Both **4** and **5** have lower pK_a values than **1**.[13] Since the K_i of **4** is similar to the K_M of **1**, the increase in acidity apparently compensates for the lack of stabilized Asx-turn structure. Reaction of peptide **4** to afford a product which is labile to the reaction conditions was considered; however, increased background hydrolysis of the lipid-linked donor is not observed. The turnover of **5** is significantly lower than that for **1**, which may reflect the diminished basicity of sulfur. This suggests that the subtle balance between the protonation and deprotonation steps is essential for efficient catalysis.

Thus, we propose that the primary sequence requirements establish neighboring-group assistance for the tautomerization process.[14] Consequently, the conformation of the peptidyl acceptor appears to establish a microenvironment surrounding the carboxamido group which may govern the exceptional specificity observed in asparagine-linked glycosylation.

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Supplementary Material Available: Synthetic details and spectroscopic data for **4** and **5** and enzyme kinetic data for all peptides analyzed (11 pages). Ordering information is given on any current masthead page.

(13) For comparison, the following pK_a values are pertinent: CH_3CONH_2, 15.1 (Bordwell, F. G. *Acc. Chem. Res.* **1988**, *21*, 456); $CH_3CH_2NH_3^+$, 10.63 (*Lange's Handbook of Chemistry*, 13th ed.; Dean, J. A., Ed.; McGraw-Hill: New York, 1985); CH_3CSNH_2, 13.4 (Walter, W.; Becker, R. F. *Justus Liebigs Ann. Chem.* **1969**, *727*, 71.).

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Intramolecular Fluorescence Enhancement: A Continuous Assay of Ras Farnesyl:Protein Transferase

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Posttranslational addition of hydrophobic moieties (fatty acid acylation and prenylation) is a functionally essential modification for many proteins involved in intracellular signaling pathways.[1] Study of the transferase enzymes that catalyze these modifications is complicated by troublesome single-point methods for assaying their activity. We have developed a continuous fluorimetric assay for one of these enzymes, farnesyl:protein transferase (FPTase), that takes advantage of a common feature among all hydrophobic modification reactions: the increase in hydrophobicity about the reaction center of the acceptor substrate that occurs following conversion of substrate to product.

FPTase catalyzes the transfer of a hydrophobic farnesyl group (C_{15}) from farnesyl diphosphate (FPP) to a specific C-terminal cysteine residue of a protein substrate, forming a thioether bond

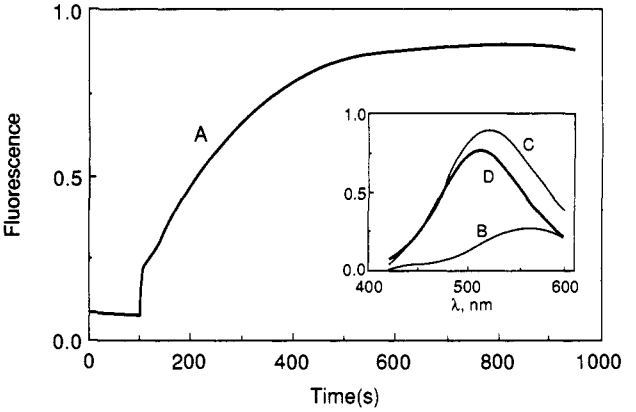
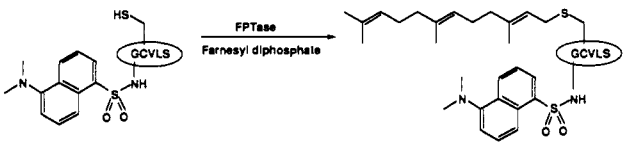


Figure 1. Fluorescence progress curve, A, of the reaction catalyzed by recombinant human farnesyl:protein transferase (0.5 nM) using Ds-GCVLS (1.0 μM) as substrate in the presence of a saturating concentration of farnesyl diphosphate (10 μM) in assay buffer (50 mM Tris-HCl, pH 7.5, 5 mM DTT, 5 mM $MgCl_2$, 10 μM $ZnCl_2$, and 0.2% octyl β-D-glucopyranoside) at 30 °C. Fluorescence data in the integration mode were obtained on a SPEX Fluorolog Model F112XI spectrofluorimeter with λ_{ex} = 340 nm (slit width = 4 nm) and λ_{em} = 505 nm (slit width = 8 nm) using 4 mm square microcells. Concentrations of stock solutions of Ds-GCVLS (in 20 mM Tris-HCl, pH 7.5, 10 mM EDTA) were calculated from the extinction coefficient of the dansyl moiety at 340 nm (ϵ = 4250 M^{-1} cm^{-1}). Inset: Fluorescence emission spectrum (λ_{ex} = 340 nm) of B, substrate Ds-GCVLS (1.0 μM in assay buffer); C, product Ds-G[f-C]VLS (1.0 μM in assay buffer); and D, difference between substrate and product.

Scheme I



and displacing inorganic diphosphate in the process[2] (Scheme I). Short peptides (≥4 residues) containing a C-terminal consensus recognition sequence (CXXX) can also serve as farnesylation acceptor substrates.[2b,c,3] Interest in FPTase has intensified because farnesylation is required for membrane association and biological function of *ras*-encoded proteins,[4] mutant forms of which play a biological role in over 20% of all human cancers and in greater than 50% of pancreatic and colon tumors.[5] Inhibition of FPTase represents a possible method for preventing relocation of mutant Ras from the cytosol to the membrane, thereby blocking its cell-transforming function. Since cytosolic mutant forms of Ras act as dominant negative inhibitors of the Ras signaling pathway, inhibition of FPTase activity would also lead to the in situ accumulation of an inhibitor specific both for the Ras pathway and for cancer cells.[6] Enzymological studies and the search for potent, specific inhibitors led us to consider alternatives to the existing single-point assays for FPTase activity, which are labor-intensive and generate radioactive waste.[7]

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The continuous FPTase activity assay is based on the enhancement of fluorescence and the accompanying shift to lower wavelength emission maximum of certain fluorophores, like dansyl, upon change from a polar to nonpolar molecular environment.[8] We designed an acceptor peptide substrate with an environmentally sensitive fluorescence probe positioned proximally to the reaction center. Substrate pentapeptide *N*-dansyl-GCVLS (Ds-GCVLS) and expected product *N*-dansyl-G[*S*-farnesyl-C]VLS (Ds-G[f-C]VLS) were synthesized by standard methods,[9,10] and the purified peptide derivatives were characterized analytically.[11] Incubation of Ds-GCVLS with recombinant human FPTase (hFPTase) and FPP results in a time-dependent increase in fluorescence at 505 nm with excitation at 340 nm (Figure 1, curve A). Covalent attachment of the farnesyl moiety to the cysteine thiol of Ds-GCVLS places the nonpolar, hydrophobic group near to the dansyl moiety, altering (intramolecularly) the local chemical environment of the reporter group and causing a dramatic change in its fluorescence properties. Fluorescence emission spectra taken before addition of enzyme (Figure 1, curve B) and after complete conversion to product (Figure 1, curve C) show a decrease of the emission maximum wavelength from 565 to 515 nm together with a 13-fold enhancement of fluorescence intensity at 505 nm (see difference curve D). Spectra of authentic product Ds-G[f-C]VLS and of product resulting from FPTase catalysis using Ds-GCVLS as substrate are superimposable (not shown). Fluorescence enhancement and shift of the emission maximum depend upon the relative distance and chemical nature of the residue side chains between the cysteine group and the N-terminal dansyl group[12] as well as upon the detergent content in the buffer.[13]

Using Ds-GCVLS as substrate, FPTase follows Michaelis-Menten kinetics, with k_{cat}(Ds-GCVLS) = 0.5 s^{-1} and K_M(Ds-GCVLS) = 1.4 μM. The value K_M(FPP) = 30 nM in the presence of Ds-GCVLS is the same as that determined previously using Ras as substrate.[14] The time-dependent change in fluorescence (reaction velocity) is linearly dependent upon enzyme concentration. As a mimic of the normal Ras substrate, Ds-GCVLS can also be used to evaluate FPTase inhibitors. CIFM is a competitive inhibitor of Ds-GCVLS binding with K_i(CIFM) = 9 nM, which agrees with results found using Ras as acceptor substrate.[14]

To our knowledge, this is the first continuous fluorescence assay that monitors the progress of a transferase or ligase type of reaction. Here, the fluorescence enhancement results from the change in local chemical environment that occurs upon covalent attachment of two substrates. Other continuous fluorescence assays have been devised for hydrolases, lyases (proteases, lipases, phosphatases, etc.), or isomerases, where the reporter group changes its fluorescence properties after the parent substrate has been cleaved or isomerized.[15] In addition to simplifying enzyme mechanistic studies, the assay, adapted to a 96-well plate format, will facilitate high-volume drug-screening efforts. Monitoring a product-associated change in the local molecular environment of a fluorescence reporter group should be applicable to other enzymes that catalyze hydrophobic postttranslational modifications, such as geranylgeranyl, palmitoyl, and *N*-myristoyl transferases.

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Supplementary Material Available: Experimental details for the synthesis of Ds-GCVLS and Ds-G[f-C]VLS and plot showing the inhibition of hFPTase by peptide CIFM (3 pages). Ordering information is given on any current masthead page.

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(9) Dansylated peptides were prepared using standard *t*-Boc solid-phase methods using an ABI 430A peptide synthesizer. Cleavage and purification of the dansylated peptides from the PAM resin was achieved by HF hydrolysis and reverse-phase (C_{18}) HPLC, respectively (see supplementary material for details).

(10) Peptide farnesylation was achieved by treating the cysteinyl thiol with farnesyl bromide (1 equiv, 0.02 M) and diisopropylethylamine (3 equiv, 0.06 M) in DMF at 20 °C. Farnesylated peptides were purified by reverse-phase HPLC (see supplementary material for details).

(11) Compounds were characterized by ^{1}H NMR, high-resolution fast atom bombardment mass spectrometry, and elemental analysis (see supplementary material for details).

(12) CVLS is the naturally occurring C-terminal sequence for Harvey-Ras.[16] To optimize the sensitivity of the assay, a number of other N-dansylated peptides were synthesized [note: the factor by which fluorescence intensity was increased upon conversion of substrate to product follows each substrate in parentheses]: Ds-CVIM (7), Ds-CVLS (5), Ds-GCVLS (13), Ds-KCVLS (5), Ds-GKCVLS (10), and Ds-SKCVLS (4).

(13) Fluorescence is extremely sensitive to solvent conditions. For example, reactions in Tris-HCl yielded higher fluorescence enhancements than in HEPES. The fluorescence of product compared to substrate increased with increasing detergent (octyl-β-D-glucopyranoside) content, up to a concentration of 0.6% (w/v) detergent. However, at concentrations above 0.2% (w/v) detergent, the enzyme activity appeared to decrease.

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Kedarcidin, a New Chromoprotein Antitumor Antibiotic: Structure Elucidation of Kedarcidin Chromophore

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In the course of our fermentation lead discovery program, we have discovered a number of cytotoxic antitumor antibiotics which belong to the enediyne class.[1,2] Kedarcidin is a new chromoprotein antitumor antibiotic discovered during our search for new lead substances.[3] As with the known chromoprotein neocarzinostatin (NCS),[4] the antitumor activity of kedarcidin is due primarily to the chromophore. The structure determination of kedarcidin chromophore is disclosed herein.

Kedarcidin was recovered from broth filtrate by adsorption to a QAE anion exchanger, followed by gel filtration and ion exchange chromatography.[3b] The noncovalently bound chromophore was obtained by ethyl acetate extraction of concentrated aqueous chromoprotein solution, followed by silica gel vacuum liquid chromatography. The compound is a buff-colored amorphous solid

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