsp
 p HL-4(47)
 122 GGTGACAGATCTACCGACTACGGTATCTTCCAAATCAACTCCAGATACTGGTGTAAACGAC
 CCACTGTCTAGATGGCTGATGCCATAGAAGGTTTAGTTGAGGTCTATGACCACATGCTG
 HL-12*(47) p¹
 GlyLysThrProGlyAlaValAsnAlaCysHisLeuSerCysSerAlaLeuLeuGlnAsp
 p HL-5(47) p
 182 GGTAAGACCCCAGGTGCTGTAAACGCTTGTCACCTTGTCCTGTTCTGCTTTGTTGCAAGAC
 CCATTCTGGGTCCACGACAATTGCGAACAGTGAACAGGACAAGACGAAACAACGTTCTG
 HL-13*(47) p HL-14*(47)-
 AsnIleAlaAspAlaValAlaCysAlaLysArgValValArgAspProGlnGlyIleArg
 HL-6(47) p
 242 AACATCGCTGACGCTGTCGCCTGTGCTAAGAGAGTTGTTAGAGACCCACAAGGTATCAGA
 TTGTAGCGACTGCGACAGCGGACGATTCTCTCAACAATCTCTGGGTGTTCCATAGTCT
 p HL-15*(47)
 AlaTrpValAlaTrpArgAsnArgCysGlnAsnArgAspValArgGlnTyrValGlnGly
 HL-7(47) p HL-8(42)-
 302 GCTTGGGTGCTTGGAGAAACAGATGTCAAAACAGAGACGTTAGACAATACGTCCAAGGT
 CGAACCCAAACGAACCTCTTTGTCTACAGTTTGTCTCTGCAATCTGTTATGCAGGTTCCA
 p HL-16*(47) p¹
 CysGlyValOC
 362 TGTGGTGTTTAAG
 ACACCACAAATTCAGCTG
 HL-17*(23)

FIGURE 2. Human lysozyme amino acid- and synthetic gene sequences.

introduce non-radioactive labels (such as horse radish peroxidase) and to immobilize DNA to solid supports (5) (See Scheme 1).

A complete gene for human lysozyme (373 bp) was assembled from oligonucleotides that were chemically phospho-

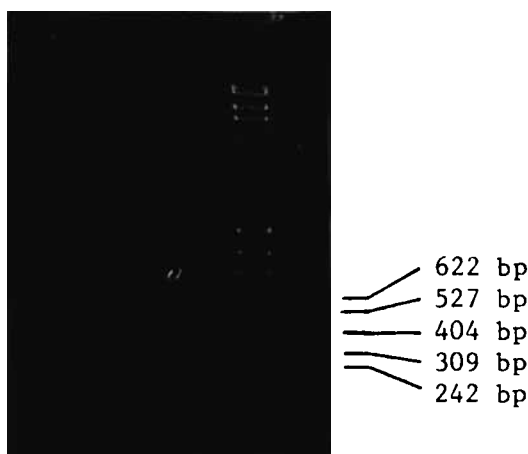


FIGURE 3. Agarose gel analysis of synthetic human lysozyme ligation (lane 1). Lane 2 is pBR322 cut with Hpa2; the sizes are shown on the right. Lane 3 is ϕ X174 DNA cut with Hae3.

rylated with the new reagent. The gene was designed from the amino acid sequence using yeast preferred codons (6). The sequences of the 17 oligomers employed (17 to 48 nucleotides long) are shown in Figure 2, where the chemically phosphorylated 5' ends are indicated with "p". Analysis in a 1.5% agarose gel of the "one-shot" ligation of the 17 fragments shows one band of the appropriate size (Figure 3). Through restriction map analysis, 10 out of 12 clones were shown to contain the correct size insert. Subsequent DNA sequence analysis of two of these clones was in complete accord with the designed oligonucleotides.

REFERENCES

1. T. Horn and M.S. Urdea, *DNA* 5, 421 (1986).
2. E. Uhlmann and J. Engels, *Tetrahedron Letters* 27, 1023 (1986)
3. T. Horn and M.S. Urdea, *Tetrahedron Letters* (1986), in press
4. W.J. Stec, G. Zon, W. Egan and B. Stec, *J. Am. Chem. Soc.* 106, 6078 (1984).
5. M.S. Urdea, unpublished results.
6. J.L. Bennetzen and B.D. Hall, *J. Biol. Chem.* 257, 3026 (1982)