Computer Calculation of Molecular Crystal Structures

Abstract. *A new method of calculating molecular crystal structures is proposed and tested. The repulsive portion of the nonbonded potential energy is fitted by a quadratic equation to yield a sum-of-squares description of the repulsive lattice energy. Minimization of this sum by full-matrix least squares yields good fits to the observed crystal structures of several hydrocarbons. The convergence properties of the method are markedly superior to those of the steepest-descent method, and calculation times are greatly decreased.*

If the molecular shape is known, or can be predicted from expected interatomic distances and angles, the packing of the molecules in the crystal can be calculated by minimization of the lattice energy by use of known nonbonded interatomic potential energies (*1*). As soon as the packing positions of the molecules in the crystal are known, the diffraction-phase problem is solved. Diffraction data can then be used to verify the molecular packing position and to refine the molecular shape.

Barriers to utilization of packing analysis have been the lack of quantitative nonbonded potential parameters, the large number of terms to be considered in the lattice sums, and the relatively slow convergence of the steepest-descent method of minimizing the lattice energy (*2*). Simple grid-point calculations searching for the minimum lattice energy have been successful in solving the diffraction-phase problem in some instances (*3*). Recently, better nonbonded (exp-6) potential parameters for hydrocarbons have become available (*4*). These potentials can be used for calculation of the lattice constants and other quantities depending on intermolecular interactions, as well as the molecular packing position.

Since in practice the lattice constants can be quickly and easily determined, we can simplify the packing-analysis problem by using the observed lattice constants. When this is done, the attractive nonbonded potentials can be neglected, since their effect is indirectly included by effective restriction of the molecular volumes in the crystal.

The repulsive potential E_r between atoms in different molecules can be approximated by the equation

$$E_r = w(d_0^n - d_c^n)^2 \quad d_c < d_0$$

where w is a scaling or weighting factor for the atom-pair type, d_0 is a constant much like a van der Waals diameter, and d_c is a calculated interatomic distance for a trial model. The crystal-packing problem for dibenzolmethane (*5*) has already been solved by use of $w = 1$, $n = 2$, and very approximate d_0 values.

We now find that a good approximation to the observed nonbonded repulsion curves (*4*) can be obtained by taking $n = 1$ and finding the best least-squares values for w and d_0. A least-squares fit was made on the basis of the first derivatives (force) to refer the bottom of the potential well to zero energy. For H...H, C...H, and C...C interactions I obtained the following repulsive potentials (kilocalories per mole):

$$E_{HH} = 1.00(2.88\text{-}d_{HH})^2 \quad 2.2 \leq d_{HH} \leq 2.9$$

$$E_{CH} = 1.56(3.03\text{-}d_{CH})^2 \quad 2.5 \leq d_{CH} \leq 3.0$$

$$E_{CC} = 1.87(3.65\text{-}d_{CC})^2 \quad 3.2 \leq d_{CC} \leq 3.6\text{Å}$$

These functions approximate the (exp-6) repulsive potentials within about 0.03 kcal/mole if they are normalized to the (exp-6) well depths at $d = d_0$.

The lattice repulsive energy, obtained by pair-wise summation, is a sum of squares when these potentials are used. The derivatives of the lattice repulsive energy, with respect to molecular translations and rotations, are easily obtained. In the general case, we obtain $6n$ linear equations in $6n$ unknowns, where n is the number of crystallographically independent molecules each having three translational and three rotational parameters. The solution of these equations yields parameter shifts leading to minimization of the lattice repulsive energy.

Thus we have approximated the lattice repulsive energy by a sum of squares, making possible a least-squares minimization. In addition, the full-matrix least-squares method includes cross terms between parameters, such as between molecular translation and rotation. The inclusion of such cross terms greatly speeds the rate of convergence of the calculation relative to the steepest-descent method, which may be likened to a diagonal-matrix approximation. The new method has the additional advantage that only a relatively small number of atom pairs need be considered—only those for which $d < d_0$; the number of interactions to be considered is thus reduced by about an order of magnitude.

We have tested the new packing method with several hydrocarbon crystal structures. For objective evaluation of the convergence properties, we used trial models in which the molecule was initially oriented in 12 ways. With this coarse angular grid the initial angles were approximately ± 20 and ± 60 deg with respect to the *X*, *Y*, and *Z* cartesian reference axes.

In the case of benzene (*6*), where no translations need be found, rapid convergence was obtained to the correct packing structure in every instance. The calculated packing position found by this method had a maximum carbon-atom deviation of 0.11 Å from the position in the observed structure. For comparison I made a similar calculation by the steepest-descent method (*7*); the calculation was slower by a factor of 12 and failed to converge in four of the 12 starting orientations.

For napthalene (*8*), convergence to the observed packing structure was obtained for two of the 12 starting models; the maximum carbon-atom deviation for the calculated packing structure was 0.07 Å. The other ten starting models led to one or another of five false minima. The correct packing structure was easily identifiable among the six minima found, since it had the lowest calculated repulsive energy. Examination of the false minima showed that in each instance an incorrect molecular interlocking scheme was obtained. The models would not refine from these false minima because refinement would require very close interatomic approaches in passage from one mode of interlocking to another. Thus, for convergence, the correct molecular interlocking scheme must be accessible from at least one of the initial molecular orientations.

To test the ability of the new method in finding molecular translations, we considered the crystal structure of phenanthrene (*9*), in which two translations are required as well as three angular coordinates. We found that a coarse grid of 1 Å in each translational direction was sufficient for obtaining the correct packing structure from the 12 trial angular orientations. Convergence was obtained from as many as four of the starting angular orientations, depending on the translational grid starting point. The correct packing model again had the lowest calculated packing energy and a maximum

carbon-atom deviation of 0.11 Å from the observed structure.

In addition to solving crystal-packing structures, this technique can be adapted to nonbonded interactions between different parts of the same molecule. Such calculations are of considerable interest, for example, in establishing the molecular conformation of proteins (*10*).

DONALD E. WILLIAMS
Department of Chemistry,
University of Louisville,
Louisville, Kentucky 40208

References

1. D. E. Williams, *Science* **147**, 605 (1965).
2. ———, *J. Chem. Phys.* **45**, 3770 (1966).
3. A. Daminani, E. Giglio, A. M. Liquori, L. Mazzarell, *Nature* **215**, 1161 (1967); E. Giglio and A. M. Liquori, *Acta Cryst.* **22**, 437 (1967); D. Rabinovich and G. M. J. Schmidt, *Nature* **211**, 1391 (1966).
4. D. E. Williams, *J. Chem. Phys.*, in press.
5. ———, *Acta Cryst.* **21**, 340 (1966).
6. G. E. Bacon, N. A. Curry, S. A. Wilson, *Proc. Roy. Soc. London Ser. A* **279**, 98 (1964).
7. D. E. Williams, *AEC Rept. IS-1042* (1964).
8. D. W. J. Cruickshank, *Acta Cryst.* **10**, 504 (1957).
9. J. Trotter, *ibid.* **16**, 605 (1963).
10. R. A. Scott and H. A. Scheraga, *J. Chem. Phys.* **45**, 2091 (1966); P. DeSantis, E. Giglio, A. M. Liquori, A. Ripamonti, *Nature* **206**, 456 (1965).

20 November 1967

Immunologic Defenses against Metastases: Impairment by Excision of an Allotransplanted Lymphoma

Abstract. *Hamsters grafted with an allotransplantable lymphoma that does not metastasize develop a state of concomitant immunity which renders them refractory to reinoculation with cells of the same tumor. Removal of the tumor 7 days after transplantation rapidly leads to a decrease in immunity, the production of enhancing antibodies, and the appearance of metastatic deposits which are probably derived from preexisting tumor cells in the blood and lymphoid tissues.*

Since the existence of tumor-specific antigens was first demonstrated, much evidence has accumulated to show that cells from a number of different types of cancer are antigenic in genetically similar animals (*1*). The immunity induced by these new antigens has been demonstrated by several methods which share one common feature: in all experiments of this kind, measures have been taken to prevent progressive growth of the immunizing tumor (*1*). The possible immunosuppressive effect which the presence of a primary tumor may exert is emphasized by studies in which no immune response could be detected unless the immunizing tumor was excised (*2*). There are, however, certain genetically incompatible (nonspecific) tumors where antigens other than those that are tumor-specific interact with the host and induce high degrees of immunity even though the primary tumor remains in place and continues to grow—a phenomenon known as concomitant immunity (*3, 4*). This difference in behavior between specific and nonspecific tumors may be explained on a quantitative basis in that the primary tumor may always suppress the immune response to some degree, and only when strong antigenic differences exist, such as those present on a nonspecific tumor, can such immunity be detected. Alternatively, different tumors may evoke qualitatively different responses. One way to distinguish between these interpretations is to study the effect of removal of the tumor in a system where strong immunity is expressed while the primary tumor is present. If the first alternative is correct, excision of the tumor should lead to heightened immunity; evidence suggestive of such a process with nonspecific tumors was produced many years ago by Andervont (*5*). We now describe the opposite situation in which resection of a primary allotransplanted tumor causes a depression of immunity. This change is of particular interest since the decline in immunity coincides with the appearance of metastases from a tumor which does not normally form secondary deposits.

The tumor which constitutes our experimental model has been described elsewhere (*4, 6, 7*). It is a lymphoblastic lymphosarcoma that occurred spontaneously, and it has been maintained by serial transplantation in randomly bred hamsters for several years. It induces a state of concomitant immunity whereby, at its height, a tumor-bearing animal may reject more than 100,000 times the number of tumor cells which is required to produce tumors in normal hamsters. Although viable tumor cells soon appear in the blood and lymph nodes of animals with actively growing primary tumors, metastases do not normally develop (*4, 6, 7*).

To perform cell transfer studies, we used young Syrian golden hamsters (75 to 90 g) from an inbred line (MHA) (*8*). Cell suspensions of the lymphoma were prepared according to a method described previously (*7*), and 1×10^7 cells were injected subcutaneously into the right flank of 16 donor animals. Seven days later, when the tumors weighed 1 to 2 g, the animals were divided into two equal groups. In the first group, the tumor grafts were excised; in the second group, the tumors were left in place, and a segment of skin (3 by 1 cm) was removed from the flank close to the tumor graft. Two days after the operation, peritoneal exudates were raised in all 16 hamsters with 6 ml of 3 percent starch solution by the method of Bennett (*9*). Forty-eight hours later, the animals were exsanguinated, and cell suspensions of the spleens and peritoneal exudates were prepared in a manner previously described (*4*). The cells as well as the serum from these animals were mixed with a suspension of the lymphoma containing 2×10^6 cells per milliliter, and 1 ml of the mixture was inoculated subcutaneously into the flanks of 48 isogenic recipients; this procedure is the Winn cell-transfer test (*10*). Cells and serums from untreated MHA hamsters were used as controls.

Growth of lymphoma cells in isogenic recipients was suppressed when the tumor cells were inoculated with splenic or peritoneal cells (but not serum) from sham-operated animals in which the immunizing tumor was preserved (Fig. 1 and Table 1). Similar cells from hamsters in which the immunizing tumor was previously resected were considerably less effective; serum from hamsters in which the original tumor had been resected potentiated tumor growth (Fig. 1).

Further investigations were carried out to answer the following questions: (i) Is the decrease in immunity produced by resection of the primary tumor (and demonstrated by the Winn test) accompanied by increased susceptibility to regrafting with the same tumor? (ii) If such susceptibility is demonstrable, can it be correlated with the appearance of metastases? (iii) Is the potentiating effect of serum from animals whose tumors have been excised due to enhancing antibody?

Thirty-two young, randomly bred