Peptidomimetic inhibitors of Ras farnesylation

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Chemistry & Biology 15 April 1994, Introductory issue

The Ras protein and related members of the growing superfamily of small molecular weight GTP-binding proteins have been the subject of intense study over the past decade [1]. First discovered as the product of the transforming gene of a sarcoma virus, the Ras protein forms part of the intracellular network responsible for the control of cell growth and differentiation. In response to the binding of extracellular growth factors to specific cell surface receptors, Ras functions as a 'molecular switch', interconverting between an active and an inactive form through the binding and hydrolysis of GTP. The transforming activity of Ras is induced by a single point mutation which reduces the GTPase activity of the protein, so that it remains locked in the active or GTP-bound state. The resulting unregulated stimulation of cell growth is thought to contribute to the development of malignant tumors. Mutations in Ras are associated with ~50% of colon tumors and ~90% of pancreatic carcinomas, making it a suitable target for the development of new therapeutic agents.

Membrane localization is critical to Ras function, yet the protein has no transmembrane domain. Instead, it is farnesylated at the carboxyl terminus by a series of post-translational modifications [2]. Ras shares with other farnesylated proteins a carboxy-terminal sequence motif, CA_1A_2X, in which cysteine is followed by two aliphatic amino acids (A) and either methionine or serine (X). Mutation of this motif prevents membrane association and reverses the transforming effects of oncogenic Ras. The enzyme farnesyltransferase [3] binds to the CA_1A_2X sequence and transfers a C_{15} farnesyl group from farnesylpyrophosphate (an intermediate in the biosynthesis of cholesterol) to cysteine via a thioether linkage. Farnesyltransferase is a heterodimeric protein composed of a ~49 kDa α-subunit and a ~46 kDa β-subunit.

The CA_1A_2X motif has been used as a starting point in the design of inhibitors of farnesyltransferase [3,4]. Tetrapeptides such as Cys-Val-Ile-Met, which conform to the CA_1A_2X motif, are substrates for the enzyme and can act as competitive inhibitors (IC_{50} ~0.1 μM). Non-substrate inhibitors have been developed by substitution of an aromatic residue at the third amino acid (A_2). The tetrapeptide Cys-Val-Phe-Met binds tightly to the enzyme (IC_{50} ~0.05 μM) yet is not itself farnesylated. Studies on related peptide inhibitors have shown that only peptides that have a positive charge on the amino-terminal cysteine and an aromatic residue at A_2 are resistant to farnesylation [4].

The key recognition elements for tetrapeptide binding to the enzyme *in vitro* were identified using inhibitors that had a free thiol at the cysteine residue, two central hydrophobic amino acids, and a carboxy-terminal carboxylate at the methionine position. The three-dimensional relationship of these elements was explored using constrained amino acids; the results suggest that the tetrapeptide adopts a turn-like structure, allowing the cysteine thiol and methionine carboxylate to coordinate to a common metal site (see Fig. 1). A similar structure is observed in the Cys-X-X-Cys loops of aspartate transcarbamylase. We thus replaced the central two residues with a rigid, hydrophobic scaffold (3-methylamino-1-carboxymethyl-5-phenyl-benzodiazepin-2-one, or (N-Me)BZA), holding the Cys and Met residues in a conformation that facilitates metal coordination [5]. The resulting peptidomimetic, Cys-(N-Me)BZA-Met, has a 150-fold higher affinity for farnesyltransferase (IC_{50} ~0.3 nM) than the initial tetrapeptide.

Farnesylation experiments using intact cells showed that Cys-(N-Me)BZA-Met blocks intracellular farnesylation at a concentration of 250 μM. Treatment of Ras-transformed rat-1 fibroblasts with the inhibitor for five days caused reversal of the transformed phenotype [5]. A tetrapeptide analog showed similar effects upon intracellular farnesylation [6]. Cys-(N-Me)BZA-Met was modified at the carboxyl terminus to give the methyl ester, improving cell uptake; this prodrug inhibitor slowed the rapid growth of Ras-transformed rat-1 cells at a concentration of 25 μM yet had no effect on the parental rat-1 line [5]. Thus, inhibitors of farnesyltransferase show promise for the future as cancer therapeutics.

References

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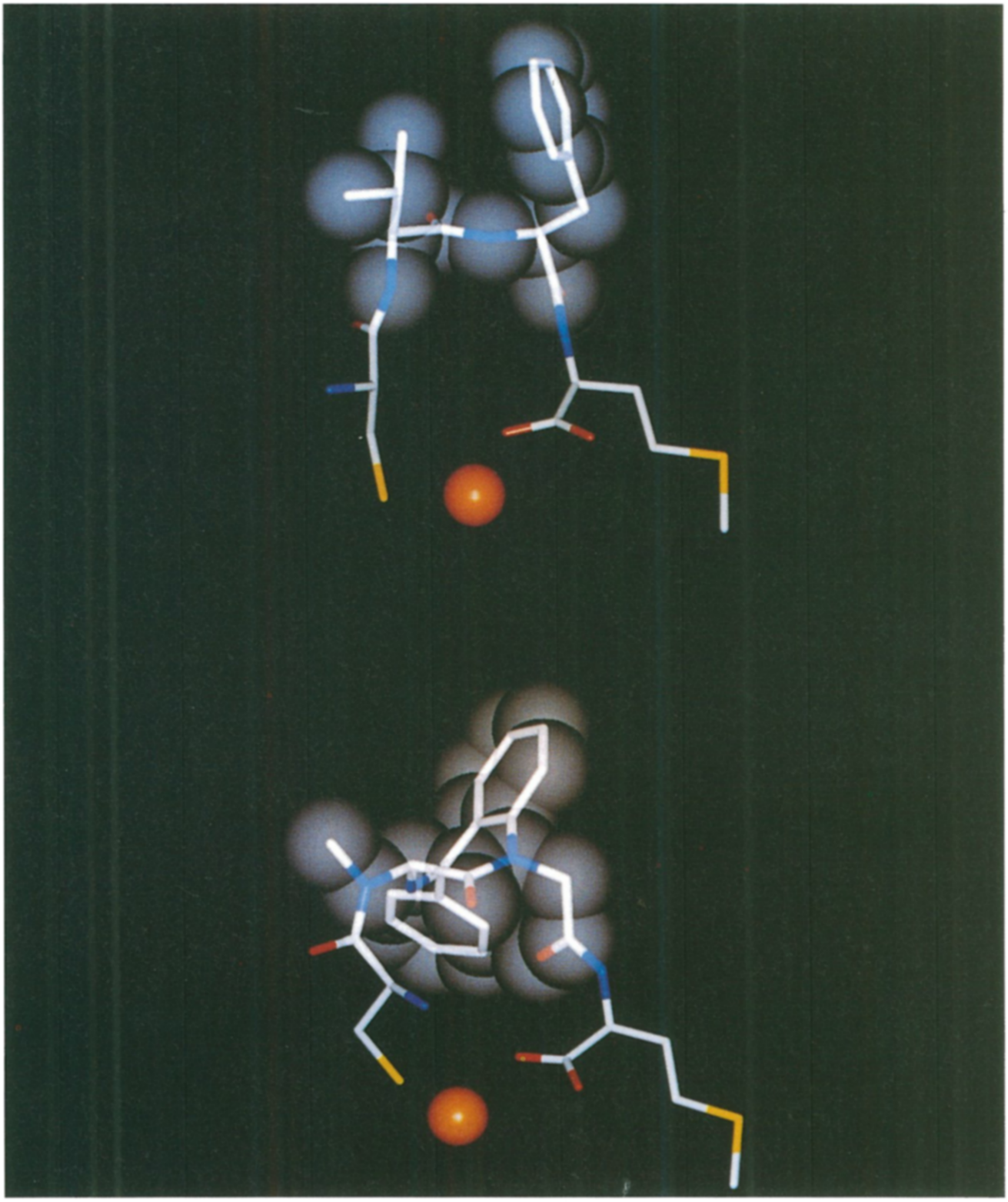

Fig. 1. (Top): 'Turn-like' conformation of the tetrapeptide inhibitor Cys-Val-Phe-Met illustrating the amino-terminal cysteine sidechain and carboxy-terminal methionine carboxylate coordination to Zn^{2+} (orange sphere) in a manner similar to the coordination observed by Cys-X-X-Cys sequences in zinc-binding proteins. (Bottom): Similar conformation enforced by the rigid benzodiazepine core of the peptidomimetic Cys-(N-Me)BZA-Met. The van der Waals surfaces illustrate how the two molecules, though different in composition, have similar presentations of the metal binding groups and hydrophobic moieties. (Illustration prepared with the assistance of Kerrie Andow, Genentech Inc.)