Genetically Engineered Fluoropolymers. Synthesis of Repetitive Polypeptides Containing *p*-Fluorophenylalanine Residues

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Received April 1, 1994; Revised Manuscript Received June 22, 1994®

ABSTRACT: Genetic engineering offers the potential for creating new proteins with novel materials properties. The aim of this study is to develop strategies for incorporating the non-natural amino acid *p*-fluorophenylalanine (pfF), which is known to be utilized by the *Escherichia coli* protein synthesis apparatus, into repetitive polypeptides. pfF has been successfully incorporated into target protein by *in vivo* substitution of pfF for phenylalanine in a phenylalanine auxotroph of the *E. coli* BL21(DE3) expression strain containing a chromosomal copy of the bacteriophage T7 RNA polymerase gene under *LacUV5* promoter control. The auxotroph was generated by transposon mutagenesis using P1-mediated transduction. The repetitive protein $-[(Ala\text{-}Gly)_3\text{-}pfF\text{-}Gly]_{13}-$ (1) was expressed from an artificial gene under T7 promoter control. Expression of 1 was carried out by shifting the host culture to pfF-containing medium after induction of T7 RNA polymerase for 10 min with isopropyl β-D-thiogalactopyranoside in medium containing the 20 natural amino acids. Replacement of 95–100% of phenylalanine by pfF was confirmed by ^1H NMR spectroscopy and amino acid analysis. Fourier transform infrared spectroscopy and X-ray scattering suggest that both 1 and the phenylalanine variant (2) adopt antiparallel β-sheet structures in the solid state.

Introduction

Protein-based polymeric materials derived from genetic engineering are characterized by their structural homogeneity and by precisely controlled chemical functionality. Structural homogeneity in terms of chain length, composition, sequence, and stereochemistry facilitates controlled growth of macromolecular crystals[1–4] and the development of unique crystalline and surface arrays.[5] With respect to chemical functionality, genetic strategies offer opportunities for chemical modification of appropriately designed protein chains to produce new materials with useful optical, electronic, or liquid crystalline properties. However, to the extent that biological protein synthesis is limited to the 20 natural amino acids normally encoded by messenger RNA templates, many desirable functional groups, e.g., halogens, silyl groups, alkenes, and alkynes, cannot be incorporated directly in the translation step. In principle, there are three direct routes to proteins that contain non-natural amino acids: (1) *in vivo* synthesis that utilizes the natural protein synthesis apparatus to replace a natural amino acid with a chemically related analogue, (2) a mix of chemical and biological synthesis requiring chemical acylation of a suppressor transfer RNA and *in vitro* translation,[6–11] and (3) purely chemical synthesis of peptides. Each method has strengths and limitations. Chemical synthesis has the fewest constraints in terms of amino acid specificity but lacks the superb control over polymer structure that is the greatest advantage of biological protein synthesis. Cell-free *in vitro* synthesis utilizing chemically acylated tRNA is currently the best approach for circumventing the specificity of biological amino acylation. However, at present, the limitations to this approach are the low suppression efficiency (in the best case ≤50%), which limits the utility of the method for multiple-site replacements by non-natural amino acids, and the difficulty of chemical tRNA acylation, which limits protein production to small sample sizes. *In vivo* synthesis offers the best combination of precise control of product structure, ease of scale-up, and overall cost. However, the high specificity and fidelity of the biological synthesis apparatus limit the range of non-natural amino acids that can be utilized.

More than 20 non-natural amino acids are known to be incorporated into bacterial or eucaryotic cell proteins,[12–14] and three (selenomethionine, trifluoroleucine, and dihydrophenylalanine) have been reported to support growth of the corresponding *E. coli* amino acid auxotrophs.[15–18] We and others have utilized this approach to produce recombinant proteins containing selenomethionine in place of methionine with complete or near-complete substitution *in vivo*.[19,20] Other analogues that do not support growth can be accommodated by the translational machinery and are incorporated into proteins. These analogues do not interfere with translation *per se* and, in principle, genetically engineered protein materials could be produced effectively *in vivo* with high levels of analogue incorporation in the absence of growth by using an inducible expression system.

The particular analogue that we discuss herein is *p*-fluorophenylalanine (pfF). pfF has been shown to be incorporated into *E. coli* alkaline phosphatase *in vivo* by analysis of radiolabeled protein,[21] and incorporation has been demonstrated *in vitro* using an *E. coli* cell-free system.[22] We have confirmed the translational activity of pfF through its incorporation into plasmid-encoded protein in a coupled transcription–translation system *in vitro*.[23] These results make pfF an excellent candidate for the preparation of genetically engineered polymers containing fluorine. Periodic polypeptides of sequence $[(Ala\text{-}Gly)_n\text{-}X\text{-}Gly]_m$ (X = Phe in sequence 2 or pfF in 1) were chosen as targets for incorporation of *p*-fluorophenylalanine. These and related polymers might be expected to exhibit many of the useful characteristics of conventional fluoropolymers, e.g., low surface energy, low coefficient of friction, excellent solvent resistance, and good hydrolytic stability, while retaining the precise structural control provided by the bacterial protein synthesis machinery.

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® Abstract published in *Advance ACS Abstracts*, August 15, 1994.

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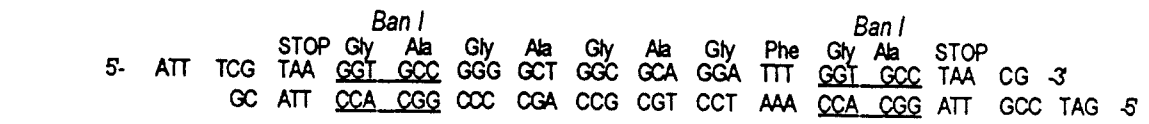

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The experiments reported herein illustrate an efficient *in vivo* synthesis strategy for incorporation of pfF into genetically engineered protein-based materials.

Experimental Section

Materials. *E. coli* strain NK6024 was obtained from the *E. coli* stock center, Yale University (New Haven, CT). *p*-Fluoro-L-phenylalanine was supplied by PCR Inc. (Gainesville, FL). [^{3}H]-Glycine was purchased from Dupont/NEN (Wilmington, DE). Formic acid-d_1 (98% DCOOH) was from Cambridge Isotope Laboratories (Woburn, MA). All other materials were from standard sources.

General Methods. ^{1}H NMR spectra were run at room temperature on a Bruker AC 200 instrument at an operating frequency of 200 MHz. Polymers (5–10 μg) were dissolved in 500 μL of 98% DCO_2H for NMR measurments. Fourier transform infrared spectra were obtained on a Nicolet IR/32 spectrometer. X-ray diffraction patterns were recorded in a Statton X-ray camera with a pinhole collimated, Ni-filtered Cu Kα X-ray source and a sample-to-film distance of 53.1 mm.

Construction of Bacterial Expression Vector. A DNA fragment (3) including a 24 base pair (bp) DNA monomer encoding one copy of the repeating unit of interest (*vide infra*) was prepared as described previously.[20] The 24-bp monomer was isolated by restriction digestion with *Ban*I and ligated to generate a population of multimers. The population of multimers was then ligated into the unique *Ban*I restriction site of pMD3a,[20] and the resulting recombinant plasmids were used to transform *E. coli* strain HB101. A plasmid containing 13 repeats of the DNA monomer segment was selected, the size of the insert was determined by electrophoresis, and the artificial coding sequence was subcloned into the *Bam*HI site of the pET-3a[24] expression vector to yield pET-13GF.

Construction of Phenylalanine Auxotroph. Host cells of *E. coli* strain NK 6024 featuring the *pheA*::Tn10 mutation were first infected by transducing phage P1 and the transposon was transferred into the expression strain BL21(DE3) carrying the gene for T7 RNA polymerase.[24,25] The resulting auxotroph was then treated with chlorotetracycline and fusaric acid[26] to obtain the stable auxotroph designated AF. Plasmids pLysS[24] and pET-13GF were used to transform the auxotroph to yield AFpLysS/pET-13GF.

***In Vivo* Labeling.** *E. coli* strain AFpLysS/pET-13GF cells were grown in 15 mL of M9 minimal medium supplemented with 20 μg/mL of each of the 20 L-amino acids, 0.1 mM $CaCl_2$, 1 mM $MgSO_4$, 1 μg/mL thiamine chloride, 0.2% glucose, 200 μg/mL ampicillin, and 25 μg/mL chloramphenicol at 37 °C. When the cell density reached ca. 6 × 10^8 cells/mL, isopropyl β-D-thiogalactopyranoside (IPTG) was added to final concentration of 0.4 mM. After 10 min, cells were centrifuged at 10000*g* for 5 min at 4 °C and washed with distilled, deionized water. Cells were resuspended in 15 mL of M9 minimal medium with 19 L-amino acids (all except phenylalanine) plus 100 μg/mL of *p*-fluoro-L-phenylalanine and [^{3}H]glycine (150 μCi). Aliquots of cells (1 mL) were periodically removed and harvested by centrifugation at 14000*g* for 1 min at 4 °C. The cells were washed with M9 minimal salts solution, lysed in 100 μL of sample buffer (50 mM Tris-Cl pH 7.5, 2% SDS, 2 mM EDTA, 1% β-mercaptoethanol, 0.05% bromophenol blue, and 10% glycerol), and heated at 95 °C for 5 min. Whole cell lysates corresponding to equal number of cells were separated on a 10% SDS-polyacrylamide gel by the method of Laemmli.[27] The gel was stained with Coomassie Brilliant Blue G-250 for 3 h, destained, then soaked for 30 min in Enlightening enhancer (Dupont/NEN), transferred onto Whatman 3MM paper, and dried at 60 °C for 3 h. The dried gel was exposed to X-ray film for 48 h at −80 °C.

Large-Scale Expression and Purification. AFpLysS/pET-13GF cells were grown to a cell density of ca. 8 × 10^8 cells/mL in 6 L (four 1.5 L batches) of M9 minimal medium supplemented with 40 mg/L of each of the 20 L-amino acids, 0.1 mM $CaCl_2$, 1 mM $MgSO_4$, 1 mg/L thiamine chloride, 0.2% glucose, 200 mg/L ampicillin, and 25 mg/L chloramphenicol at 37 °C. The cells were then induced with IPTG at a final concentration of 0.4 mM. After 10 min the cells were collected by centrifugation at 10000*g* for 10 min at 4 °C and washed twice with M9 minimal salts solution. The cells were then resuspended in 6 L of M9 minimal medium supplemented with 40 mg/L of 19 L-amino acids (all except phenylalanine), 100 mg/L *p*-fluoro-L-phenylalanine, 0.1 mM $CaCl_2$, 1 mM $MgSO_4$, 1 mg/L thiamine chloride, 0.2% glucose, 200 mg/L ampicillin, and 25 mg/L chloramphenicol. After 3 h the cells were harvested by centrifugation at 10000*g* for 10 min at 4 °C, resuspended in 120 mL of lysis buffer (50 mM Tris-Cl pH 8.0, 1 mM EDTA, and 100 mM NaCl), and stored −20 °C for 12 h. The cells were lysed by thawing, placed on ice, and sonicated using a Branson sonifier at 40 units power for 15 min. Lysozyme (80 mg) and phenylmethanesulfonyl fluoride (80 μL, 100 mM) were added to the lysate, and the mixture was stirred for 20 min at 37 °C. Deoxycholic acid sodium salt (160 mg) was then added and stirring was continued for another 20 min at 37 °C. $MgCl_2$ was added to a final concentration of 10 mM and 1 mg each of RNase A and DNase I was added to digest nucleic acids. After 1 h of incubation at 37 °C, the mixture was adjusted to pH 7.7 with 1 M KOH, 600 mg of lipase (Sigma Chemical Co.) was added, and the mixture was incubated at 37 °C for 1 h. The pellet was collected by centrifugation at 10000*g* for 30 min at 4 °C and resuspended in 1 L of Triton X-100 buffer (50 mM Tris-Cl pH 8.0, 10 mM EDTA, 100 mM NaCl, and 2% Triton X-100), stirred for 6 h at room temperatuare, and stored at 4 °C for 24 h. The precipitate was collected by centrifugation at 10000*g* for 30 min at 4 °C and dried by lyophilization. The dried pellet was dissolved in 98% formic acid and diluted to 70% by the addition of distilled water to give a concentration of 5 mg/mL with respect to the dry weight of the pellet. Cyanogen bromide was added to a concentration of ca. 10 mg/mL. The reaction mixture was purged with nitrogen and stirred at room temperature for 24 h in the dark. Solvent was removed *in vacuo*, and the pellet was resuspended in 500 mL of distilled water and stirred for 2 days with periodic changes of the water. The pellet was lyophilized and then resuspended in 50 mL of 2% aqueous SDS and stirred for 1 h. The insoluble material was collected by filtration on a 20 μm glass filter and washed repeatedly with hot water. The cleaved protein was recovered by lyophilization and dried at 78 °C *in vacuo*. The yield of the protein 1 from 6 L was 72 mg.

Results and Discussion

Construction of the Expression Strain. A phenylalanine auxotroph was generated from *E. coli* strain BL21-(DE3)[24] by P1-mediated transduction using a donor strain (NK 6024) which had been transposed by Tn10 at the *pheA* region in the bacterial chromosome.[25] The terminal pathway of phenylalanine biosynthesis in *E. coli* is shown in Figure 1.[28] Since the recipient strain has the *recA*$^+$ phenotype, transposon mutagenesis results in loss of chorismate mutase–prephenate mustase activity through recombination events on the chromosome. The auxotroph was then treated with chlorotetracycline and fusaric acid to select for loss of tetracycline resistance derived from Tn10.[26] This procedure results in the isolation of a more stable auxotroph in which chorismate mutase–prephenate mutase activity is completely lost. One mutant, designated AF, showed complete dependence on phenylalanine, and no revertants were observed under typical growth condi-

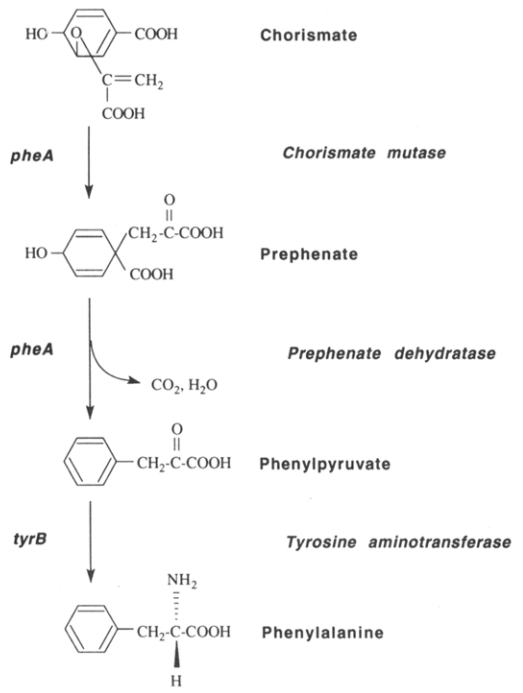

Figure 1. Terminal pathway of phenylalanine biosynthesis.

tions over 96 h. Strain AF was then transformed with plasmids pLysS[24] and pET-13GF to yield the expression strain.

Effect of pfF on the Synthesis of T7 RNA Polymerase. The T7-based expression system[24] takes advantage of the highly processive RNA polymerase from bacteriophage T7 and its selectivity for T7 promoter sequences. Typically, the T7 polymerase is expressd under *lacUV5* control from a prophage on the *E. coli* chromosome, and the gene for the target protein is under the control of the ϕ10 promoter carried on a plasmid vector.

A potential problem with the T7 system for expressing analogue-containing proteins is that transcription of the target gene requires the prior synthesis of active T7 RNA polymerase. If incorporation of the analogue yields an inactive polymerase, then the presence of the analogoue at the onset of induction could severely limit the extent of target protein synthesis. To examine this possibility, induction of the synthetic protein was compared in the presence and absence of the analogue (Figure 2). Protein expression was monitored via incorporation of [^{3}H]glycine. Induction by IPTG for 10 min in the presence of phenylalanine prior to a shift to pfF-containing medium (lanes 1–6, Figure 2) afforded more of the target polymer (arrows, Figure 2) than when *p*-fluorophenylalanine was present from the onset of induction (lanes 7–12, Figure 2).[29] Thus, in subsequent experiments, the expression of the target protein was preceded by a 10 min induction period in the absence of pfF, which appears to allow sufficient time for synthesis of active T7 polymerase.

Synthesis of Fluorinated Protein. Figure 3 shows the results of experiments in which protein synthesis was monitored by the *in vivo* incorporation of [^{3}H]glycine in different media, following induction for 10 min in M9 minimal medium containing the 20 natural amino acids. In the negative control experiment in which cells were shifted to a medium lacking both phenylalanine and pfF, only a very low level of protein synthesis was observed. This result is consistent with the fact that the expression strain is a tight auxotroph and is not able to grow if deprived of phenylalanine. When the induced cells were shifted to a medium containing phenylalanine but without the

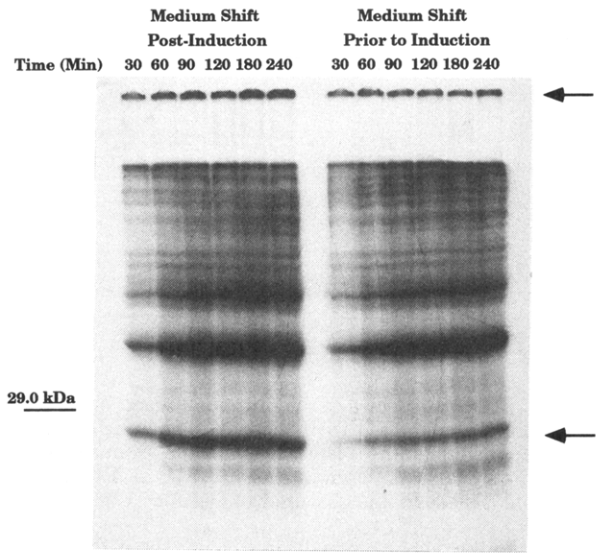

Figure 2. Effect of timing of medium shift on product yield. Fluorograph of 10% SDS–polyacrylamide gel showing expression of protein labeled with [^{3}H]glycine *in vivo*. Two protocols were used: Lanes 1–6, cultures (OD_{600nm} = 0.6) were induced for 10 min in the presence of 20 natural amino acids, washed, and shifted to the pfF-containing medium (see Experimental Section); lanes 7–12, cultures (OD_{600nm} = 0.6) were washed and shifted directly to a pfF medium containing IPTG for induction (see Experimental Section). The arrows indicate the target protein. Time points are relative to medium shift.

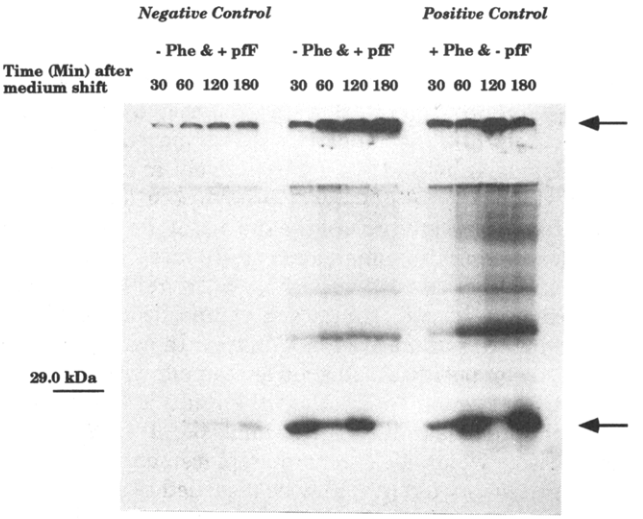

Figure 3. *In vivo* synthesis of 1 and 2. Protein yields were compared after cultures were shifted to three different media 10 min after induction in the presence of the 20 natural amino acids: Lanes 1–4, medium lacking phenylalanine and pfF (negative control); lanes 5–8, medium containing pfF but without phenylalanine; lanes 9–12, medium containing phenylalanine but without pfF (positive control). Protein accumulation was monitored by the *in vivo* incorporation of [^{3}H]glycine and analyzed on a 10% SDS–poyacrylamide gel. Arrows show the target protein. Time points indicated are relative to medium shift.

analogue (positive control), prominent new protein bands appeared, indicating that the target protein accumulates with time. Finally, shifting to medium containing *p*-fluorophenylalanine clearly demonstrates product accumulation at the same migration locus as the positive control. Comparison of the intensity of the band of aggregated protein (top arrow) with that observed in the positive control suggests that the solubility of the product is reduced by incorporation of pfF.

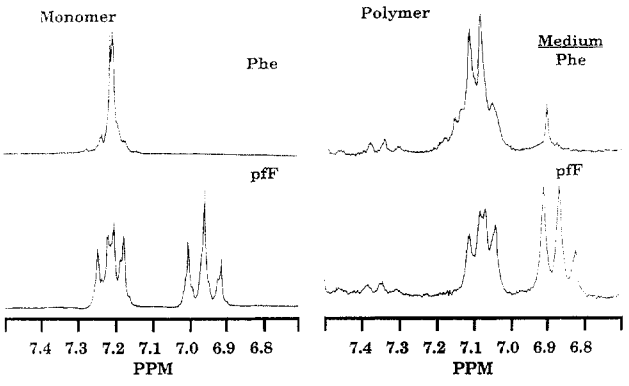

Figure 4. ^{1}H NMR spectra of the proteins 1 and 2 in DCOOH. The spectra are compared with those of L-phenylalanine and *p*-fluoro-L-phenylalanine, respectively, in the aromatic proton region. The signal at 6.9 ppm in the spectrum of 2 arises from the solvent. This signal is obscured in the spectrum of 1.

Table 1. Amino Acid Compositional Analysis of Proteins 1 and 2

amino acid	mol % (theor)	mol % (obs) 1	mol % (obs) 2
glycine	50.0	51.0	50.5
alanine	37.7	37.0	36.4
p-fluorophenylalanine	12.3[a]	10.3	
phenylalanine	12.3[b]	0.6	11.2
aspartic acid		1.1	1.0
leucine			0.5
valine			0.4

[a] Expected for 1. [b] Expected for 2.

Level of *p*-Fluorophenylalanine Incorporation. Isolated proteins 1 and 2 were analyzed by ^{1}H NMR spectroscopy and their spectra were compared with those of L-phenylalanine and L-pfF, respectively, in the aromatic region (Figure 4). A high level of incorporation of pfF is indicated by the similarity of the spectra of the fluorinated polymer and pfF monomer, and careful integration of the spectrum of 1 is consistent with 98 ± 2% replacement of phenylalanine by pfF. Amino acid compositional analyses of 1 and 2 are summarized in Table 1. In each case, the observed composition is in good agreement with expectation. Of particular interest are the relative fractions of pfF and phenylalanine in the analysis of 1. A direct comparison yields an extent of replacement of 94.5%; however, this should probably be regarded as a minimum value, as some contribution from background amino acids (e.g., aspartic acid, valine, and leucine) is apparent. Taken together, the NMR and amino acid analyses indicate near-perfect (95–100%) replacement of phenylalanine by pfF.

Solid-State Properties. Isolated proteins 1 and 2 were analyzed by FTIR spectroscopy (Figure 5). Proteins were gelled in 40 mg/mL solutions in 70% formic acid, washed with methanol and water, dried, and then dispersed in KBr.[30] Both polymers show evidence of antiparallel β-sheet structure, in that the amide I bands appear at ca. 1700 and 1625 cm^{-1} and the amide II bands appear at ca. 1525 cm^{-1}.[31-33] Absorptions at ca. 1506 and 1222 cm^{-1} (apparent in 1 (Figure 5A) but weak or absent in 2 (Figure 5B) arise from pfF, with the absorption at 1506 cm^{-1} due to out-of-plane deformation of the para-substituted aromatic ring and that at 1222 cm^{-1} corresponding to a vibration of the carbon–fluorine bond.[34] Wide-angle X-ray scattering patterns of oriented mats of proteins 1 and 2, cast from formic acid, also confirm the presence of β-sheet structure (data not shown).

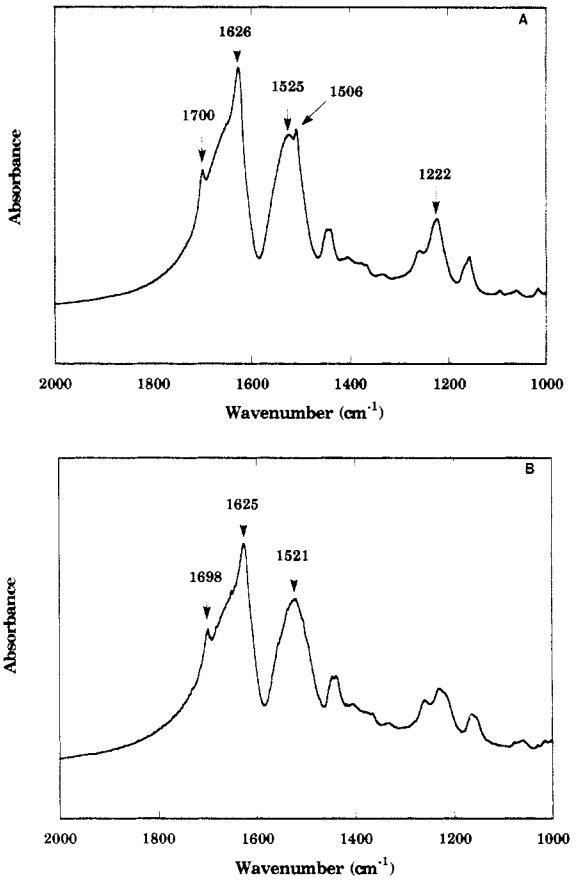

Figure 5. Infrared spectra of proteins of 1 (A) and 2 (B).

Conclusion

We establish in this study an *in vivo* synthesis strategy for high-level incorporation of the non-natural amino acid *p*-fluorophenylalanine into genetically engineered protein-based polymeric materials. This strategy should be applicable to many other amino acid analogues and should thereby broaden the range of materials properties that can be attained via biological synthesis of artificial proteins.

Acknowledgment. This work was supported by the NSF Materials Research Laboratory at the University of Massachusetts and by a grant from the U.S. Army Research Office. We thank Kobe Steel, Ltd., for a leave of absence granted to E.Y.

References and Notes

(1) McGrath, K. P.; Tirrell, D. A.; Kawai, M.; Mason, T. L.; Fournier, M. *Biotechnol. Prog.* **1990**, *6*, 188–192 and references therein.
(2) McGrath, K. P.; Fournier, M. J.; Mason, T. L.; Tirrell, D. A. *J. Am. Chem. Soc.* **1992**, *114*, 727–733.
(3) Creel, H. S.; Fournier, M. J.; Mason, T. L.; Tirrell, D. A. *Macromolecules* **1991**, *24*, 1213–1214.
(4) Krejchi, M. T. Atkins, E. D. T.; Waddon, A. J.; Fournier, M. J.; Mason, T. L.; Tirrell, D. A. *Science*, in press.
(5) Zhang, G.; Fournier, M. J.; Mason, T. L.; Tirrell, D. A. *Macromolecules* **1992**, *25*, 3601–3603.
(6) Noren, C. J.; Anthony-Cahill, S. J.; Griffith, M. C.; Schultz, P. G. *Science* **1989**, *244*, 182–188.
(7) Ellman, J. A.; Mendel, D.; Schultz, P. G. *Science* **1992**, *255*, 197–200.
(8) Mendel, D.; Ellman, J. A.; Schultz, P. G. *J. Am. Chem. Soc.* **1993**, *115*, 4359–4360.
(9) Bain, J. D.; Glabe, C. G.; Dix, T. A.; Chamberlin, A. R. *J. Am. Chem. Soc.* **1989**, *111*, 8013–8014.
(10) Bain, J. D.; Diala, E. S.; Glabe, C. G.; Wacker, D. A.; Lyttle, M. H.; Dix, T. A.; Chamberlin, A. R. *Biochemistry* **1991**, *30*, 5411–5421.
(11) Bain, J. D.; Switzer, C.; Chamberlin, A. R.; Benner, S. A. *Nature* **1992**, *356*, 537–539.

(12) Wilson, M. J.; Hatfield, D. L. *Biochem. Biophys. Acta* **1984**, *781*, 205–215
(13) Dittmer, K. *Ann. N.Y. Acad. Sci.* **1950**, *52*, 1274–1301.
(14) Richmond, M. H. *Bacteriol. Rev.* **1962**, *26*, 398–420.
(15) Tuve, T.; Williams, H. *J. Am. Chem. Soc.* **1957**, *79*, 5830–5831.
(16) Cowie, D. B.; Cohen, G. N. *Biochem. Biophys. Acta* **1957**, *26*, 252–261.
(17) Rennert, O. M.; Anker, H. S. *Biochemistry* **1963**, *2*, 471–476.
(18) Pine, M. J. *Antimicrob. Agents Chemother.* **1978**, *13*, 676–685.
(19) Hendrickson, W. A.; Horton, J. R.; LeMaster, D. M. *EMBO J.* **1990**. *9*, 1665–1672.
(20) Dougherty, M. J.; Kothakota, S.; Mason, T. L.; Tirrell, D. A.; Fournier, M. J. *Macromolecules* **1993**, *26*, 1779–1781.
(21) Richmond, M. H. *J. Mol. Biol.* **1963**, *6*, 284–294.
(22) Dunn, T. F.; Leach, F. R. *J. Biol. Chem.* **1967**, *242*, 2693–2699.
(23) Kothakota, S.; Yoshikawa, E.; Murphy, O. J.; Mason, T. L.; Tirrell, D. A.; Fournier, M. J., submitted for publication.
(24) Rosenberg, A. H.; Lade, B. N.; Chui, D.; Lin, S.; Dunn, J. J.; Studier, F. W. *Gene* **1987**, *56*, 125–135.
(25) Miller, J. H. *Experiments in Molecular Genetics*; Cold Spring Harbor Laboratory: New York, 1972.
(26) Bochner, B. R.; Huang, H.; Schieven, G. L.; Ames, B. N. *J. Bacteriol.* **1990**, *143*, 926–933.
(27) Laemmli, U. K. *Nature* **1970**, *227*, 680–685.
(28) Im, S. W. K.; Pittard, J. *J. Bacteriol.* **1971**, *106*, 784–790.
(29) The product partitions into two bands: a solubilized fraction that runs just ahead of the 29 kDa standard, and an insoluble fraction that remains in the loading well (see arrows in Figure 2). The identity of the insoluble fraction is confirmed by the time course of its appearance following induction of protein synthesis (cf. Figure 3) and by the absence of this band in electrophoretic analyses of control cultures in which synthesis of 1 does not occur. The expected molar mass of the protein is 10 635 Da; the anomalously low electrophoretic mobility of such artificial proteins is well-established (Beavis, R. C.; Chait, B. T.; Creel, H. S.; Fournier, M. J.; Mason, T. L.; Tirrell, D. A. *J. Am. Chem. Soc.* **1992**, *114*, 7585–7586).
(30) Parkhe, A. D.; Fournier, M. J.; Mason, T. L.; Tirrell, D. A. *Macromolecules* **1993**, *26*, 6691–6693.
(31) Moore, W. H.; Krimm, S. *Biopolymers* **1976**, *15*, 2465–2470.
(32) Bandekar, J.; Krimm, S. In *Advances in Protein Chemistry*; Anfinsen, C. B., Edsall, J. T. Richards, F. M., Eds.; Academic Press: Orlando, FL, 1986; Vol. 38; pp 183–364.
(33) Krimm, S.; Abe, Y. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*(10), 2788–2792.
(34) Colthup, N. B.; Daly, L. H.; Wiberley, S. E. *Introduction to Infrared and Raman Spectroscopy*; Academic Press: New York, 1990.