nesquehonite may form directly from the resultant solutions. Therefore, the observed mineralogy and morphology of the nesquehonite and current knowledge of nesquehonite stability are both consistent with direct formation of nesquehonite under Antarctic conditions. The formation of additional nesquehonite during curation of the meteorite can be understood as continued outward migration and evaporation of the saline solutions formed in Antarctica.

Antarctic meteorites have been on the earth for on the order of 10^4 to 10^6 years and have apparently been transported great distances by glaciers (*25, 26*). Whether chemical weathering of meteorites occurred during their encasement in ice or only after they were exposed to the atmosphere on stranding surfaces has been uncertain (*5*). Results for nesquehonite from LEW 85320 suggest that, at least for salt formation, weathering may be sufficiently rapid that most observable effects can develop in tens of years rather than over thousands of years. Rapid formation of nesquehonite suggests, but does not prove, that most weathering phenomena occur after exhumation of the meteorites from deep-glacial ice. In the simplest interpretation, ages of magnesium carbonate weathering products on Antarctic meteorites would indicate the times elapsed since surface exposure of the meteorites. The amount of rust on a surface alone cannot be used as a reliable indicator of the state of preservation of meteorites. The correlation between degrees of weathering and terrestrial-residence ages might evade detection if reliance is placed solely on the rust index.

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Herbicide Resistance in Transgenic Plants Expressing a Bacterial Detoxification Gene

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The herbicide bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) is a photosynthetic (photosystem II) inhibitor in plants. A gene, *bxn*, encoding a specific nitrilase that converts bromoxynil to its primary metabolite 3,5-dibromo-4-hydroxybenzoic acid, was cloned from the natural soil bacterium *Klebsiella ozaenae*. For expression in plants, the *bxn* gene was placed under control of a light-regulated tissue-specific promoter, the ribulose bisphosphate carboxylase small subunit. Transfer of this chimeric gene and expression of a bromoxynil-specific nitrilase in leaves of transgenic tobacco plants conferred resistance to high levels of a commercial formulation of bromoxynil. The results presented indicate a successful approach to obtain herbicide resistance by introducing a novel catabolic detoxification gene in plants.

ADVANCES IN GENETIC ENGINEERING of plants have allowed the development and transfer of agronomically important traits such as viral resistance, insect resistance, and herbicide resistance. These traits allow transfer of single dominant genes that exhibit a rapidly discernible phenotype. Herbicide resistance can be achieved by at least three different mechanisms: overproduction of a herbicide-sensitive biochemical target; structural alteration of a biochemical target, resulting in reduced herbicide affinity; or detoxification-degradation of the herbicide before it reaches the biochemical target inside the plant cell. Resistance obtained by the first two mechanisms has been developed for the herbicides glyphosate (*1, 2*), atrazine (*3*), the sulfonylureas (*4*), and phosphinothricin (*5*). A report describing the transfer to plants of a *Streptomyces* gene encoding a phosphinothricin acetyltransferase (*6*) resulted in phosphinothricin-resistant plants, establishing detoxification by conjugation as a viable strategy. Two advantages of a detoxification-degradation mechanism, as opposed to altering a biochemical target, are that specialized compartmentation of the detoxifying activity is not required and that greater herbicide resistance can be achieved with lower levels of detoxifying enzyme. Disadvantages include the potential toxicity of one or more metabolites and the possibility that detoxifying activities might react with endogenous plant compounds to impair plant function.

We have been interested in the nitrile-containing broadleaf herbicide bromoxynil (3,5-dibromo-4-hydroxybenzonitrile), a potent photosystem II (PSII) inhibitor. Although the actual chloroplast-localized biochemical target is not well defined, there is evidence that bromoxynil acts by binding a component of the quinone-binding protein complex of PSII, inhibiting electron transfer (*7, 8*). It has further been suggested that a low-affinity binding site within this complex exists in the 32-kD polypeptide (*9, 10*). Bromoxynil has a very short half-life in the environment, as microbial populations and tolerant plant species can convert the cyano moiety of bromoxynil to the corresponding amide and acid derivatives (*11–13*). A natural soil isolate, *Klebsiella ozaenae*, has been identified that transforms bromoxynil to 3,5-dibromo-4-hydroxybenzoic acid, releasing ammonia (*14*). This reaction (Fig. 1) is carried out via a bromoxynil-specific nitrilase. The nitrilase gene (*bxn*), which is plas-

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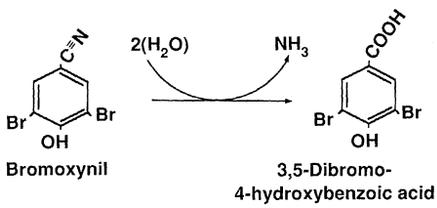

Fig. 1. Reaction depicting the conversion of the herbicide bromoxynil to the primary metabolite via the bromoxynil-specific nitrilase.

mid-encoded in *K. ozaenae*, was cloned on a 2.6-kb DNA fragment and expressed in *Escherichia coli* (*15*). The nucleotide sequence of a 1.2-kb DNA segment was subsequently determined and shown to encode a 38-kD polypeptide (*16*). The bromoxynil-specific nitrilase specified by this fragment was purified to homogeneity and characterized (*16*). A chimeric *bxn* gene has now been constructed for expression in plants. The plant promoter used for this chimeric gene is derived from a tobacco light-inducible tissue-specific ribulose bisphosphate (RuBP) small subunit gene (*17*). Genes from the RuBP small subunit family have been shown to be expressed only in photosynthetic tissue (*18, 19*). The rationale for using this promoter is the following. If the primary target of bromoxynil is photosynthesis and if the herbicide is not translocated within the plant, then detoxification of bromoxynil in green tissue should be a minimal requirement to obtain whole plant resistance. Transgenic tobacco plants expressing the bromoxynil-specific nitrilase in photosynthetic tissues are resistant to high doses of a commercial formulation of bromoxynil, and the trait is stably inherited in the succeeding generation.

Nicotiana tabacum cv. 'Xanthi' plants were maintained axenically through shoot transplants and used as tissue donors. Disks (2 mm in diameter) were excised from young leaves and placed in Murashige and Skoog medium containing indoleacetic acid (2 mg/liter) and kinetin (2 mg/liter) and placed in the dark at 23°C. *Agrobacterium tumefaciens* strain LBA4404 (*20*) containing the shuttle vectors pBrx39 or pBrx40 (*21*) carrying chimeric genes for expression in plants were grown overnight and added to the leaf disks. Cocultivation, selection of transformed shoots on kanamycin (100 mg/liter), and regeneration of plants were essentially carried out as described (*22*). Plants were transferred to 10-cm pots containing soil and maintained in a growth chamber (25°C, 50% relative humidity, and 16-hour photoperiod) or under greenhouse conditions.

A number of independent kanamycin-resistant transgenic plants generated by cocultivation were selected for analysis. Leaf cuttings were obtained from individual plants and, in two separate experiments, were maintained photoautotrophically in the presence of increasing bromoxynil concentrations; chlorosis was monitored (Fig. 2). Control tissue is normally bleached at $10^{-6}M$ to $10^{-5}M$ bromoxynil. Leaf tissue from a number of transformed plants (39-3, 40-2, 40-4, 40-6, and 40-7) appeared normal at $10^{-5}M$ bromoxynil, and tissue from plants 40-1 and 40-5 appeared normal at $10^{-4}M$ bromoxynil. Plants 39-2 and 40-3 were bleached at the same rate as control samples and subsequently were shown not to be transformed.

To further characterize these plants with respect to integration of the chimeric *bxn* gene and expression of the bromoxynil-specific nitrilase, we analyzed individual plants judged to be bromoxynil-resistant from the leaf section experiment. DNA blot analysis of six of the transformed plants indicated that these plants contained copies of *bxn* gene sequences (Fig. 3). Five of the plants contained the intact 4.5-kb Hind III fragment, the expected size of the small subunit–*bxn*–octopine synthase (*ocs*) chimeric gene. Plants 40-1 and 40-4 had an extra DNA segment containing *bxn* sequencing, and plant 40-5 had DNA fragments of two different sizes, indicating rearrangements of the chimeric gene during *Agrobacterium*-mediated transfer and integration into plant DNA. Copy number analysis indicates that one to three copies of the *bxn* gene are present, depending on the transgenic plant analyzed. Leaf samples from these selected plants were subjected to immunoblot analysis (Fig. 3). Plants 40-1, 40-5, and 40-7 had high levels of the 38-kD nitrilase polypeptide, whereas plants 40-4 and 40-6 had much lower levels of nitrilase. Expression of the enzyme in plant 39-3 is very low and can only be detected by overexposing the blot. Antiserum to the bromoxynil-specific nitrilase is not cross-reactive with any major plant components (although a nonspecific interaction of the immunoglobulin G fraction can be seen). The appearance of only the 38-kD polypeptide and no degradation products indicates stability of this polypeptide in plant cells. These differential protein levels observed for the independent transformants correlate with the level of bromoxynil resistance obtained in the leaf section assay described in the legend to Fig. 2. Leaf tissue from plants 40-1 and 40-5 contained the highest levels of nitrilase, and no chlorosis was observed at $10^{-4}M$ bromoxynil. These data suggest that small subunit promoter–controlled expression of the bromoxynil-specific nitrilase in leaf tissue results in protection of leaf tissue from photosynthetic damage. Messenger RNA levels were determined for one of these transgenic plants (40-1). Chimeric mRNA of the expected size (1.8 kb) was observed at a high level in leaf tissue, a low level in stem tissue, and was undetectable in roots (*23*). This finding indicates that the small subunit promoter was functioning in a tissue-specific manner.

Four of the primary (T_1) transformants were subjected to both open pollination and backcross experiments for genetic analysis tests. These transformants were chosen because they exhibited a range of nitrilase levels and contained a nonrearranged 4.5-kb small subunit–*bxn*–*ocs* chimeric gene. Plants (T_2) were grown from seed and subjected to

Table 1. Inheritance of the *bxn* gene in transformed plants. Approximately 200 T_2 progeny plants were obtained from both open pollination (OP) and backcross experiments of four primary (T_1) transformants and analyzed by spraying at the three to four leaf stage with a commercial formulation of bromoxynil (Buctril) at 0.5 lb per acre. Segregation ratios and χ^2 values were calculated on the basis of the expected values. Qualitative determination of nitrilase levels was derived from scanning protein immunoblots of T_1 transformed leaf tissue (Fig. 3). Values are expressed as a percentage of total leaf protein. Approximately 300 T_2 progeny plants were obtained by open pollination of the original plant. Plants were divided into lots of 30 each and sprayed with the following concentrations of Buctril: 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 lb per acre. Maximum resistance level was determined as the concentration at which chlorotic lesions appeared. Segregants that did not carry the gene were not scored.

Cross	Genetic analysis			Loci	Nitrilase level (%)	Maximum resistance level (lb/acre)
	Bromoxynil-resistant	Bromoxynil-sensitive	Ratio (χ^2)			
39-3 OP	102	48	3/1 (3.920)			
39-3 × 'Xanthi'	106	98	1/1 (0.156)	1	0.0007	1.5 to 2.0
40-1 OP	189	11	15/1 (0.192)			
40-1 × 'Xanthi'	170	34	3/1 (3.777)	2	0.01	>4.0
40-6 OP	160	47	3/1 (0.583)			
40-6 × 'Xanthi'	103	95	1/1 (0.602)	1	0.002	3.0
40-7 OP	178	62	3/1 (0.088)			
40-7 × 'Xanthi'	102	77	1/1 (3.755)	1	0.005	4.0

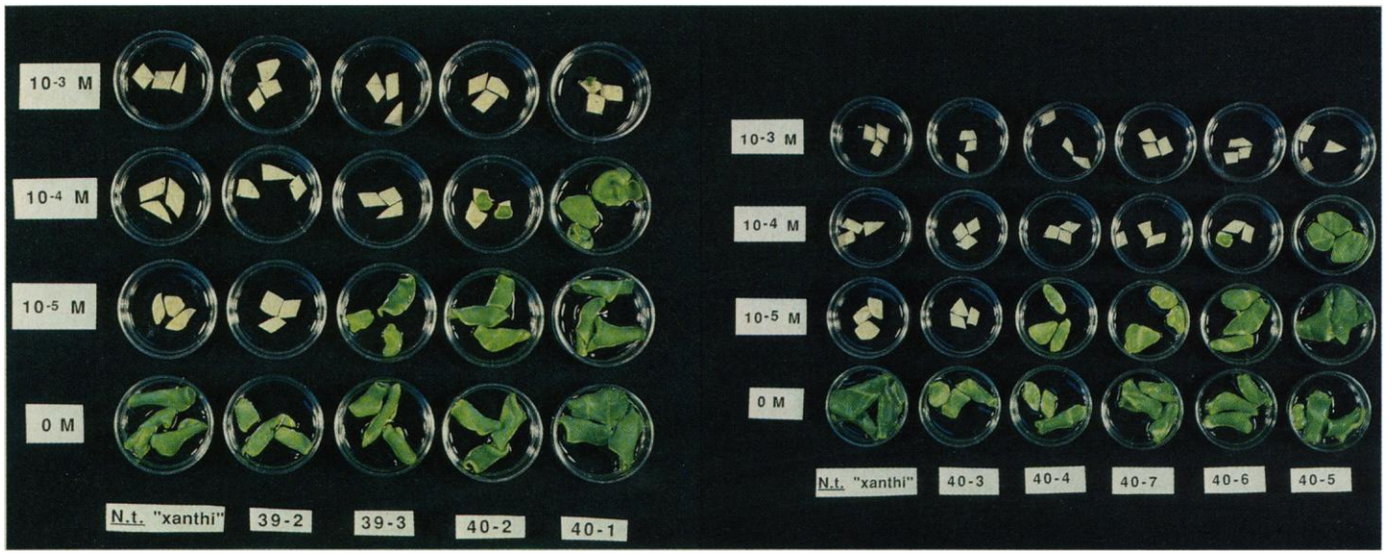

Fig. 2. Leaf section analysis of transgenic tobacco plants. Leaf cuttings were obtained from selected transformed plants, surface-sterilized, placed in the concentrations of bromoxynil indicated, and maintained photoautotrophically (*27*); chlorosis was monitored. Photographs were taken 11 days after initiation of the experiment. *N.t.* 'Xanthi' refers to control leaf sections.

genetic analysis by spraying with a commercial formulation of bromoxynil (Buctril, Rhône-Poulenc) at 0.5 lb (0.23 kg) per acre (normally a high field rate). Another set of plants was subjected to Buctril spraying at 0.5 lb per acre increments to determine maximum level of tolerance before chlorotic lesions appeared (Table 1). The bromoxynil resistance trait segregated as a single genetic locus in progeny of plants 39-3, 40-6, and 40-7, and as two independent loci in progeny of 40-1 in both open pollination and backcross experiments. The maximum Buctril resistance levels exhibited by the progeny of these four plants also correlated with the protein level observed in leaf tissue obtained from the respective primary (T_1) transformant. Progeny from 39-3, which contained the lowest level of nitrilase, exhibited symptoms at lower rates (1.5 to 2.0 lb per acre), whereas progeny of 40-1, which contained high nitrilase levels, exhibited no symptoms even at 4.0 lb per acre. This result suggests that enzyme levels determined in the primary transformants are maintained in their progeny and that the greater the level of nitrilase in the leaves of transgenic plants, the higher the level of bromoxynil resistance. Selected plants from the progeny (T_2) of plant 40-1 that were sprayed with bromoxynil at increasing rates are shown in Fig. 4. These transgenic plants expressing nitrilase grow, flower, and set seed normally, even when sprayed with concentrations of bromoxynil eightfold higher than the highest field rate normally used. The enzyme appears to be localized in the plant cell cytosol, as chloroplasts prepared from leaf tissue contain no detectable nitrilase polypeptide nor can the bromoxynil-specific ni-

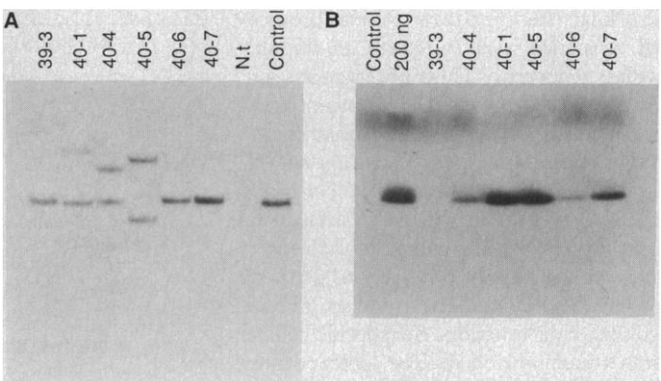

Fig. 3. (**A**) Plant DNA was prepared as described (*28*) from 1-g leaf samples and digested to completion with Hind III, and 5 to 7 μg of total plant DNA were electrophoresed in a 1% agarose gel. The DNA was transferred to nitrocellulose, and the blot was hybridized with a 1.2-kb Bam HI–Eco RI *bxn* gene fragment labeled with ^{32}P (*29*). *N.t.* refers to DNA isolated from nontransformed tobacco tissue. Control contains the isolated 4.5-kb Hind III fragment (two copies per genome equivalent) added to DNA isolated from nontransformed plant. (**B**) Immunochemical detection of the enzyme from leaf tissue of selected transformants. Blotting procedure is described in (*30–33*). Control indicates nontransformed leaf tissue. The second lane contains 200 ng of purified nitrilase (*16*) spiked into a nontransformed leaf sample and immunoprecipitated.

Fig. 4. Phenotype of transformed plants. T_2 seed from primary transformant 40-1 was grown in 10-cm pots (three plants per pot) to the three to four leaf stage and sprayed with a commercial formulation of bromoxynil (Buctril) at the amounts indicated. Spray blank contained the formulation without the active ingredient. The photograph was taken 6 days after spraying.

trilase be incorporated into chloroplasts in vitro (*24*).

Expression of the *K. ozaenae* bromoxynil-specific nitrilase in photosynthetic tissue of transgenic plants confers high-level resistance to bromoxynil. The herbicide is converted to a nontoxic metabolite, 3,5-dibromo-4-hydroxybenzoic acid, by the enzyme. A low level of nitrilase expression (approximately 0.0007% total leaf protein) is required to obtain high commercial resistance levels; the higher the expression level of nitrilase, the greater the degree of resistance that can be achieved (although this is not a direct linear relation). Expression of the enzyme in plants can be achieved under the control of a tissue-specific light-regulated promoter to attain whole plant resistance. Protection of the herbicide-sensitive photosystem II target (chloroplast located) can be obtained by expressing the bromoxynil-specific nitrilase in a separate cellular compartment (cytosol). The trait is dominant and heritable, and the enzyme levels obtained in the primary transformants relate to bromoxynil resistance levels observed in the succeeding generation. These observations suggest that in the case of the bromoxynil-specific nitrilase and the phosphinothricin acetyltransferase (*6*), detoxification-degradation is superior to altering a biochemical target to obtain herbicide resistance in plants by gene transfer technology. Complex pathways and mutant proteins and genes do not have to be engineered nor are specialized targeting functions for specific cellular compartments needed. The sole requirement consists of a single-step conversion of the herbicide to a nontoxic metabolite.

Although extensive analysis of residues in these transgenic plants has not been completed, it will be interesting to determine the fate of the metabolite (3,5-dibromo-4-hydroxybenzoic acid) produced by the action of nitrilase. If the bromine atoms of 3,5-dibromo-4-hydroxybenzoic acid are hydrolyzed by an endogenous plant dehalogenase, then the product of this reaction would be *p*-hydroxybenzoic acid, a normal constituent of plant metabolism. In this case, no metabolites of bromoxynil would be detected. Alternatively, 3,5-dibromo-4-hydroxybenzoic acid could be concentrated in the vacuole or transported out of the cell or both may occur. The bromoxynil-specific nitrilase is active in vitro only as a dimer (*16*), suggesting that this polypeptide must undergo formation in the plant cell into an active complex. Supporting this phenomenon is the appearance of a faint undenatured band at approximately 72-kD on immunoblots (*25*). An analogous result was observed on simultaneous expression of the bacterial *luxA* and *luxB* genes in transgenic plants (*26*) for detection of an active luciferase. These results indicate that bacterial proteins can form active enzymes in a diverse plant cell environment.

The gene encoding the bromoxynil-specific nitrilase can be used as a selective marker for rapid screening at the whole plant level. Leaf sections can be excised from the plant and screened photoautotrophically, or individual leaves can be treated with commercial bromoxynil-formulations without harm to the remainder of the plant. At high bromoxynil concentrations, symptoms can be scored within 24 to 36 hours after application. Finally, expression of the *K. ozaenae* bromoxynil-specific nitrilase in transgenic plants to obtain a bromoxynil-resistant phenotype is another example that agronomically important germplasm can be derived from a nonplant source and that novel and diverse biochemical pathways can be introduced into plants without deleterious effects.

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21. Construction of a chimeric small subunit–*bxn*–*ocs* gene for expression in plants. The nucleotide sequence of a 1212-bp Pst I–Hinc II DNA segment encoding the bromoxynil-specific nitrilase has been described (*16*). This sequence contains 65 bp of 5′ untranslated nucleotides. To facilitate removal of a portion of these excess nucleotides, plasmid pBrx9 (*15*) was digested with Pst I and treated with nuclease Bal 31 and Bam HI linkers added to the resulting ends. Bam HI–Hinc II fragments containing a functional *bxn* gene were cloned into the Bam HI–Sma I sites of pCGN565 (a pUC18 derivative containing a chloramphenicol resistance marker). A plasmid, pBrx25, was generated that contained only 11 bp of 5′ untranslated bacterial sequence. A Bam HI–Sac I DNA fragment containing the *bxn* gene was excised from pBrx25 and cloned to the Bam HI–Sac I sites of pCGN1510 to create plasmid pBrx36. Plasmid pCGN1510 (K. E. McBride, unpublished data) contains a 1.4-kb segment of DNA from a tobacco small subunit gene (TSSU 3-8) 5′ untranslated region (*17*) and a 2.6-kb DNA fragment from the *ocs* 3′ region of the transferred DNA (T-DNA) [R. F. Barker, K. Idler, D. Thompson, J. Kemp, *Plant Mol. Biol.* **2**, 335 (1983)]. These DNAs provide the necessary initiation and termination signals, respectively, for expression of the chimeric mRNA in plants. A 4.5-kb Hind III fragment was excised from plasmid pBrx36 and cloned in both orientations into the Hind III site of the *Agrobacterium* binary vector plasmid pCGN783 (C. M. Houck, D. K. Shintani, V. C. Knauf, *Frontiers Appl. Microbiol.*, in press) to generate plasmids pBrx39 and pBrx40. Plasmid pCGN783, a pRK290-based broad host range vector, has a chimeric gene encoding neomycin phosphotransferase (35S-npt-tr7) for selection of kanamycin-resistant transformed plants, border segments of the T-DNA to facilitate transfer to plant DNA, and a gentamycin resistance marker for *Agrobacterium* selection.
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27. Leaf sections were surface-sterilized, suspended in medium containing Murashige and Skoog salts, naphthaleneacetic acid (0.93 mg/liter), and benzylaminopurine (0.11 mg/liter) and placed in a sealed box where a 15% O_2, 5% CO_2, and 80% N_2 air flux was circulated at about 1 liter/min. The box was placed at 25°C under 5000 lux with an 18-hour daily light exposure.
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30. For each sample, 1 g fresh weight of leaf material was ground to a powder in liquid nitrogen. Polyvinylpyrrolidone (0.3 g) was added, then 2 ml of an extraction buffer containing 0.1*M* potassium phosphate, *p*H 6.8, 0.15*M* NaCl, 10 m*M* EDTA, bovine serum albumin (25 mg/ml), 0.3% Tween 20, 0.05% NP-40, 10 m*M* dithiothreitol, 10 m*M* thiourea, 1 m*M* phenylmethylsulfonyl fluoride, and 10 μ*M* leupeptin. Samples were centrifuged at 15,000*g* for 15 min; 15 μl of nitrilase antiserum obtained from rabbits and 125 μl of 10% (w/v) suspension of cleaned *Staphylococcus aureus* (Calbiochem) were added to each supernatant. After 1 hour incubation at room temperature, samples were centrifuged and the pellet was washed twice with 3 ml of a solution containing 50 m*M* tris, *p*H 7.5, 1 m*M* EDTA, 0.5*M* NaCl, and 0.05% NP-40. The pellets were resuspended in 30 μl of a solution containing 125 m*M* of tris, *p*H 6.8, 4% SDS, 20% glycerol, 10% β-mercaptoethanol, and 0.05% bromophenol blue. The samples were then heated for 5 min at 95°C, spun in the microfuge for 5 min, and the supernatants electrophoresed in 10% acrylamide gels (*31*). The resolved polypeptides were transferred to nitrocellulose filters as described (*32*). Filters were incubated in Blotto (*33*) overnight at 4°C or for 1 hour at room temperature and then incubated for 30 min at room temperature in Blotto containing a 1:200 dilution of antiserum to nitrilase. Filters were washed for 10 min in 20 m*M* tris, *p*H 7.5, and 150 m*M* NaCl and then for 20 min in the same buffer containing 0.05% Tween 20, and for another 10 min in buffer without Tween 20. Then, 50 ml of Blotto containing 25 μCi of ^{125}I-labeled protein A was added to the filters and incubated at room temperature with agitation for 2 hours. The filters were washed 2 hours in 50 m*M* tris, *p*H 7.5, 1*M* NaCl, and 0.5% Sarkosyl, and then 1 to 2 hours in 50 m*M* tris, *p*H 7.5, 5 m*M* EDTA, 150 m*M* NaCl, 0.5% Triton X-100, and 0.1% SDS. After rinsing and drying, filters were exposed to Kodak AR x-ray film.
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Single-Chain Antigen-Binding Proteins

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Single-chain antigen-binding proteins are novel recombinant polypeptides, composed of an antibody variable light-chain amino acid sequence (V_L) tethered to a variable heavy-chain sequence (V_H) by a designed peptide that links the carboxyl terminus of the V_L sequence to the amino terminus of the V_H sequence. These proteins have the same specificities and affinities for their antigens as the monoclonal antibodies whose V_L and V_H sequences were used to construct the recombinant genes that were expressed in *Escherichia coli*. Three of these proteins, one derived from the sequence for a monoclonal antibody to growth hormone and two derived from the sequences of two different monoclonal antibodies to fluorescein, were designed, constructed, synthesized, purified, and assayed. These proteins are expected to have significant advantages over monoclonal antibodies in a number of applications.

TYPICAL ANTIBODIES ARE COMPOSED of four polypeptide chains, two light chains of about 220 amino acid residues and two heavy chains of about 440 amino acids (*1*). These chains fold into domains of approximately 110 amino acids, assuming a conserved three-dimensional conformation (*2*). The domains associate to form discrete structural regions. The antigen binding or variable regions are formed by the interaction of the variable light (V_L) and variable heavy (V_H) domains at the amino termini of the chains. The first constant regions are formed by interaction of the remainder of the light chain and the first constant domain of the heavy chain. Two or three additional constant regions are formed by interaction of the two heavy chains. The heavy chain constant regions are responsible for effector functions, such as complement fixation and binding to receptors (*3*).

The high background present when intact antibodies have been used to image tumors is in large part due to the binding of the antibody to nontumor cells via the heavy chain constant regions (*4*). When Fab fragments, consisting of the variable region and the first constant region, have been used the background problem has been partially circumvented (*4*). Therefore, a better molecule to use as an imaging or delivery agent would be the Fv fragment, which consists of only the V_L and V_H domains. Unfortunately, there have been few reports of the successful isolation of Fv fragments by proteolytic digestion of intact antibody molecules (*5*).

The idea for the design and synthesis of single-chain antigen-binding proteins was conceived during attempts to circumvent problems encountered when expressing antibody genes in *Escherichia coli* and to avoid problems associated with reassociation of Fv fragments. These proteins consist of the V_L and V_H sequences synthesized as a single polypeptide chain, with the carboxyl terminus of the V_L linked by a designed peptide to the amino terminus of the V_H. Both chains are therefore expressed in equimolar concentrations, and the covalent linking of the two chains facilitates the association of the V_L and V_H domains after folding.

Determination of the three-dimensional structures of antibody fragments by x-ray crystallography has led to the realization that variable domains are each folded into a characteristic structure composed of nine strands of closely packed β-sheets. This structure is maintained despite sequence

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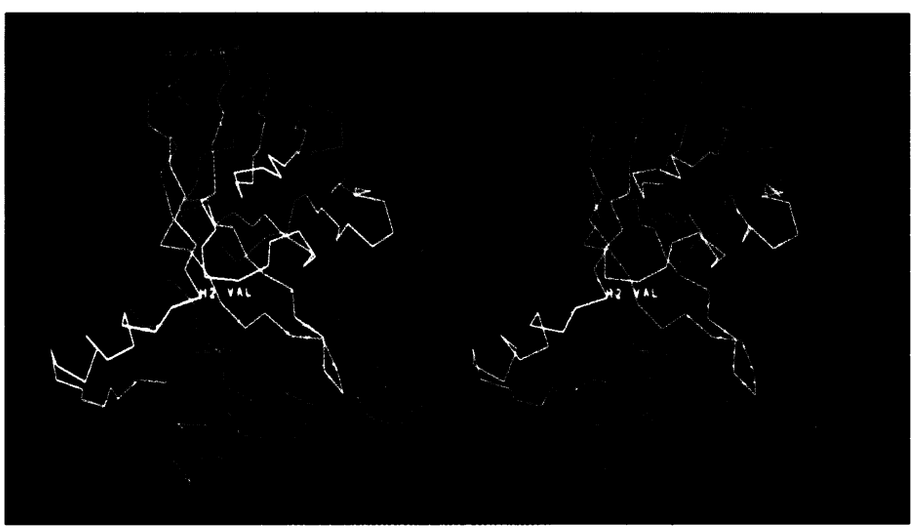

Fig. 1. An alpha-carbon model of the anti-BGH 3C2/59 single-chain antigen-binding protein. The V_L is red, the V_H is blue, and the linker is green. The model was generated by superimposing the sequences of the 3C2 V_L and V_H domains of the single-chain antigen-binding protein onto the structure of the variable region of MCPC603 (*9*). The sequence of the protein depicted is MENV LTQSPAIMSASPGEKVTMTCRASSSVS SSYLHWFQQKSGASPKLWIYSTSNLA SGVPARFSGSGSGTSYSLTISSVEAEDA ATYYCQQYSGYPLTFGAGTKLKESGS VSSEQLAQFRSLDVQLVESGGDLVKP GGSLKLSCAASGFTFISYGMSWVRQT PDKRLEWVATISSGSTYTYYPDSVKG RFTISRDNAKNTLYLQMSGLKSEDTA MYYCARRITTVVLTDYYAMDYWGQ GTSVTVS; with the linker region underlined. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. The linker, which in this case is derived from a segment of human carbonic anhydrase, spans from residue 105 of the V_L to residue 2 of the V_H with the use of the Kabat numbering system (*7*). The carboxyl terminus corresponds to residue 116 of the V_H, after which a stop codon was introduced. A methionine was inserted in front of the V_L for expression in *E. coli*. The single-chain antigen-binding proteins have been designated by the monoclonal name (for example, 3C2) with the number of the designed linker sequence after the diagonal mark (for example, 3C2/59).