excellent agreement with experiment and rival the costly first-principles calculations for accuracy. In addition, the conditions for which large values of B are obtained are apparent from Eq. 1; that is, minimized d and I. Because small atomic radii imply small d, the first-row atoms of the periodic table are good candidates for building blocks of large B solids. For example, the radii of B, C, and N are 0.88 Å, 0.77 Å, and 0.70 Å respectively. These yield bulk moduli for diamond and BN in agreement with experiment. Because these radii yield d = 1.47 Å for C-N bonds and $I < 1$, Eq. 1 suggests (*2*) that carbon nitride compounds have high bulk moduli.

A prototype crystal structure for C_3N_4 can be chosen with knowledge of the known structure of β-Si_3N_4. The average coordination number for this structure is N_c = 3.43. Using estimates of I and d, we see that Eq. 1 yields B ~ 410 to 440 GPa which brackets the calculated value for diamond. A first-principles calculation (*3*, *10*) predicts a similar result, B = 427±15 GPa. In contrast, a first-principles calculation (*11*) for β-Si_3N_4 yields B = 265 GPa. This smaller value for B results from the larger bond length and larger charge transfer from Si to N.

Niu *et al.* (*1*) examined the question of charge transfer in β-C_3N_4 by measuring the 1*s* binding energy for C and N. They conclude that the C-N bond in β-C_3N_4 is covalent with relatively little charge transfer, which is in agreement with the theoretical predictions. As stated before, according to pseudopotential theory, there is a repulsive Pauli potential keeping the valence electrons away from the core region. For β-Si_3N_4, the p electrons in the Si cores repel the valence p electrons, whereas the N cores, which are only *s*-like, do not contribute a strong p repulsion. The result is considerable charge transfer from Si to N and a much more ionic bond in β-Si_3N_4 than in β-C_3N_4, where both C and N have the same core structure. The resulting C-N covalent bond is strong and somewhat similar to the C-C bond in diamond.

The general agreement between values of B calculated with Eq. 1, first-principles calculations, and experiments lends support to the usefulness of Eq. 1 for the calculation of trends and specific values for covalent systems. In addition, Eq. 1 suggests that the bulk modulus of diamond, unlike the speed of light, can be exceeded. Specifically, C-N compounds may break the diamond barrier. At present, the bulk modulus and hardness of β-C_3N_4 are yet to be measured. However, Niu *et al.* (*1*) state, on the basis of qualitative observations, that their C-N films are "thermally robust and hard." Because of the high Debye temperature of carbon nitride compounds, these materials should also be excellent heat conductors.

More generally, the study of hardness or bulk modulus is a good touchstone for theory. When the general prediction (*2*) of how short, nonionic covalent bonds increase bulk moduli and specific predictions for prototype materials are tested, there will be feedback to the theory. Subsequent modifications of the theory or suggestions for new classes of materials to be tested can broaden the domain of the models. In any case, at this point, the confirmation of theory implied by the measurements of Niu *et al.* (*1*) indicate that we have entered an era in which it is possible to use theory to design materials with predictable properties.

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Vancomycin Resistance: Decoding the Molecular Logic

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The 1990s may come to be remembered as a decade in which infectious diseases made a dramatic worldwide resurgence, largely because of the appearance of antibiotic-resistant microbes (*1*). In the United States alone, the unrecognized yearly cost of antibiotic resistance is estimated to be $100 million (*2*). The deciphering of drug resistance mechanisms will not only allow a better understanding of incipient clinical crises but may also suggest strategies for reversing resistance and preventing the appearance of new resistant microbes. Recent work has shed light on the development of resistance to vancomycin, a glycopeptide antibiotic that for 25 years has been an effective treatment for Gram-positive bacterial infections.

Vancomycin is the drug of choice for infections caused by streptococcal or staphylococcal strains that are resistant to β-lactam antibiotics (such as penicillin) and for patients who are allergic to the latter drugs. β-Lactam antibiotics and vancomycin interfere with separate but contiguous steps in the biosynthesis of bacterial cell walls—β-lactams by blocking crosslinking steps that strengthen the wall and vancomycin by forming a complex with the substrate for the penicillin-sensitive reaction. Because about 95% of *Staphylococcus aureus* isolates are resistant to the β-lactam methicillin (*3*), vancomycin has found expanded usage in situations where *S. aureus* is particularly problematic, such as in long-term care facilities, burn centers, among narcotics abusers, in infective endocarditis, and in patients with indwelling intravenous lines (*4*). A major advantage of vancomycin treatment had been the virtual absence of resistant strains; thus, the recent emergence of vancomycin resistance in the clinic, observed first in Europe and now globally (*5*), has been met with great apprehension.

Vancomycin is a modified heptapeptide with a cup-shaped architecture. The "cavity" of the drug, which is created by posttranslational crosslinks of tyrosine residues, binds tightly to peptidoglycan (PG) strands in the bacterial cell wall that terminate in D-Ala-D-Ala (*6*). Complex formation between vancomycin and D-Ala-D-Ala termini at the exterior surface of the cell blocks transglycosylation and transpeptidation of nascent PG strands. As a result, the crosslinking of PG strands and the tensile strength of the PG layer are reduced, rendering the bacteria susceptible to osmotic lysis.

The genes responsible for high-level vancomycin resistance in pathogenic enterococci have recently been cloned and sequenced (*7*). Resistance involves the action of nine genes contained within the transposable element Tn 1546, which is carried on a plasmid (*8*). The gene products include a transposase and a resolvase, which, by promoting mobilization of Tn 1546 from one DNA locus to another, allow the rapid spread of vancomycin resis-

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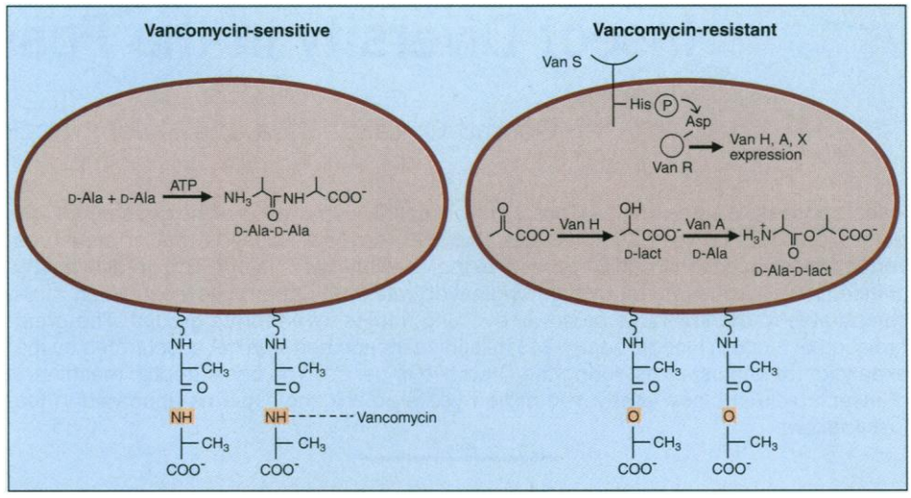
tance through bacterial populations. Of the remaining seven genes in the transposon (termed *Van R, S, H, A, X, Y,* and *Z*), only *Van Y* and *Van Z* are dispensable.

DNA sequence analysis and studies of biochemically purified gene products (*9–12*) have begun to reveal the molecular logic of vancomycin resistance. The *Van S* and *Van R* genes are homologous with several two-component sensor-response regulator genes used by bacteria to monitor external signals and transduce that information for selective gene regulation (*13, 14*).

The seven vancomycin resistance genes are expressed only in the presence of the antibiotic. Exactly how the bacteria sense the presence of vancomycin—whether the drug itself or some autolytic degradation product of the cell wall signals damage—is not yet clear but is likely to involve specific ligand recognition by the "sensor" homolog, Van S. Van S has an extracellular domain and a cytoplasmic domain that shares sequence features with histidine kinases. In the presence of adenosine triphosphate (ATP), a histidine residue within its cytoplasmic domain becomes autophosphorylated. Thus, Van S bears a striking resemblance to eukaryotic transmembrane hormone receptors and growth factor receptors, which undergo tyrosine autophosphorylation in their cytoplasmic domain in response to extracellular ligand binding. The phosphorylated Van S can then transfer the phosphoryl group to Van R, the "response regulator" homolog, on an aspartic acid residue that is highly conserved in this family of proteins (*15*). Van R is itself a DNA binding protein (*16*) that functions as a transcriptional activator controlling expression of the adjacent *Van H, A, X, Y,* and *Z* genes. Although it remains to be proven that the phosphorylated form of Van R activates the structural genes, this is the case for several other phosphorylated response regulators (*17, 18*).

How does expression of *Van H, A,* and *X* alter the peptidoglycan structure of the cell wall to render the bacteria drug-resistant? Characterization of the Van H and A proteins has revealed a simple but clever mechanism in which the resistant cells have replaced the normal D-Ala-D-Ala PG termini with D-Ala-D-lactate termini that are not recognized by vancomycin. The function of *Van X* is still mysterious.

Normally the D-Ala-D-Ala moiety used in PG synthesis is supplied as a dipeptide by the action of the chromosomally encoded enzyme D-Ala-D-Ala ligase. The *Van A* gene encodes a homologous ligase but with an altered specificity in that it recognizes D-α-hydroxy acids (D-lactate, D-hydroxybutyrate) rather than D-Ala as the COOH-terminal partner. Van A catalyzes synthesis of esters or "depsipeptides" such as D-Ala-D-lactate. The resistant cell is supplied with D-lactate or D-hydroxybutyrate by means of the *Van H* gene product, which is an α-keto acid reductase that produces these compounds from the normal metabolites pyruvate and α-ketobutyrate (*10*). Van A and Van H work in concert to provide depsipeptide D-Ala-D-lactate in place of dipeptide D-Ala-D-Ala. The next enzyme in PG assembly can add either the D-Ala-D-Ala unit to produce the normal PG strands with D-Ala-D-Ala termini in vancomycin-sensitive bacteria, or the D-Ala-D-lactate unit to produce altered PG termini in resistant bacteria (*19, 20*) (see figure).



Molecular logic of vancomycin resistance. Vancomycin-sensitive and -resistant bacteria differ in a critical component of their cell wall. Sensitive bacteria (**left**) synthesize PG strands that terminate in D-Ala-D-Ala; vancomycin binds avidly to these termini, thereby disrupting cell wall synthesis and leading to cell lysis. Resistant bacteria (**right**) harbor a transposable element encoding nine genes that contribute to the resistance phenotype. The gene products include a transmembrane protein (Van S) that senses the presence of the drug and transmits a signal—by transfer of a phosphoryl group—to a response regulator protein (Van R) that activates transcription of the other resistance genes. The combined activities of Van H and Van A lead to synthesis of a depsipeptide, D-Ala-D-lactate, which can be incorporated into the PG strands of the cell wall. The altered PG termini do not affect the structural integrity of the cell wall, but substantially reduce its affinity for vancomycin, thereby rendering the bacteria resistant to the drug.

In vitro binding studies have shown that the affinity of vancomycin for *N*-acyl-D-Ala-D-lactate is 1000 times less than its affinity for *N*-acyl-D-Ala-D-Ala, paralleling the 1000-fold reduced sensitivity of vancomycin-resistant bacteria to drug (*10*). This observation is consistent with evidence from nuclear magnetic resonance studies, which suggest that the D-Ala-D-Ala amide N-H forms a crucial hydrogen bond with a carbonyl group in the vancomycin backbone. The presence or absence of this N-H determines whether vancomycin will bind to the cell wall. The structural integrity of the PG meshwork is not compromised by the depsipeptide building-block substitution. Indeed, the ester linkage is more reactive than the D-Ala-D-Ala linkage in the subsequent transpeptidation and strand crosslinking steps.

Thus, clinical resistance to vancomycin has emerged as a result of a simple switch of an ester bond for an amide bond. An understanding of this molecular logic has revealed specific target proteins (for example, Van A) for the design of drugs to reverse the vancomycin resistance phenotype.

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