path of measurement. Most line shape measurements would not require such a detailed study and expenditure in time, but the three-dimensional aspect of the measurement should be acknowledged in the interpretation of the results. A second path of measurement through the diffraction maxima may indicate if there are any problems encountered from broadening in the other directions.

The authors would like to acknowledge the support of this study by the National Science Foundation.

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Acta Cryst. (1965). **18**, 686

Macroscopic Characteristics of Ribonuclease Crystals of Modifications I and II*

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(*Received* 7 *March* 1963 *and in revised form* 20 *July* 1964)

The morphology and mechanical properties of crystals of modifications I and II of bovine pancreatic ribonuclease are described and discussed in the light of proposed molecular packings.

It is of interest to correlate such macroscopic characteristics of protein crystals as their morphology, etch symmetry, and cleavage or fracture with postulated models of the molecular packing within the crystals. Some electron-microscope studies (*e.g.* Labaw & Wyckoff, 1957; Labaw, 1959) of large molecules such as the viruses have directly revealed the packing of the molecules on various crystal faces. However, an electron-microscope study of a smaller protein, bovine pancreatic ribonuclease (Dawson & Watson, 1959) showed some detail in the form of striation of the faces, but did not give complete resolution of neighboring molecules. In such a case, we may find it especially useful to infer the molecular packing from X-ray diffraction data, and correlate this packing model with the observed morphology and other macroscopic characteristics.

The present study is concerned with the morphology, cleavage, and fracture of bovine pancreatic ribonuclease crystals of modifications I and II, which have been previously reported (Fankuchen, 1941; Fankuchen, 1945; Carlisle & Scouloudi, 1951; King, Magdoff, Adelman & Harker, 1956; the latter reference is hereinafter denoted as KMAH).

Harker (1957) has proposed molecular packing models for these modifications on the basis of symmetry and steric packing considerations, and has kindly supplied by private communication the values of the molecular parameters on which his packing diagrams are based. These values are corroborated by the arrangement of peaks in the low-resolution (10 Å) three-dimensional Patterson functions of these modifications. For form I, the molecular centroids are in positions 4(*a*) of space group $P2_12_12_1$ with $x=0{\cdot}00$, $y=0{\cdot}00$, $z=0{\cdot}25$, as corroborated by Patterson peaks at $0\frac{1}{2}0$, $\frac{1}{2}0\frac{1}{2}$, and $\frac{1}{2}\frac{1}{2}\frac{1}{2}$. For form II, the molecular centroids predicted by packing considerations are in positions 2(*a*) of space group $P2_1$, with $x=0{\cdot}17$, $y=0{\cdot}50$, $z=0{\cdot}74$.

For convenience, the following abbreviations will be used: RNase = bovine pancreatic ribonuclease; MPD = 2-methyl-2,4-pentanediol.

Experimental

The bulk of the crystals studied here were prepared and preserved by standardized methods described elsewhere (King, 1964*a*).

Observations of morphology and cleavage or fracture were made upon crystals immersed in a portion of their preserving solution held in a microscope slide having a flat-bottomed well; the specimens were kept covered with cover glasses at all times to prevent evaporation, except when the crystals were being manipulated mechanically. Interfacial angles were measured under a polarizing microscope having a rotating stage.

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For measurements of interfacial angles in the zone of the axis of elongation of form I (the *a* axis), a crystal was cleaved normal to this axis and the fragments were stood up on their cleavage faces (while immersed in the preserving solution).

Characteristics of modification I

In contrast to the crystals studied earlier (KMAH), samples prepared according to the standardized method generally produced crystals of I bounded by the forms {101}, {010}, and {011}, normally platy on (010).

While the {*h*0*l*} form observed on standard crystals was generally {101}, with an observed angle $(101)\wedge(10\bar{1})=99°\pm2°$ (calculated: 99·05°), other {*h*0*l*} forms occasionally replace it, as evidenced by discrepant values of $(h0l)\wedge(h0\bar{l})$. For the most part, these high-index forms have appeared in samples prepared under non-standard conditions, but when manifested, they occur at both ends of the given crystal. Also, in so far as examination has shown, the high-index forms are shown by different crystals from the same sample tube. Various preparations have shown angles of $115°\pm2°$ corresponding to {304} as reported in KMAH (calculated: 114.76°), and $107°\pm1°$ corresponding to {708} (calculated: 106·51°), or even curved faces replacing {101}. These high-index faces cannot be ascribed to rapid growth, but conversely, are sometimes found in samples showing especially long growth times of many months.

The form {100} reported in KMAH appears only on crystals grown near *p*H 7, *i.e.* near the alkaline end of the stability range of I.

On the other hand, the form {001} reported in KMAH is probably inexistent, the faulty identification resulting from observation of very thin crystals. All observations of sufficiently thick crystals of I have shown an {0*kl*} form beveling the plates, {001} being absent. This {0*kl*} form proved to be {011} in the cleavage fragment studied, with $(011)\wedge(0\bar{1}1)=51°\pm2°$ (calculated: 52·79°).

Crystals of modification I show the expected parallel extinction between crossed nicols.

The porous character of protein crystals gives rise to cleavage phenomena of differing types. On the one hand, certain of these crystals can be cleaved by application of external stress, just as simpler crystals can. However, an analogous phenomenon can also arise from internally developed strains in the form of parallel cracks along crystallographic planes. Such internal stresses are due to non-uniform expansion or contraction of the crystal, as occasioned by drying or by exposure of the crystals to a new medium. In such cases, the crystals may crack along planes not exhibiting cleavage under external stress.

Under the pressure of a glass point applied to the broad (010) face, crystals of I show perfect cleavage on (100). The cleavage fragments generally show unimpaired X-ray diffraction patterns. Thus cleavage provides a useful method for obtaining specimens of a desired size for diffraction studies.

Under internal strain, crystals of modification I crack along {101} or {001}. Cracking along {101} has been observed during drying, even in crystals bounded by the form {304}.

The dominant morphology of modification I can be interpreted in terms of the molecular packing proposed by Harker (1957). Fig. 1 compares the observed habit of the crystals with a packing model. The circles indicate the distribution of centroids of the molecules, without implication as to their shapes (Harker suggests that they are better represented as prolate spheroids of low eccentricity). The differing orientations of the four molecules in the unit cell are also neglected here. In this packing, each molecule has four nearest neighbors about 29 Å away, related by translations $\pm\frac{1}{2}a$ $\pm\frac{1}{2}c$, two neighbors related by translations $\pm\frac{1}{2}b$ ($37\frac{3}{4}$ Å), and two neighbors related by $\pm c(37\frac{1}{2}$ Å). The chemical and steric nature of the molecular contacts is not revealed at this resolution, but continuity of the structure requires molecular contacts between neighbors in the $\pm\frac{1}{2}a\pm\frac{1}{2}c$ and the $\pm\frac{1}{2}b$ directions. The existence of physical molecular contacts in the $\pm c$ directions is more problematical; the tendency of crystals of form I to shrink in this direction under chemical modification (King, 1964*b*) would cast doubt on the existence of these contacts, although the existence of the perfect {100} cleavage would favor their existence.

In contrast, the high-index forms that have appeared sporadically must involve stepped faces, either with regularly occurring setbacks, as in the {304} form, or with varying intervals, as in the curved faces. The occurrence of stepped faces here recalls those reported by Labaw & Wyckoff (1957) in electron micrographs

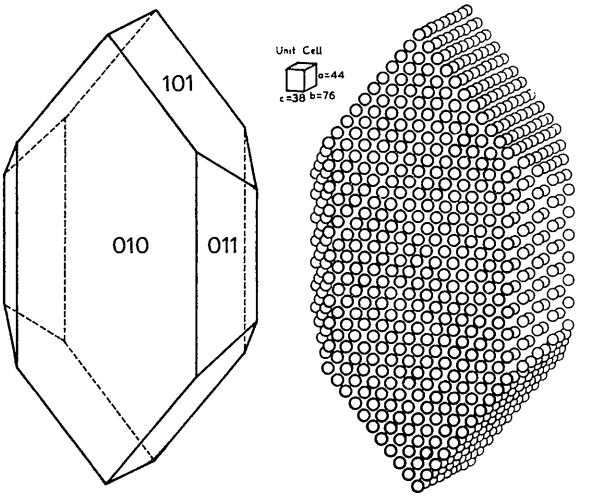

Fig. 1. The morphology and molecular packing of crystals of ribonuclease I. Left: Typical face development of ribonuclease I. Right: Interpretation of the morphology in a packing diagram.

of southern bean mosaic virus crystals, or by Labaw (1959) in tobacco necrosis virus.

Most of the crystals of I in any sample tube are usually intergrown, only a minority being single and thus usable in X-ray diffraction studies. The intergrowths are usually composed of a series of individual crystals of I related by rotation about the *c* axis by about 6° 20′, as shown in Fig. 2. While no simple twinning law was found to account for these intergrowths, their occurrence and that of the high-index faces may be related phenomena.

Characteristics of modification II

The crystals of modification II have conformed to previous descriptions in being bounded by the forms {100}, {011}, {0$\bar{1}$1}, and {001}. Crystals equilibrated with 70 vol. % MPD show an interedge angle [011] ∧ [01$\bar{1}$] = 109·5° ± 1·0° (calculated: 108·6°). The {001} form has been very rarely absent in crystals grown from aqueous alcohols, but is always present in standard preparations (King, 1964*a*). Crystals grown from aqueous methyl or ethyl alcohol are elongated along *a*, as described by Carlisle & Scouloudi (1951), or more often equidimensional. In contrast, the crystals of II grown under the standard conditions (from a solution 50 vol.% in MPD, 5 vol.% in methyl alcohol) are usually somewhat tabular on {100}, while crystals grown from various other alcohols and glycols (as in KMAH) are even more flattened, those from the higher alcohols often being quite thin plates.

Crystals of II show extinction parallel to *b*, as expected.

Fig. 3 compares the observed morphology of modification II with a model drawn on the basis of the packing proposed by Harker (1957). The packing on (011) and (0$\bar{1}$1) is not distinguished in the drawing, since the distinction depends on the differing profiles of the two ends of the RNase molecule. The {100} form contains sets of exposed molecules of opposite orientations occurring at two different heights. This feature recalls the striations in the electron micrographs of Dawson & Watson (1959), although the lack of resolution of individual molecules in these micrographs prevents the drawing of conclusions on the packing.

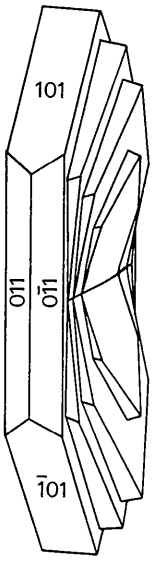

Fig. 2. An intergrowth of ribonuclease I crystals.

Crystals of II do not cleave under external stress, but fracture irregularly. The fragments give diffraction patterns with broadened or split maxima indicating distortion. However, cracking along {001} planes has been observed under internal stresses, as in slow drying.

The proposed packing for modification II is more complex and also denser than for I. Here each molecule is surrounded by ten neighbors at distances ranging from 30 to 38 Å. Thus, regardless of the structural details of the RNase molecules, their pattern of contacts must be more close-knit and intricate in modification II than in I. This is in line with the absence of cleavage in form II.

However, while the habits of both modifications and the cleavage of modification I can be readily pictured on the basis of the packing models, they could not be predicted on this basis. In particular, there seems to be little correlation between the closeness of packing of the nets of molecules exposed on the various faces and the relative development of the different forms. However, we should expect the growth kinetics of the faces to be governed more by the fine details of the molecular contacts than the gross packing pattern. Thus, the study of low-resolution

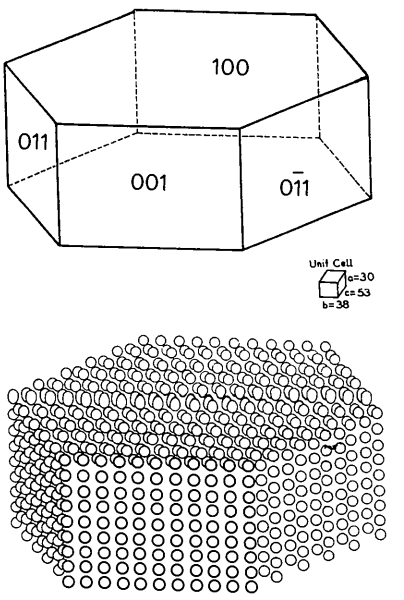

Fig. 3. The morphology and molecular packing of crystals of ribonuclease II. Top: Typical face development of ribonuclease II. Bottom: Interpretation of the morphology in a packing diagram.

packing models provides us with a crude picture, but not a dynamic model, for the morphological and mechanical properties of these protein crystals.

The author wishes to thank Mrs Edith H. Pignataro for the X-ray diffraction measurements, Miss Elaine DeJarnette for some of the microscopic measurements, and Mrs Virginia Hong Wei, Miss Menilyn Merrick, Mr Edward Thomas Bisiakowski, Mr Herbert Kwasnik, and Mr Edward Mordia for valuable assistance in the crystal preparations. He further wishes to express indebtedness to Dr David Harker for numerous discussions on this problem.

It is also a pleasure to express appreciation to the several institutions whose continued support has made possible the work of the Protein Structure Project. They are: The Dean Langmuir Foundation, The Rockefeller Foundation, The Damon Runyon Memorial Foundation, The New York Foundation, The Polytechnic Institute of Brooklyn, and the International Business Machines Corporation.

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Acta Cryst. (1965). 18, 689

Position and Thermal Parameters of Oxygen Atoms in Calcite*

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(*Received* 25 *June* 1964)

The position and thermal parameters of the oxygen atoms in calcium carbonate (calcite) at room temperature, 215 °K and 130 °K, have been determined. Reflections to which only oxygen atoms contribute were measured by counter methods. Parameters were refined by least-squares methods. The C–O bond length is 1·283 ± 0·002 at room temperature and does not change significantly between room temperature and 130 °K.

Calcite, one of the polymorphic modifications of calcium carbonate, crystallizes in the rhombohedral system, in space group $R\bar{3}c$, with two molecules of $CaCO_3$ per unit cell. The carbon atoms and the calcium ions lie along the threefold axis, the former at 0, 0, 0, and $\frac{1}{2}$ $\frac{1}{2}$, $\frac{1}{2}$, and the latter at $\frac{1}{4}$, $\frac{1}{4}$, $\frac{1}{4}$ and $\frac{3}{4}$, $\frac{3}{4}$, $\frac{3}{4}$. The three oxygen atoms of each carbonate group are arranged symmetrically about the carbon atoms in planes normal to the threefold axis; each oxygen atom lies on a twofold axis. Successive carbonate groups are rotated 180° relative to one another as shown in Fig. 1.

The carbon atoms and the calcium ions are in body centered positions. The atomic arrangement deviates from a completely body centered one only by virtue of 60° rotations of successive carbonate groups about the body diagonal; *i.e.* oxygen atoms are not in body centered positions. As a result, only the oxygen atoms contribute to reflections with indices of the type $h+k+l=2n+1$.

The calcite structure may be conveniently described in terms of a triply primitive hexagonal unit cell with $a=4{\cdot}990$ and $c=17{\cdot}00$ Å (at room temperature). There is only one variable position parameter: that of an oxygen atom in one of the (*e*) positions of the hexagonal cell:

$x, 0, \frac{1}{4}$; $0, x, \frac{1}{4}$; $\bar{x}, \bar{x}, \frac{1}{4}$; $\bar{x}, 0, \frac{3}{4}$; $0, \bar{x}, \frac{3}{4}$; $x, x, \frac{3}{4}$.

The length of the C–O bond, which has been the subject of many experimental investigations, is deter-

* This work was supported by the Office of Scientific Research, U.S. Air Force, Contract AF-49-(638)-827 and in part by the U.S. Atomic Energy Commission.

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