Transition State Analogs for Protein Farnesyltransferase

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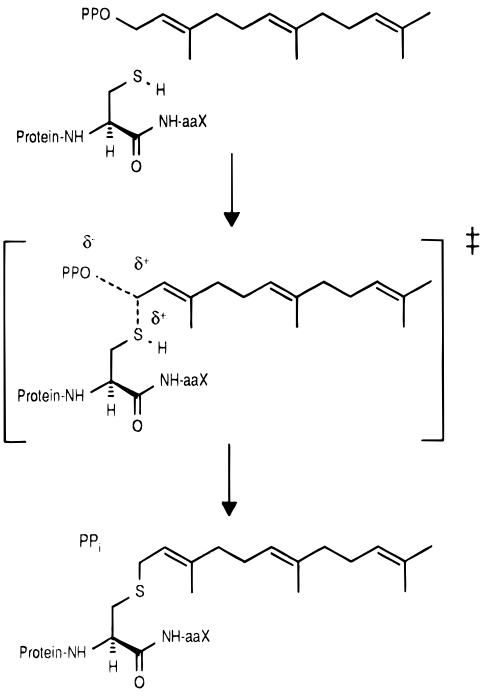
Received April 12, 1996

Addition of an isoprenoid residue to the sulfhydryl moiety of a cysteine is an essential posttranslational modification for many physiologically important proteins.[1] Three different enzymes are responsible for the modifications. Protein farnesyltransferase (PFTase) and protein geranylgeranyltransferase-I (PGGTase-I) catalyze the alkylation of C-terminal cysteines in proteins ending in CaaX, where a is usually an aliphatic amino acid and X is variable. The cysteine in CaaX motifs is farnesylated when X is alanine, methionine, serine, glutamine, or cysteine[2−4] and geranylgeranylated when X is leucine or isoleucine.[3,5] CaaX proteins are further modified by proteolytic removal of the aaX tripeptide and methylation of the new carboxy terminus.[6] Protein geranylgeranyltransferase-type-II (PGGTase-II) modifies both cysteines in C-terminal XXCC, XCXC, or CXCX sequences.[7] These modifications enhance the lipophilicity of the proteins and promote their association with membranes.

The oncogene products of the Ras family of proteins (pRas) are among the CaaX proteins bearing a farnesyl moiety. Approximately 30% of human cancers are associated with mutations in pRas.[8] The discovery that farnesylation is required for oncogenic forms of pRas to manifest their transforming activity has stimulated an intense search for inhibitors of PFTase as potential chemotherapeutic agents.[9] Tetrapeptides containing CaaX sequences are alternate substrates for the enzyme and were the first class of inhibitors of the enzyme to be studied.[10] Nonhydrolyzable peptidomimetics containing cysteine and methionine residues separated by a variety of spacers were reported shortly afterward.[11−14] Some of these compounds are potent inhibitors of PFTase *in vivo* and disrupt the growth of Ras-transformed cells. Nonhydrolyzable analogs of farnesyl diphosphate (FPP) such as α-(hydroxyfarnesyl)phosphonic acid are also potent inhibitors of the enzyme.[15] Other compounds such as chaetomellic acid A and zargazoic acid were identified through microbial screens and do not closely resemble either substrate.[16]

Scheme 1. Electrophilic Mechanism for Protein Prenyltransferases



Recent work with fluorinated analogs of FPP indicates that alkylation of the sulfhydryl moiety in cysteine by FPP is an electrophilic displacement, as illustrated in Scheme 1.[17] While several different structural classes of PFTase inhibitors, including bisubstrate analogs,[18] have been studied, none were designed to duplicate the topological and electrostatic features of the transition state for the reaction. We now report the synthesis and evaluation of L-β-farnesylaminoalanine (faA) and tetrapeptide faAVIA (see Figure 1) as transition state analogs for the electrophilic alkylation of the sulfhydryl group in cysteine by FPP.

faA was prepared by treatment of the Cbz derivative of L-serine lactone[19] with farnesylamine[20] followed by removal of the blocking group, as shown in Scheme 2. The farnesylated amino acid was then incorporated into tetrapeptide faAVIA by

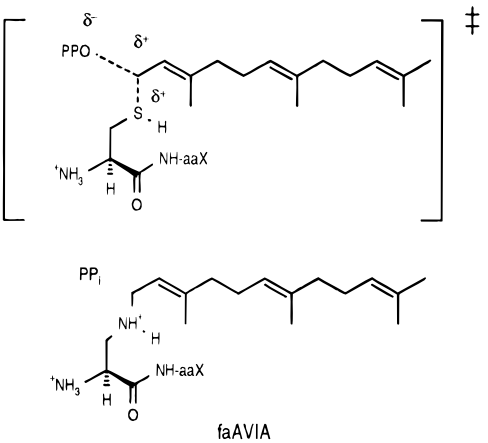


Figure 1.

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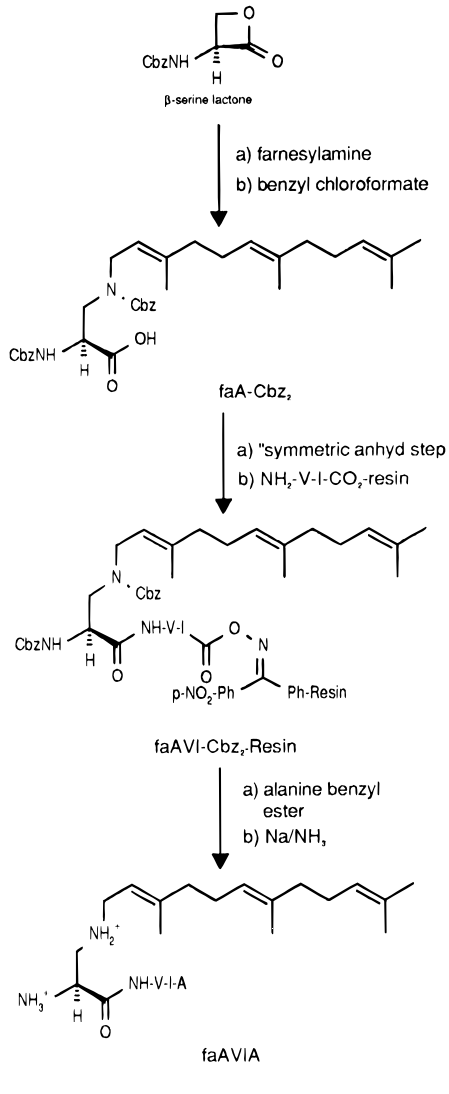
S0002-7863(96)01214-0 CCC: $12.00

Scheme 2. Synthesis of faAVIA



solid phase procedures using the oxime resin developed by DeGrado and Kaiser.[21] Dipeptide fragment Val-Ile, linked to the resin through the isoleucine carboxyl as a hydroxamate ester, was coupled to faA by treatment of the dipeptide with the symmetric anhydride[22] of Cbz$_2$-faA. The resulting polymer-bound tripeptide was cleaved from the solid support with concomitant formation of the final amide linkage by treatment with the benzyl ester of alanine to give Cbz$_2$-faAVIA-Bz.[23] The blocked tetrapeptide was purified on silica gel (R_f = 0.57, 7:3 EtOAc/hexanes), and the protecting groups were removed with sodium in liquid ammonia to give faAVIA.[24]

Farnesylamine, β-aminoalanine, transition state analogs faA and faAVIA, and product analogs *S*-farnesylcysteine and *S*-farnesyl-CVIA were all examined as inhibitors of recombinant yeast PFTase[25] using a fluorescence assay[26] to measure the rate of condensation of FPP with dansyl-GCVIA. IC$_{50}$s for the compounds are listed in Table 1. β-Aminoalanine was not an inhibitor (IC$_{50}$ > 1 mM). Farnesylamine had an IC$_{50}$ of 70 μM. This observation is consistent with a previous report that the amine inhibited farnesylation of proteins *in vitro* in a cell-free extract and *in vivo* in a cell culture assay.[27] Addition of a β-alanyl moiety to the amino group in farnesylamine slightly improved the potency of the inhibitor, while addition of the full tetrapeptide unit reduced the IC$_{50}$ 5-fold. In contrast, S-farnesylcysteine and *S*-farnesyl-CVIA were not inhibitors at concentrations up to the limits of their solubility (ca. 200 μM).

Table 1. Inhibitors of PFTase

compound	IC$_{50}$[a] (μM)
β-aminoalanine	>1000
farnesylamine	70
faA	51
faA-VIA	14
S-farnesylcysteine	> 200
S-farnesyl-CVIA	> 200

[a] IC$_{50}$ values were determined in the presence of 2 μM FPP and 2.5 μM dansyl-GCVIA.

Inorganic pyrophosphate (PP$_i$) was a poor inhibitor of yeast PFTase (K_i = 2.4 mM). However, when faAVIA was incubated with yeast PFTase in buffer containing 1 mM PP$_i$, the IC$_{50}$ for the ammonium analog decreased 35-fold to 370 nM. The synergism seen between faAVIA and PP$_i$ was also observed for other ammonium-based transition state analog inhibitors of enzymes in the isoprenoid biosynthetic pathway, including squalene synthase[28,29] and trichodiene synthase.[30] In these cases, the synergistic effects for the ammonium derivatives in the presence of PP$_i$ were larger than observed for yeast PFTase, although the analogs themselves were less potent than faAVIA. The relative magnitude of the synergism may reflect the extent of charge separation between the developing carbocation and the PP$_i$ leaving group in the transition state structures for the four different enzyme-catalyzed reactions. For the three enzymes showing potent synergism, the nucleophilic partner is a carbon−carbon double bond, and one might anticipate a high degree of cationic character in the farnesyl moiety at the transition state. In contrast, the thiol, or perhaps thiolate, nucleophile for protein prenylation might react by an enforced mechanism through a less highly developed electrophilic transition state. In summary, the faA analogs represent an important new class of PFTase inhibitors whose biological activity supports the hypothesis that prenylation of proteins occurs by an electrophilic mechanism.

Acknowledgment. This work was supported by Grant GM 21328 from the National Institutes of Health.

JA961214C

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(23) HRMS (CI) calcd for $C_{55}H_{76}N_5O_9$: 950.5643. Found: 950.5619. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45−7.22(m, 15H, aromatic Hs), 7.21−7.10 (m, 2H, NH), 6.96 (d, 1H, J = 8.7 Hz, NH), 5.25−5.04 (m, 9H, benzyl Hs, H at C2, C6, C10), 4.62 (apparent t, 1H, J = 7.3, 7.2 Hz, ala-α), 4.49−4.37 (m, 2H, α-Hs), 4.35−4.26 (m, 1H, α-H), 3.85 (d, 1H, J = 5.8 Hz, H at C1), 3.70 (dd, 1H, J = 14.8, 8.5 Hz, faA-β), 3.50 (d, J = 14.8 Hz, faA-β), 2.24−2.09 (bs, 1H, val-β), 2.10−1.69 (m, 9H, ile-β, H at C4, C5, C8, C9), 1.65 (s, 3H, CH_3 at C3), 1.60−1.40 (m, 10H, ile-γ, CH_3 at C7, C10, H at C12), 1.36 (d, 3H, J = 7.2 Hz, ala-β), 1.20−0.98 (bs, 1H, ile-γ), 0.95−0.72 (m, 12H, val-γ, ile-γ, δ); ^{13}C NMR (75MHZ, $CDCl_3$) δ 172.4, 170.7, 170.5, 170.2, 157.9, 156.7, 140.1, 136.2, 137.1, 135.3, 135.2, 131.2, 128.4, 128.4, 128.2, 128.1, 128.0, 127.9, 127.7, 124.2, 123.6, 118.9, 67.6, 67.0, 67.0, 58.9, 57.7, 56.6, 48.6, 48.0, 45.6, 39.7, 39.7, 36.5, 30.0, 26.7, 26.3, 25.7, 24.8, 19.2, 18.0, 17.7, 16.2, 16.0, 15.4, 11.4.

(24) HRMS (CI) calcd for $C_{32}H_{57}N_5O_5$: 592.4406. Found: 592.4438. ^{1}H NMR (300 MHz, DMSO-d_6 and CD_3COOD) δ 8.45 (d, 1H, J = 8.5 Hz, NH), 8.07 (d, 1H, J = 6.6 Hz, NH), 7.98 (1H, J = 8.7 Hz, NH), 5.25 (t,1H, J = 6.8 Hz, H at C2), 5.16−5.02 (m, 2H, H at C6, C10), 3.76 (dd, 1H, J = 7.8, 5.8 Hz, faA-α), 3.42 (d, 2H, J = 6.8 Hz, H at C1), 3.00 (dd, J = 12.5, 5.8 Hz, faA-β), 2.82 (dd, 1H, J = 12.5, 7.8 Hz, faA-β), 2.13−1.83 (m, 9H, val-β, H at C4, C5, C8, C9), 1.82−1.69 (m, 1H, ile-β), 1.68−1.61 (m, 6H, CH_3 at C3, C7), 1.59−1.51 (m, 6H, CH_3 at C10, H at C12), 1.25 (d, 3H, ala-β), 1.15−1.03 (m, 1H, ile-γ), 0.97−0.75 (m, 12H, val-γ, ile-γ, δ); ^{13}C NMR (75MHZ, DMSO-d_6 and CD_3COOD) δ 174.1, 170.5, 170.3, 141.1, 134.8, 130.7, 124.1, 123.6, 118.0, 57.8, 56.6, 51.2, 49.0, 47.8, 45.1, 39.2, 36.7, 30.6, 26.2, 25.9, 25.4, 24.2, 19.2, 17.8, 17.5, 16.3, 15.7, 15.2, 10.9.

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