PROTEINS TO ORDER. USE OF SYNTHETIC DNA TO GENERATE SITE-SPECIFIC MUTATIONS

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Introduction

The ability to cause specific changes in the amino acid sequences of proteins would greatly advance studies on the influence of protein structure on biochemical function. If the desired changes can once be made in the nucleic acid which encodes the protein, one can use cloning in an appropriate microorganism to produce essentially limitless quantities of the mutant protein. We describe here the application of oligonucleotide-directed site-specific mutagenesis (1,2) to accomplish this objective for the enzyme β-lactamase, the gene for which is contained in the plasmid pBR322 (3,4). The method uses a procedure to screen for mutant clones which depends on the DNA in the various colonies and not on the properties of the mutant protein; the method can, therefore, be widely applied and does not require, in each separate case, the development of a screening procedure which depends on some phenotypic difference between mutant and wild-type protein.

Methods

Oligonucleotide Synthesis:
The 15-base deoxyoligonucleotide 5'-A ATG ATG ACC TCT TTT-3' was synthesized by the triester method on a solid phase support (5).

Isolation and Cleavage of Plasmid:
Plasmid pBR322 was grown in E. coli strain LS1, a Lac^+, $RecA^+$ derivative of HB101. The supercoiled pBR322 DNA was nicked with restriction endonuclease Hpa II in the presence of ethidium bromide. Phenol extraction removed both protein and ethidium bromide.

Exonuclease Treatment, Heteroduplex Formation and Transformation:
The nicked circles were treated with E. coli exonuclease III. The partially single stranded circles thus generated were annealed to the synthetic deoxyoligonucleotide and used for in vitro DNA synthesis in the presence of the Klenow fragment of E. coli polymerase I and T_4 DNA ligase.

The resulting DNA was used to transform competent E. coli LS1.

Colony Screening by Primer-probe Hybridization:
The synthetic deoxyoligonucleotide was labelled with ^{32}P. Replicas of the colony collection (6) were prehybridized at 65° C to denatured, sonicated salmon sperm DNA and then hybridized to the ^{32}P-probe at room temperature for 16-18 hours. The resulting filters were washed and radioautographed.

Results

The primer used in this site-specific mutagenesis was designed to have a double mismatch and to cause an inversion of the ser-thr sequence characteristic of the active site of β-lactamase (7,8); ser 70→thr; thr 71→ser. Because of the double mismatch, mutant and wild-type colonies can be readily distinguished in the screening procedure. The method is general since one can, invariably, have a double mismatch even when mutating only a single amino acid residue by making phenotypically silent base changes.

The screening procedure is simple and thousands of colonies can be easily assayed. By using the procedures outlined we have isolated mutants with a frequency of about $1/10^3$.

To confirm that the anticipated mutations had, in fact, been introduced, the plasmid DNA was sequenced (9). As expected, the mutant gave the sequence 5'•••A ATG ATG ACC TCT TTT•••3' in contrast to the wild-type sequence 5' A ATG ATG AGC ACT TTT•••3' (asterisks denote the sites of mutation).

The enzyme, β-lactamase (ser 70→thr, thr 71→ser), was catalytically inactive.

Discussion

This procedure opens a new approach to studies of the mechanism by which proteins function by allowing a rational approach to the determination of the influence of amino acid sequence on three dimensional structure and on biochemical properties of enzymes, polypeptide hormones, transport proteins, immunoglobulins, cell surface receptors and the many other types of proteins found in biological systems. The procedure requires: (i) that the relevant gene be cloned and expressed, (ii) that the nucleotide sequence of the gene be known and (iii) that one have a convenient source of chemically synthesized deoxyoligonucleotides. Fortunately, all these requirements can now be met for many systems. The microbial expression of even mammalian proteins is now becoming commonplace, DNA sequencing is routine and rapid, and chemically synthesized DNA will soon become widely available.

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