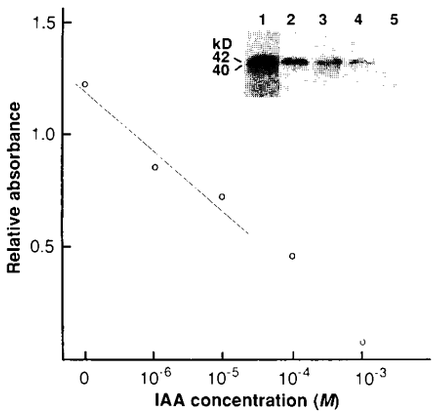

Fig. 3. Reduction of 40- and 42-kD polypeptide labeling in microsomes of VFN8 stems by IAA. Inset: Fluorograph displaying polypeptide labeling in the presence of (1) zero, (2) $10^{-6}M$, (3) $10^{-5}M$, (4) $10^{-4}M$, or (5) $10^{-3}M$ added IAA. Polypeptides of VFN8 stem microsomes were labeled as described in Fig. 1. Line drawing shows results of a densitometer scan of the inset fluorograph. Relative absorbance (in arbitrary units) of the 42-kD band was plotted as a function of the molar concentration of added IAA.

ing, which prevents normal membrane insertion or folding of the polypeptides or reduces the affinity of the binding site for auxin in stem tissue.

It is believed that there are at least three plasma membrane–bound auxin receptors; an uptake symport, an asymmetrically distributed efflux carrier, and a receptor associated with an outwardly directed proton pump thought to be involved in elongation growth (*1*). Present data do not allow us to distinguish between these possible receptor types. On the one hand, zucchini hypocotyl plasma membrane vesicles show a specificity for competition of azido-IAA labeling by auxin analogs that is similar to the specificity demonstrated for auxin uptake into both membrane vesicles and hypocotyl segments via the symport (*9*). In addition, the ability of VFN8 shoots to normalize *dgt* roots could indicate that the *dgt* lesion alters either the uptake or efflux of auxin during cell-to-cell transport. However, other experiments indicate that the rate of polar auxin transport (for example, from shoot apex to base) is unimpaired in *dgt* stems as compared to that in VFN8 (*13*). In addition, since *dgt* hypocotyl sections do not grow in response to externally applied auxin (7), the receptor responsible for auxin-stimulated growth may be affected by this lesion. The identification of the two polypeptides that appear to be affected by the *dgt* lesion may make it possible to dissect the mechanism of auxin action.

REFERENCES AND NOTES

1. P. H. Rubery, *Annu. Rev. Plant Physiol.* **32**, 569 (1981); M. A. Venis, *Hormone Binding Sites in Plants* (Longman, New York, 1985); K. R. Libbenga and A. M. Mennes, in *Plant Hormones and Their Role in Plant Growth and Development*, P. J. Davies, Ed. (Nijhoff, Dordrecht, 1987), pp. 194–221.
2. J. W. Cross, *Plant Cell Environ.* **8**, 351 (1985).
3. R. W. Zobel, *J. Hered.* **63**, 94 (1972).
4. ———, *Plant Physiol.* **52**, 385 (1973).
5. D. W. Fujino, S. J. Nissen, A. D. Jones, D. W. Burger, K. J. Bradford, *ibid.* **88**, 780 (1988).
6. M. B. Jackson, *Physiol. Plant.* **46**, 347 (1979); K. J. Bradford and S. F. Yang, *Plant Physiol.* **65**, 327 (1980).
7. M. O. Kelley and K. J. Bradford, *Plant Physiol.* **82**, 713 (1986).
8. L. L. Melhado, A. M. Jones, N. J. Leonard, L. N. Vanderhoef, *ibid.* **68**, 469 (1981); A. M. Jones, L. L. Melhado, T-H. D. Ho, N. J. Leonard, *ibid.* **74**, 295 (1984); A. M. Jones, L. L. Melhado, T-H. D. Ho, C. J. Pearce, N. J. Leonard, *ibid.* **75**, 1111 (1984).
9. G. R. Hicks, D. L. Rayle, A. M. Jones, T. L. Lomax, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
10. We have observed that while the doublet can be labeled in different plant species, such as *Arabidopsis*, corn, and several conifers, the relative intensity of polypeptide labeling varies. For example, in zucchini the polypeptides label with equal intensity, whereas in VFN8, the 42-kD band is more intense than the 40-kD band (Fig. 1B).
11. F. Wightman, E. A. Schneider, K. V. Thimann, *Physiol. Plant.* **49**, 304 (1980).
12. R. W. Zobel, *Can. J. Bot.* **52**, 735 (1974).
13. S. G. Daniel, D. L. Rayle, R. E. Cleland, *Plant Physiol.*, in press.
14. T. Spector, *Anal. Biochem.* **86**, 142 (1978).
15. J. Heukeshoven and R. Dernick, *Electrophoresis* **6**, 103 (1985).

16. We thank K. Bradford for supplying the original stock of VFN8 and *dgt* seeds, A. M. Jones for generously providing the [^{3}H]5N$_3$-IAA, and R. Hopkins and I. Gelford for assistance with membrane preparation. Supported by National Science Foundation grant DCB-8718731 and National Aeronautics and Space Administration grants NAGW 1253 (to T.L.L.) and NAGW 230 (to D.L.R.).

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Influence of Interior Packing and Hydrophobicity on the Stability of a Protein

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Protein interiors contain many tightly packed apolar atoms in a nearly crystalline state. Both shielding of apolar atoms from solvent and efficient interior packing arrangements affect protein stability, but their relative importance is unclear. To separate these effects, the stabilities of wild-type and mutant gene V proteins from bacteriophage f1 were studied by measuring resistance to denaturation. The effects of subtle interior packing changes, both separate from and combined with changes in buried side chain hydrophobicity, were measured. For the interior apolar-to-apolar substitutions studied, the two effects were of the same magnitude and alteration of packing without accompanying hydrophobicity changes substantially destabilized the protein.

PROTEINS ARE ONLY MARGINALLY stable, with their active, folded forms favored by as little as 5 to 15 kcal/mol as compared with their denatured forms (*1, 2*). Apolar amino acid side chains that are buried in the interiors of proteins are likely to affect protein stability in two general ways. The first effect is that their shielding from the external aqueous solvent stabilizes the folded protein through the hydrophobic effect (*3*). The magnitude of this effect has been compared with scales of amino acid hydrophobicity derived from partitioning of small apolar molecules between water (representing the unfolded protein) and apolar solvents or the vapor phase (representing the interior of the folded protein) (*4–6*). These studies suggest that burying larger apolar side chains should increase protein stability.

Protein interiors have some properties unlike those of apolar liquids or the vapor phase, however (*7, 8*). Protein interiors are more densely packed than apolar liquids, and their packing has been likened to that of crystals of small molecules (7). This packing can be quite fixed; for example, in a lysozyme mutant in which an interior methyl group was replaced by a hydrogen, all other atoms were still in the same positions as the wild type and a cavity remained in the place of the methyl group (*6*). Moreover, not all interior atoms in proteins are nonpolar, and water molecules are sometimes buried inside proteins (*1*). A second class of effects, then, are those due to differences between protein interiors and, for example, an apolar liquid. Because these effects are generally related to the rigidity and tight packing of protein interiors, we refer to them as "packing" effects. As used here, packing encompasses the combined effects of close packing, distortion of the remainder of the protein, and the polarity of the protein interior on protein stability, and thus differs slightly from the traditional usage of the term (*8*). For example, close packing is expected to increase favorable van der Waals interactions, but disruption of the remainder of the protein from an otherwise optimal conformation might decrease its stability (*6, 7*).

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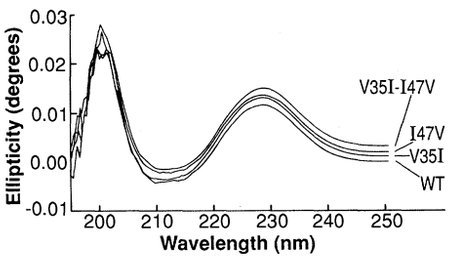

Fig. 1. Gene V protein CD. The proteins were suspended at concentrations of 70 μM of monomers in 0.02M NaH_2PO_4, 0.15M $NaClO_4$, pH 7.1, using an extinction coefficient of $\varepsilon_{276\ nm} = 7100M^{-1}$ for all four proteins (*17*). The spectra were recorded at 25.0°C, using a cuvette with a path length of 0.05 cm. A background spectrum from the buffer alone was subtracted from all other spectra. Spectra for the three mutant proteins are slightly offset for clarity, as indicated.

Table 1. Estimated free energies of unfolding in the absence of denaturant ($\Delta G°_{H_2O}$) and in the presence of 2.25M GuHCl ($\Delta G°_{2.25M}$). The differences between the free energies of unfolding of the mutants and WT are also given ($\Delta\Delta G°_{H_2O}$ and $\Delta\Delta G°_{2.25M}$). Error estimates (2 SD) were obtained from the errors in the parameters used to fit the data to a two-state unfolding model. We investigated possible systematic errors due to the use of a two-state model by constructing a set of data based on a three-state model (folded dimer, folded monomer, and unfolded monomer) in which up to 9% of the protein was in the intermediate state (folded monomer). This model probably overestimates the amount of intermediate form present. Interpretation of the test data with a two-state model yielded estimates of free energies of unfolding that were in error by −1 kcal/mol for $\Delta\Delta G°_{H_2O}$ and by only −0.2 kcal/mol for $\Delta\Delta G°_{2.25M}$.

Protein	Free energy difference (kcal/mol)			
	$\Delta G°_{H_2O}$	$\Delta G°_{2.25M}$	$\Delta\Delta G°_{H_2O}$	$\Delta\Delta G°_{2.25M}$
WT	16.46 ± 0.6	7.97 ± 0.1		
V35I	16.03 ± 0.5	7.52 ± 0.1	−0.4 ± 0.8	−0.4 ± 0.1
I47V	14.04 ± 0.6	5.55 ± 0.2	−2.4 ± 0.8	−2.4 ± 0.2
V35I-I47V	13.70 ± 0.5	5.03 ± 0.2	−2.8 ± 0.8	−2.9 ± 0.2

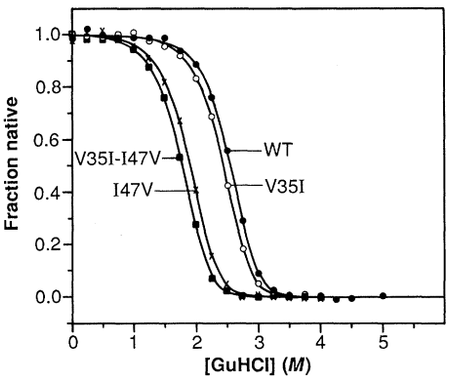

Fig. 2. Equilibrium between native and unfolded gene V protein in solutions containing GuHCl. The calculated fraction of each protein that is in the folded state is shown as a function of denaturant concentrations, after corrections have been introduced to the CD measurements as described below. Solid lines are model curves drawn using a two-state model of the unfolding equilibrium. The fraction of each protein that was in the native state was calculated using a simple model to describe the CD exhibited by the gene V protein as a function of denaturant concentration. The equilibrium between folded (F) and unfolded (U) forms of the protein is given by $F \rightleftarrows 2U$. For a given concentration, P_o, of polypeptide chains, at some concentration of denaturant, C_m, one-half of the chains are in the folded form and one-half are denatured. The free energy of unfolding at this concentration of denaturant, $\Delta G°$, is then given by $\Delta G° = -RT\ln P_o$, where R is the gas constant and T is the absolute temperature. If $\Delta G°$ depends linearly on the concentration of denaturant, C, then $\Delta G° = -RT\ln P_o + m(C - C_m)$ where m is an empirically determined constant. Finally, the measured CD, θ, is related to the concentrations of folded and unfolded protein by $\theta = [F](\alpha + \beta C) + [U]\{(\gamma + \delta(C - 5M)\}$, where the constant α is the molar CD of the folded protein in the absence of denaturant, β is a constant defining the dependence of this CD on denaturant concentration, and γ and δ are similar constants for the unfolded form of the protein in 5M GuHCl. For CD measurements, gene V proteins were suspended at a fixed concentration of 10 μM in a solution containing 0.05M MOPS [2-(N-morpholino)propanesulfonic acid], 0.1M NaCl, 1 mM EDTA, at pH 7.0, and the indicated concentration of GuHCl. The CD was measured at 25.0°C after at least 1 hour of equilibration, using a cuvette with a path length of 1.0 cm, and the CD from the buffer alone was subtracted.

Our goal was to separate the contributions of packing effects from those of the hydrophobic effect. We used the gene V protein of bacteriophage f1 as a model system (*9*). This protein is a small dimer containing 87 amino acids per subunit with no disulfides and a single cysteine buried within each momomer (*10*). The crystal structure, refined at a resolution of 2.3 Å, consists entirely of strands of β structure connected by turns (*11*). The two subunits make extensive contact and form an intermolecular β barrel at the dimer interface. The gene V protein is reversibly denatured in solutions containing guanidine hydrochloride (GuHCl) (*12*). One can monitor the unfolding at equilibrium by using either the chemical reactivity of the buried Cys residues, which become exposed as the protein unfolds, or the strong circular dichroism (CD) at 229 nm, due to Tyr residues, exhibited only by the folded protein (*12, 13*). The two techniques yielded nearly identical measurements of the extent of denaturation as a function of denaturant concentration for the wild type and each mutant protein in this study (*14*). The dependence of the folding equilibrium on protein concentration in solutions containing denaturants is consistent with that expected for a simple transition between a folder dimer and two unfolded monomers. We estimate that these two forms of the protein constitute at least 90% (and probably nearly all) of the gene V protein present at equilibrium, and thus a two-state model can be used (*1, 2, 12*).

We made gene V proteins with substitutions at Val^{35} and Ile^{47} to measure the effects of side chain packing separately from those of hydrophobicity. These residues are in contact with each other and are completely shielded from solvent by the main chain and external side chain atoms of the monomer polypeptide, representing a locus where changes in the volume and disposition of hydrophobic side chains might have the greatest effects on protein stability (*11*). Residues differing from each other by single methylene groups were chosen to minimize perturbations in the overall structure of the protein. The four proteins studied were the wild type (WT), the single mutants Val^{35}→Ile (V35I) and Ile^{47}→Val (I47V), and the "reversed" double mutant Val^{35}→Ile and Ile^{47}→Val (V35I-I47V). The three mutant genes were obtained by directed mutagenesis of the WT gene in a plasmid (*15–18*). All three mutant genes supported phage growth at 34°C. The plaques were the same size as those formed by the WT, indicating that at this temperature the mutant proteins are fully active and thus are likely to have structures very similar to that of the WT. The similarity of the CD spectra of these proteins in the range from 195 to 250 nm (*13*) supports this conclusion (Fig. 1).

We estimated free energy differences between the folded and unfolded forms of the four proteins by CD measurements of their resistance to GuHCl-induced denaturation (*19, 20*) (Fig. 2) and by making three assumptions. (i) Only the folded dimer and the unfolded monomer are substantially present at equilibrium. (ii) The free energy of unfolding is linearly related to the concentration of GuHCl in the solution. This assumption is important only for estimation of free energy differences in the absence of denaturant (*1, 20*). (iii) The CD of that portion of the protein that is folded (or unfolded) is a linear function of denaturant concentration, even in the transition region where the CD of the two forms cannot be separately measured. The effect of GuHCl on CD is small in the present experiments, so this assumption probably introduces only

a small error (*2*). The CD data were fitted to a simple model by least squares (Fig. 2) (*21*).

The free energies of unfolding of the three mutant proteins were 0.4 to 2.9 kcal/mol less than the free energy of unfolding of WT (Table 1). The free energy differences from WT determined in the presence of 2.25*M* GuHCl, where the equilibrium constants can be measured directly, agreed closely with those extrapolated to the absence of denaturant (Table 1).

The single mutant V35I has an additional methylene group added to each subunit, differing from WT both in hydrophobicity and in packing. The sum of hydrophobicity and packing effects caused a decrease of 0.4 kcal/mol in stability relative to WT. The contribution of packing can be deduced by subtracting the expected effect due to the change in hydrophobicity from this sum. The difference in free energy of transfer from water to octanol between Val and Ile residues is ~0.8 kcal/mol (*4*). Considering hydrophobicity alone, V35I containing two additional methylene groups per dimer would be expected to be ~1.6 kcal/mol more stable than WT. Thus ~2.0 kcal/mol of unfavorable packing interactions exist in V35I compared with WT, probably reflecting a distortion of the protein required to introduce the extra groups.

The mutant I47V is the converse of V35I; it differs from WT by the deletion of one methylene group per subunit. Thus I47V would be ~1.6 kcal/mol less stable than WT based on the hydrophobic effect. This mutant was indeed less stable, but by 2.4 kcal/mol. In this case, packing differences lead to an additional destabilization of ~0.8 kcal/mol. The destabilization of the protein upon replacement of methyl groups by hydrogens is probably due in part to the creation of cavities where the methyl groups originally resided (*6*). In such a cavity a number of potential van der Waals contacts are not made. In extreme cases, a protein might be destabilized by up to ~2 kcal/mol per cavity beyond the loss of stability due to the reduction of buried hydrophobic surface (*22*). That the additional destabilization is not this large in the case of the I47V mutant suggests either that this cavity is partially collapsed or that the methyl group that was removed had not made ideal van der Waals contacts in the WT protein.

The reversed mutant V35I-I47V incorporates both changes made in the two single mutants, leading to a change in packing but not in hydrophobicity as compared with WT. The reversed mutant was the least stable of the three studied (by 2.9 kcal/mol of dimers compared with WT), indicating that packing changes by themselves can substantially destabilize the protein. Poorer packing leading to destabilization could include a net loss of van der Waals contacts, contacts that are too close, strain and distortion induced in the remainder of the protein, or even a gain or loss of internal water molecules. Importantly, the decrement in stability of the double mutant was essentially the sum of the packing effects seen in the single mutants. This result is not unique for Val and Ile at positions 35 and 47. In other experiments, double mutants such as V35L-I47L, V35I-I47L, and V35L-I47V (where L is Leu) had stability decrements that were roughly the sum of the destabilizations of their constituent single mutants (*14*). This additivity of stability effects indicates that the destabilization caused by substitutions at positions 35 and 47 is not due primarily to interactions between these two residues but rather is due to unfavorable contacts with the rest of the protein.

It is conceivable that the mutations studied here affect the stability of the unfolded rather than the folded form of the protein. Either would result in a net change in the free energy of unfolding. In the case of the mutant with the double substitution, this appears unlikely. The WT protein and the double mutant differ in the positions of two amino acids but have identical composition, and in the flexible denatured state these would be expected to have similar environments.

Packing effects appear to destabilize these three mutant proteins by 0.8 to 2.9 kcal/mol, whereas hydrophobic effects stabilize or destabilize them by only 0 to 1.6 kcal/mol (Fig. 3). Therefore, both effects must be considered in order to predict correctly the stabilities of proteins with apolar-to-apolar amino acid substitutions at interior residues. A different way in which to view these results is to note that all of the mutations destabilized the gene V protein. Thus, simply adding buried hydrophobic groups to proteins may not generally increase their stabilities. Our results indicate that it is not simple to predict the stability of proteins with substitutions at buried sites. More stable forms of many proteins can probably be engineered in this way, but only after we learn how to repack their interiors without structural distortion (*23*).

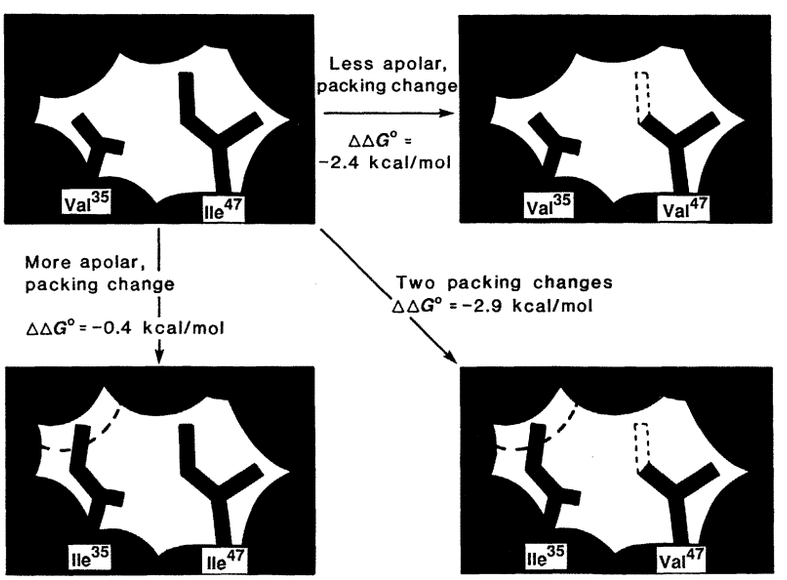

Fig. 3. Schematic illustration of stability changes induced by amino acid substitutions.

REFERENCES AND NOTES

1. T. E. Creighton, *Proteins* (Freeman, New York, 1984).
2. C. Ghelis and J. Yon, *Protein Folding* (Academic Press, New York, 1982).
3. W. Kauzmann, *Adv. Protein Chem.* **14**, 1 (1959); C. Tanford, *The Hydrophobic Effect* (Wiley, New York, ed. 2, 1980).
4. J.-L. Fauchère and V. Pliška, *Eur. J. Med. Chem. Chim. Ther.* **18**, 369 (1983).
5. W. P. Jencks, *Catalysis in Chemistry and Enzymology* (Dover, New York, 1987); C. Chothia, *Annu. Rev. Biochem.* **53**, 537 (1984); D. Eisenberg and A. D. McLachlan, *Nature* **319**, 199 (1986); Y. Nozaki and C. Tanford, *J. Biol. Chem.* **246**, 2211 (1971); R. Wolfenden, L. Andersson, P. M. Cullis, C. C. B. Southgate, *Biochemistry* **20**, 849 (1981); K. Yutani, K. Ogasahara, T. Tsujita, Y. Sugino, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 4441 (1987); J. T. Kellis, Jr., K. Nyberg, D. Šali, A. R. Fersht, *Nature* **333**, 784 (1988).
6. M. Matsumura, W. J. Becktel, B. W. Matthews, *Nature* **334**, 406 (1988).
7. C. Chothia, *ibid.* **254**, 304 (1975); G. E. Schulz and R. H. Schimer, *Principles of Protein Structure* (Springer-Verlag, New York, 1979).
8. F. M. Richards, *Annu. Rev. Biophys. Bioeng.* **6**, 151 (1977); J. W. Ponder and F. M. Richards, *J. Mol. Biol.* **193**, 775 (1987).
9. B. Alberts, L. Frey, H. Delius, *J. Mol. Biol.* **68**, 139 (1972); J. S. Salstrom and D. Pratt, *ibid.* **61**, 489 (1971).
10. H. T. Pretorius, M. Klein, L. A. Day, *J. Biol. Chem.* **250**, 9262 (1975); S. J. Cavalieri, K. E. Neet, D. A. Goldthwait, *J. Mol. Biol.* **102**, 697 (1976); S. C. Kowalczykowski, D. G. Bear, P. H. von Hippel, in

The Enzymes, P. D. Boyer, Ed. (Academic Press, New York, 1981), vol. 14, p. 373.
11. G. D. Brayer and A. McPherson, *J. Mol. Biol.* **169**, 565 (1983).
12. H. Liang and T. C. Terwilliger, unpublished observations.
13. L. A. Day, *Biochemistry* **12**, 5329 (1973).
14. W. Sandberg and T. C. Terwilliger, unpublished observations.
15. T. C. Terwilliger, *Gene* **71**, 41 (1988).
16. ———, W. D. Fulford, H. B. Zabin, *Nucleic Acids Res.* **16**, 9027 (1988).
17. T. C. Terwilliger, *Gene* **69**, 317 (1988).
18. The genes were expressed in *Escherichia coli* and the protein was purified in the same way as WT (*9*). All four proteins had Stokes radii corresponding to a dimer with the expected molecular mass of 20 kD as determined by size-exclusion chromatography [R. J. T. Corbett and R. S. Roche, *Biochemistry* **23**, 1988 (1984)], and each purified protein was homogeneous as assessed by SDS–gel electrophoresis [U. K. Laemmli and M. Favre, *J. Mol. Biol.* **80**, 575 (1973); K. Ito, T. Date, W. Wickner, *J. Biol. Chem.* **255**, 2123 (1980)].
19. C. N. Pace, *Crit. Rev. Biochem.* **2**, 1 (1975).
20. J. A. Schellman, *Biopolymers* **26**, 549 (1987).
21. P. R. Bevington, *Data Reduction and Error Analysis for the Physical Sciences* (McGraw-Hill, New York, 1969).
22. A. R. Fersht, *Enzyme Structure and Mechanism* (Freeman, New York, ed. 2, 1985).
23. H. Nojima, A. Ikai, T. Oshima, H. Noda, *J. Mol. Biol.* **116**, 429 (1977); M. F. Perutz and H. Raidt, *Nature* **255**, 256 (1975); J. E. Walker, A. J. Wonacott, J. I. Harris, *Eur. J. Biochem.* **108**, 581 (1980).
24. We thank T. Chatman, S. Choi, and S. Wilcoxen for technical assistance; H. Tager, H. Zabin, and H. Liang for helpful discussions; and P. Gardner and D. Steiner for generous gifts of oligodeoxynucleotides. We acknowledge support (to T.C.T.) from the Bristol Myers Company, the Duchussois Foundation, NIH grant GM38714, and NSF Presidential Young Investigator Award DMB 8657754. W.S.S. was supported by NIH grant PHS 5732 GM07281.

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Purification and Complementary DNA Cloning of a Receptor for Basic Fibroblast Growth Factor

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Basic fibroblast growth factor (bFGF) participates in many processes including early developmental events, angiogenesis, wound healing, and maintenance of neuronal cell viability. A 130-kilodalton protein was isolated on the basis of its ability to specifically bind to bFGF. A complementary DNA clone was isolated with an oligonucleotide probe corresponding to determined amino acid sequences of tryptic peptide fragments of the purified protein. The putative bFGF receptor encoded by this complementary DNA is a transmembrane protein that contains three extracellular immunoglobulin-like domains, an unusual acidic region, and an intracellular tyrosine kinase domain. These domains are arranged in a pattern that is different from that of any growth factor receptor described.

THE FIBROBLAST GROWTH FACTOR (FGF) family consists of polypeptide growth factors characterized by amino acid sequence homology, heparin-binding avidity, the ability to promote angiogenesis, and mitogenic activity toward cells of epithelial, mesenchymal, and neural origin. Members of the FGF family appear to have roles in development, tissue repair, maintenance of neurons, and the pathogenesis of disease (*1–6*). Aberrant expression of FGFs may cause cell transformation by an autocrine mechanism (*7, 8*). Moreover, FGFs may enhance tumor growth and invasiveness by stimulating blood vessel growth into the tumor (*4*) or by inducing production of proteases such as plasminogen activator (*9*).

The FGF family includes acidic and basic FGFs (*10*), the *int-2* gene product (*11*), the *hst* gene product (Kaposi sarcoma–FGF) (*6, 12*) FGF-5 (*13*), and the keratinocyte growth factor (*14*). The actions of acidic and basic FGF are mediated through binding to high-affinity cell surface receptors of 145 and 125 kD (*15*). It is not known, however, whether each FGF interacts with a different receptor or whether the different forms of FGF share the same receptor. We now describe an attempt to characterize the primary structure of the receptor for basic FGF (bFGF).

The bFGF receptor was purified by a lectin-affinity chromatography step followed by a specific ligand-affinity chromatography procedure with bFGF that had been biotinylated on cysteine residues. The modified bFGF was indistinguishable from unmodified bFGF in its ability to inhibit the binding of ^{125}I-labeled bFGF to high-affinity bFGF receptors in Swiss 3T3 cells (Fig. 1A) and its ability to stimulate the phosphorylation of a 90-kD protein (*16*), known to be a substrate of bFGF-induced tyrosine kinase activity (*17*). The biotinylation reaction modified 90 to 95% of the bFGF molecules as measured by binding to avidin-conjugated agarose (Fig. 1A). Less than 5% of control bFGF, which had been subjected to a mock biotinylation reaction, bound to the avidin-agarose (Fig. 1A). Iodine-125–labeled biotin-bFGF bound to bFGF receptors in Swiss 3T3 cells with high affinity (dissociation constant = 1 n*M*) and could

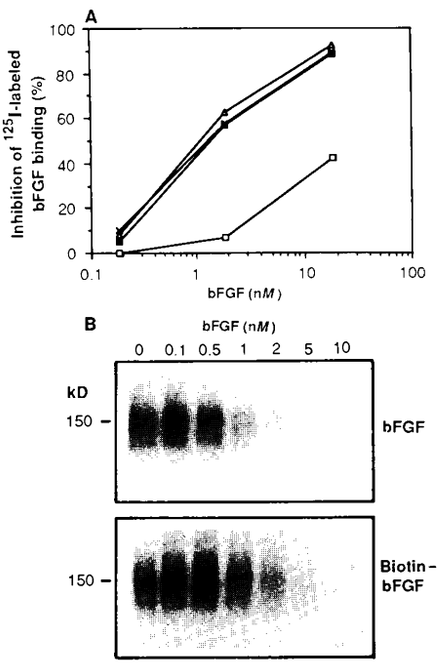

Fig. 1. (**A**) Competitive binding of ^{125}I-labeled bFGF to Swiss 3T3 cells. Iodine-125–labeled bFGF (2 Ci/μmol) was added to confluent cells (6 fmol of ^{125}I-labeled bFGF per 10^5 cells) in the presence of the indicated concentrations of: unmodified bFGF (x); biotin-bFGF (■); the unbound fraction after biotin-bFGF was incubated with avidin-agarose (□); the unbound fraction after bFGF was incubated with avidin-agarose (△). Binding was performed for 30 min at 37°C in culture media (DME H21) containing 0.2% gelatin and heparin (15 U/ml). The cells were washed three times with a buffer containing 20 m*M* Hepes (*p*H 7.4), 0.2% gelatin, and 150 m*M* NaCl. The radioactivity present was determined in a Beckman gamma counter. Maximal binding (0% inhibition) represents 5700 cpm of specific binding (nonspecific binding was 600 cpm). All determinations were made in triplicate. Recombinant human bFGF (*37*) was iodinated using iodogen (Pierce). bFGF was biotinylated with iodoacetyl-LC-biotin (Pierce) at a 4:1 molar excess of cysteine residues in 10 m*M* tris-HCl (*p*H 8.0) for 5 hours at 4°C (*38*). Unreacted biotin was removed by gel filtration with PD 10 columns (Pharmacia). (**B**) Whole-cell cross-linking. ^{125}I-labeled biotin-bFGF or ^{125}I-labeled bFGF (0.1 pmol) was added to Swiss 3T3 cells (5×10^5 cells) in the presence or absence of unlabeled bFGF as indicated. The cells were washed and cross-linked with 0.15 m*M* disuccinimidyl suberate (DSS) (Pierce). The cells were then solubilized and subjected to SDS–polyacrylamide gel electrophoresis (PAGE), and ^{125}I-labeled proteins were detected by autoradiography.

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