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Orientation of Non-Crystallographic Symmetry Axes in Protein Crystals

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Abstract

A survey of 129 protein crystal structures with more than one molecule per asymmetric unit shows that local (non-crystallographic) symmetry axes are not randomly oriented. When compared to the crystal cell edges, face diagonals, body diagonal and reciprocal cell edges, 65% of the local symmetry axes are found to be parallel to one of the reference directions to within 15°; another 18% are orthogonal to within 3°; only 17% are in general orientations. In monoclinic, trigonal and hexagonal crystals, a majority of the local symmetry axes are orthogonal to the unique axis, while preferred orientations are parallel to the cell edges in orthorhombic crystals.

Introduction

Proteins often crystallize with multiple copies of the polypeptide chains in the asymmetric unit of the crystal. These copies are related by local (non-crystallographic) symmetry. Phase determination using non-crystallographic symmetry is a major tool in protein structure determination (Rossmann & Blow, 1962; Bricogne 1976). Yet, the type of symmetry encountered and the possible relationship between crystal and local symmetries have not been systematically analyzed. Here, we examine 183 elements of local symmetries in 129 protein crystals and show that, though there is no *a priori* requirement on the nature and orientation of local axes except in special cases, they are not randomly oriented with respect to the crystal axes. A strong preference is observed in protein crystals for orientations either parallel or orthogonal to the cell edges. This preference may be related to the nature of protein interactions leading to crystallization, especially in the case of oligomeric (multi-subunit) proteins where the molecule itself has symmetry.

Methods

A survey of about 1000 articles reporting protein structures determined by X-ray crystallography showed that about one-third of the crystal forms described had elements of non-crystallographic symmetry. We selected 129 crystal forms for which high-resolution structures have been determined and we tabulated parameters defining the local symmetry. When the transformation matrices relating similar molecules or subunits were reported in articles, they were used as such. Otherwise, they were obtained from authors or extracted from the file headers of the Brookhaven Protein Data Bank (Bernstein *et al.*, 1977). Alternatively, a least-squares superposition procedure (McLachlan, 1979) was applied to C^{α} coordinates. For each element of local symmetry relating a pair of molecules or subunits, we calculated a rotation angle κ, direction cosines for the rotation axis and the screw translation t along this axis. When deviations from exact symmetry were observed, they were usually too small to affect the value of these parameters. The Brookhaven Cartesian system (a, $c^* \times a$, c^*) was used unless specified.

Results

Nature of local symmetries

Parameters for 183 local symmetry elements in 129 crystal forms are listed in Table 1, ordered by space group. Four local symmetries are translations; 18 are general rotations where the angle κ is not $360°/n$ or where the screw-translation component is significant. The remaining 161 are point-group rotations.

The distribution of space groups in our sample is similar to that observed for protein crystals in general. All lattice classes are present, but only 25 space groups are represented, nine of them by a single example. The most frequent space groups are orthorhombic $P2_12_12_1$ and $P2_12_12$, monoclinic $P2_1$ and $C2$, and triclinic $P1$. These five space groups of relatively low symmetry account for 66% of the crystal forms listed in Table 1. High-symmetry space groups are less abundant. There are 12 tetragonal, 17 trigonal, seven hexagonal crystal forms and only one cubic crystal, the U1 small ribonucleoprotein A (Nagai, Oubridge, Jessen, Li & Evans, 1990). It has two molecules per asymmetric unit with a local twofold axis approximately parallel to the face diagonals.

All local symmetry axes included in Table 1 have at least one degree of freedom. Thus, we omitted cubic crystal forms of icosahedral viruses in spite of their local fivefold symmetry, the orientation of the fivefold

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Table 1. *Local symmetry in protein crystals*

(*a*) In crystals of oligomeric proteins, the asymmetric unit may have fewer subunits than the molecule itself. (*b*) A local symmetry is a translation if $\kappa < 15°$, a point-group rotation if κ is within 20° of $360°/n$ and $t < 2$ Å, a screw rotation otherwise. (*c*) α is the acute angle between the local symmetry axis and the reference axis mentioned; axes are quoted as parallel when $\alpha < 15°$, orthogonal when $90° - \alpha < 3°$, or when referred as such in publications, the value of α being unavailable. (*d*) Orthogonality is imposed by crystal symmetry.

Space group / Protein	Asymmetric unit content (*a*)	Local axis (*b*) Type	Orientation	Reference axis	Angle α (*c*)	Reference
Triclinic						
*P*1						
Alcohol dehydrogenase	Dimer	2	Parallel	$a + b$	10	Eklund *et al.* (1981)
Diphtheria toxin	Dimer	2	Parallel	$a + b$		Choe *et al.* (1992)
Glyceraldehyde-3-*P* dehydrogenase	Tetramer	2	*P* parallel	$a + c$	2	Murthy *et al.* (1980)
		2	*Q* parallel	$a - c$	2	
		2	*R* parallel	b^*	2	
Aldose reductase	Tetramer	2	*P* general			Rondeau *et al.* (1992)
		Screw	*Q* general			
		2	*R* parallel	$a - c$	8	
Lactate dehydrogenase	Tetramer	2	*P* parallel	a^*	0	Hogrefe *et al.* (1987)
		2	*Q* parallel	b^*	7	
		2	*R* orthogonal	a	0	
Cytochrome b562	2 molecules	2	Parallel	$a - c$	2	Czerwinski & Mathews (1974)
Dihydrofolate reductase	2 molecules	2	Orthogonal	b	0	Davies *et al.* (1990)
Ovalbumin	4 molecules	2	Parallel	b	4	Stein *et al.* (1991)
		2	Parallel	b	4	
Monoclinic (*b* unique)						
$P2_1$						
Creatinase	Dimer	2	Parallel	c	2	Hoeffken *et al.* (1988)
DNA polymerase III	Dimer	2	Parallel	b	12	Kong *et al.* (1992)
Ribulose bisphosphate carboxylase	Dimer	2	Parallel	$a - c$	1	Schneider *et al.* (1986)
Triosephosphate isomerase	Dimer	2	Parallel	$b - c$	6	Lolis *et al.* (1990)
Trypanothione reductase	Dimer	2	Parallel	a^*	12	Kuriyan *et al.* (1991)
Tyr-tRNA synthetase	Dimer	2	Parallel	$a + b - c$	5	Brick & Blow (1987)
Uteroglobin	Dimer	2	Parallel	c^*	10	Bally & Delettré (1989)
Glyceraldehyde-3-*P* dehydrogenase	Tetramer	2	*P* parallel	$a + c$	3	Skarzynski & Wonacott (1988)
		2	*Q* parallel	b	3	
		2	*R* parallel	$a - c$	3	
Muconolactone isomerase	Decamer	5	Parallel	a	0	Katti *et al.* (1989)
		2	Parallel	b	4	
Phage φ X174	Virion	5	Orthogonal	b	2	McKenna *et al.* (1992)
		3	Orthogonal	c	1	
Canine parvovirus	Virion	5	Parallel	a	3	Tsao *et al.* (1992)
		2	Parallel	b	3	
Actin–DNase I complex	2 complexes	Translation				Kabsch *et al.* (1985)
Cellobiohydrolase II	2 molecules	Translation				Rouvinen *et al.* (1990)
α-Chymotrypsin	2 molecules	2	Parallel	a^*	0	Blow *et al.* (1964)
β-Lactamase	2 molecules	Screw	Parallel	a^*	0	Moews *et al.* (1990)
Pepsin–renin inhibitor complex	2 complexes	2	Orthogonal	a	0	Chen *et al.* (1992)
Ca^{2+} binding protein	2 molecules	2	Orthogonal	$a - b$ c	0	Babu *et al.* (1987)
Taka amylase	2 molecules	2	Parallel	a	0	Brady *et al.* (1991)
Monellin	4 molecules	2	Orthogonal	a	1	Ogata *et al.* (1987)
		2	General			
RAS protein	4 molecules	2	Orthogonal	b	3	Brünger *et al.* (1990)
Deoxyhemoglobin S	2 $(\alpha\beta)2$	2	Parallel	a	10	Wishner *et al.* (1975)
		2	Parallel	a	9	
		Screw	Parallel	a	1	
Lactate dehydrogenase	2 tetramers	2	*P* general			Wigley *et al.* (1992)
		2	*Q* orthogonal	c	2	
		2	*R* general			
		2	*P'* general			
		2	*Q* orthogonal	c	3	
		2	*R'* general			
*C*2						
Cu,Zn superoxide dismutase	Dimer	2	Orthogonal	$a + b + c$	1	Tainer *et al.* (1982)
GCN4 leucine zipper	Dimer	2	Orthogonal	b	0	Rasmussen *et al.* (1991)
Glutathione peroxidase	Dimer	2	Parallel	$a + c$	6	Epp *et al.* (1983)
Glyceraldehyde-3-*P* dehydrogenase	Dimer	2	Parallel	c^*	0	Mercer *et al.* (1976)
Isolectin I	Dimer	Screw	Parallel	a	0	Wright (1989)
Glutamate dehydrogenase	Trimer	3	Parallel	$a + c$	1	Baker *et al.* (1992)
N-Carbamoylsarcosine amidohydrolase	Tetramer	2	*P* parallel	c	6	Romao *et al.* (1992)
		2	*Q* parallel	$a - b$	8	
		2	*R* parallel	$a + b$	7	
Glutamine synthetase	12-mer	6	Parallel	c^*	6	Almassy *et al.* (1986)
		2	Parallel	a	6	
Myoglobin	2 molecules	2	Parallel	a^*	0	Smerdon *et al.* (1990)
Plakalbumin	2 molecules	2	Parallel	c^*	1	Wright *et al.* (1990)
Thioredoxin	2 molecules	Translation				Katti *et al.* (1990)
Orthorhombic						
$P2_12_12$						
Ascorbate oxidase	Dimer	2	Parallel	$a - b$	9	Messerschmidt *et al.* (1989)
Asp–tRNA synthetase tRNA complex	Dimer	2	General			Ruff *et al.* (1991)
Concanavalin A	Dimer	2	Parallel	a	7	Shoham *et al.* (1979)
Isolectin I	Dimer	2	Parallel	$a - b$	6	Bourne *et al.* (1990)
Lactate dehydrogenase	Dimer	2	Parallel	a	13	Hackert *et al.* (1973)

Table 1 (*cont.*)

Space group / Protein	Asymmetric unit content (*a*)	Local axis (*b*) Type	Orientation	Reference axis	Angle α (*c*)	Reference
Malate dehydrogenase	Dimer	Screw	General			Birktoft *et al.* (1989)
Prealbumin	Dimer	2	Parallel	*a*	5	Blake *et al.* (1974)
Xylose isomerase	Dimer	2	Parallel	*a*	0	Farber *et al.* (1989)
Bilin binding protein	Tetramer	2	*P* parallel	$a-b$	10	Huber *et al.* (1987)
		2	*Q* parallel	$a-b$	13	
		Screw	*R* parallel	*c*	12	
Hemoglobin	$(\alpha\beta)2$	2	General			Liddington *et al.* (1988)
Glyceraldehyde-3-*P* dehydrogenase	Tetramer	2	*P* parallel	*c*	7	Leslie & Wonacott (1984)
		2	*Q* parallel	*b*	6	
		2	*R* parallel	*a*	3	
Tobacco mosaic virus disk protein	34-mer	17	Parallel	*b*	0	Champness *et al.* (1976)
Adenylate kinase Ap5A complex	2 molecules	2	Parallel	*c*	10	Müller & Schulz (1992)
Cu,Zn superoxide dismutase	2 dimers	2	Parallel	$a-b$	10	Djinovic *et al.* (1992)
		2	Parallel	$b-c$	8	
$P2_12_12_1$						
C-type lectin	Dimer	2	Parallel	$a-c$	11	Weis *et al.* (1991)
Dihydrofolate reductase	Dimer	2	Orthogonal	*b*		Matthews *et al.* (1986)
Factor for inversion stimulation	Dimer	2	Parallel	*a*	10	Kostrewa *et al.* (1992)
Cytochrome c′	Dimer	2	Orthogonal	$a-b+c$	0	Finzel *et al.* (1985)
Formate dehydrogenase	Dimer	2	General			Lamzin *et al.* (1992)
Glutathione *S*-transferase	Dimer	2	Orthogonal	*b*	1	Reinemer *et al.* (1991)
Hexokinase	Dimer	Screw	Parallel	*b*	5	Steitz *et al.* (1976)
HIV-1 protease inhibitor complex	Dimer	2	Parallel	$b-c$	8	Miller *et al.* (1989)
Lipoamide dehydrogenase	Dimer	2	Orthogonal	$a-b$	0	Schierbeek *et al.* (1989)
Pea lectin	Dimer	2	Orthogonal	$a+b-c$	2	Einspahr *et al.* (1986)
Phospholipase A2	Dimer	2	Parallel	$a-c$	8	Brunie *et al.* (1985)
Phospholipase A2 mutant	Dimer	Screw	Parallel	*a*	2	Thunnissen *et al.* (1990)
Transketolase	Dimer	2	Parallel	$a-c$	6	Lindqvist *et al.* (1992)
Triosephosphate isomerase	Dimer	2	Parallel	$a+c$	3	Wierenga *et al.* (1987)
Surface glycoprotein	Dimer	2	Parallel	$a+c$	14	Freymann *et al.* (1990)
Ascorbate oxidase	Tetramer	2	*P* parallel	*c*	7	Messerschmidt *et al.* (1989)
		2	*Q* general			
		2	*R* general			
Deoxyhemoglobin F	$(\alpha\beta)2$	2	General			Frier & Perutz (1977)
Glyceraldehyde-3-*P* dehydrogenase	Tetramer	2	*P* parallel	$a+b$	5	Rossmann *et al.* (1972)
		2	*Q* general			
		2	*R* general			
Phosphofructokinase	Tetramer	2	*P* general			Evans *et al.* (1986)
		2	*Q* parallel	*b*	0	
		2	*R* general			
Verotoxin-1 B subunit	Pentamer	5	Parallel	$a-c$	13	Stein *et al.* (1992)
Mengo virus	Virion	2	Parallel	*b*	1	Luo *et al.* (1987)
		2	Parallel	$a+c$	4	
Adenylate kinase	2 molecules	Screw	Parallel	*a*	5	Diederichs & Schulz (1990)
Amyloid beta-protein precursor	2 molecules	Translation				Hynes *et al.* (1990)
Anti-peptide antibody	2 molecules	2	Parallel	$a-c$	4	Stanfield *et al.* (1990)
GM-colony stimulating factor	2 molecules	2	Orthogonal	*c*	3	Walter *et al.* (1992)
Erabutoxin	2 molecules	2	Parallel	$a+b$	2	Saludjian *et al.* (1992)
Ribonuclease A	2 molecules	2	Parallel	$a-b+c$	11	Nachman *et al.* (1990)
T4 lysozyme M61 mutant	4 molecules	2	Parallel	$a+b$	4	Faber & Matthews (1990)
		Screw	Parallel	$a-b$	5	
Azurin H35Q mutant	4 molecules	2	Parallel	*b*		Nar *et al.* (1991)
		Screw	Parallel	*a*	6	
Neurophysin II	2 dimers	2	General			Chen *et al.* (1991)
		2	Orthogonal	*b*	1	
		2	Orthogonal	$a-c$	2	
$C222_1$						
Clam hemoglobin	Dimer	2	Parallel	$a-b-c$	4	Royer *et al.* (1990)
Relaxin	Dimer	2	Parallel	*a*	15	Eigenbrot *et al.* (1991)
Melittin	Dimer	2	Parallel	*c*	5	Terwilliger & Eisenberg (1982)
Azurin	2 molecules	2	Parallel	$a-b$	3	Baker (1988)
$\gamma\delta$ Resolvase	3 subunits	Screw	Parallel	*b*	4	Sanderson *et al.* (1990)
		2	Parallel	$a+c$	12	
$C222$						
βB2-Crystallin	2 dimers	2	Parallel	*c*	8	Driessen *et al.* (1991)
		2	Parallel	*c*	7	
$I222$						
Alkaline phosphatase	Dimer	2	Orthogonal	$a-b$	1	Sowadski *et al.* (1985)
Tetragonal						
$P4$						
Hemerythrin	2 dimers	2	Parallel	$a+b$	11	Holmes & Stenkamp (1991)
		Screw	Parallel	*c*	0	
$P4_1$						
Influenza hemagglutinin	Trimer	3	Parallel	$a+b$	12	Wilson *et al.* (1981)
[2Fe-2S] Ferredoxin I	4 molecules	2	Parallel	*c*	2	Tsukihara *et al.* (1990)
$I4$						
Muconate lactonizing enzyme	Dimer	2	General			Goldman *et al.* (1987)

Table 1 (*cont.*)

Space group Protein	Asymmetric unit content (*a*)	Local axis (*b*) Type	 Orientation	Reference axis	Angle α (*c*)	Reference
$P4_12_12$						
Mn superoxide dismutase	Dimer	2	General			Stallings *et al.* (1984)
Myeloperoxidase	Dimer	2	General			Zeng & Fenna (1992)
Insulin	Trimer	3	Orthogonal	$a+b$	(*d*)	Balschmidt *et al.* (1991)
Phosphoglucomutase	2 molecules	2	Parallel	$a+b$	8	Lin *et al.* (1986)
$P4_32_12$						
Citrate synthase	Dimer	2	Parallel	a	8	Wiegand *et al.* (1984)
Colicin A	Dimer	2	Orthogonal	b	0	Parker *et al.* (1992)
Hydroxysteroid dehydrogenase	Tetramer	2	*P* general			Ghosh *et al.* (1991)
		2	*Q* parallel	$a-b-c$	10	
		2	*R* general			
$I422$						
434 Repressor–operator complex	3 complexes	Screw	Parallel	$a+b+c$		Anderson *et al.* (1987)
Trigonal						
$P3_1$						
Protease II	2 molecules	2	Parallel	a	3	Remington *et al.* (1988)
$P3_2$						
Carboxypeptidase–inhibitor complex	2 complexes	2	Parallel	a	1	Rees & Lipscomb (1980)
Barnase	3 molecules	Screw	Parallel	a^*	4	Mauguen *et al.* (1982)
		Screw	Parallel	a^*	10	
Cro-operator complex	3 dimers	2	Parallel	c	11	Bernnan *et al.* (1990)
		Screw	Parallel	a	1	
$R3$						
Insulin	Dimer	2	Orthogonal	c	(*d*)	Dodson *et al.* (1966)
C-phycocyanin	$(\alpha\beta)2$	2	Parallel	a^*	5	Duerring *et al.* (1991)
$P321$						
Asparate carbamoyl transferase	Dimer	2	Parallel	a	12	Krause *et al.* (1987)
C-phycocyanin	3 subunits	2	Parallel	a	0	Schirmer *et al.* (1986)
$P3_121$						
Xylose isomerase	Dimer	2	Orthogonal	a	(*d*)	Henrick *et al.* (1989)
Acidic fibroblast growth factors	2 molecules	Screw	Parallel	a	15	Zhu *et al.* (1991)
Tumour necrosis factor	2 trimers	3	Orthogonal	a	1	Jones *et al.* (1991)
		3	Orthogonal	a	3	
		2	General			
$P3_221$						
Fructose-1,6-bisphosphatase	Dimer	2	Orthogonal	a	(*d*)	Ke *et al.* (1989)
Lactate dehydrogenase	Dimer	2	Orthogonal	a	(*d*)	Grau *et al.* (1981)
Lambda repressor	Trimer	3	Parallel	c	10	Pabo & Lewis (1982)
Catalase	Dimer	2	Parallel	c	14	Murthy *et al.* (1981)
Xylose isomerase	Tetramer	2	*P* general			Rey *et al.* (1988)
		2	*Q* general			
		2	*R* orthogonal	a	0	
$R32$						
Cro repressor	Tetramer	2	*P* parallel	a^*	4	Anderson *et al.* (1981)
		2	*Q* general			
		2	*R* general			
Hexagonal						
$P6_1$						
HIV-1 protease	Dimer	2	Parallel	a	0	Erickson *et al.* (1990)
Lactate dehydrogenase	Tetramer	2	*P* general			Piontek *et al.* (1990)
		2	*Q* parallel	c	7	
		2	*R* orthogonal	$a+c$	0	
Cardiotoxin VII4	2 molecules	2	Parallel	a	8	Rees *et al.* (1990)
Dihydrofolate reductase	2 molecules	2	Orthogonal	$a-c$	3	Bolin *et al.* (1982)
$P6_3$						
Thymidylate synthase	Dimer	2	Orthogonal	a	0	Montfort *et al.* (1990)
Annexin V	2 molecules	2	Orthogonal	c	1	Huber *et al.* (1992)
$P6_322$						
Indole-3-glycerol phosphate synthase	3 molecules	3	Parallel	c	1	Wilmanns *et al.* (1990)
Cubic						
$I4_132$						
U1 small nuclear ribonucleoprotein A	2 molecules	2	Parallel	$a-b$	7	Nagai *et al.* (1990)

axes being determined by the crystal symmetry. Another example is influenza virus neuraminidase. Its molecular fourfold axis coincides with a crystallographic twofold axis in several crystal forms (Varghese, Laver & Colman, 1983; Burmeister, Ruigrok & Cusack, 1992). It has no degree of freedom, and, therefore, is not relevant to our study. In contrast, we include the $P4$ crystal form of hemerythrin, an octamer with 422 point symmetry (Holmes & Stenkamp, 1991). Molecular and crystallographic fourfold axes coincide, but the orientation of the local twofold axes in the *ab* plane is free and the finding that they are parallel to the face diagonals is significant.

Oligomeric proteins account for three-quarters of our sample, with dimers and tetramers the most abundant species. Almost all dimers have twofold symmetry, tetramers having dihedral 222 symmetry. Their orthogonal twofold axes are labelled *P*, *Q* and *R* in Table 1, for convenience. Other proteins, which are monomeric in solution or contain non-identical subunits, have no internal symmetry. When these proteins crystallize with several molecules in the asymmetric unit, molecules may be related by translations or by general rotations with a screw component. The local symmetry can be rather complex when there are more than two molecules. In the 47 crystal forms that contain several monomeric molecules (or several oligomers) per asymmetric unit, the local symmetry is a translation in four cases, a general screw rotation in 18, a point-group symmetry in 25.

There are 147 examples of proper local twofold symmetry in our sample. We define twofold symmetry as having $\kappa = 180 \pm 2°$ and a screw component less than 2 Å. This includes 15 cases of tetramers with local 222 symmetry. Other point-group symmetries are much less frequent: there are eight examples of threefold, four of fivefold, one of sixfold and one of 17-fold symmetry.

Direction of local axes

We now compare the direction of local axes to that of the cell edges and of the face and body diagonals. Reciprocal cell edges are also tested in triclinic and monoclinic crystals. Directions are quoted as parallel in Table 1 when they make an angle α less than $\alpha_1 = 15°$, orthogonal when $90 - \alpha$ is larger than $90 - \alpha_2$ with $\alpha_2 = 3°$. The probability p_1 of finding two parallel directions by chance is proportional to the solid angle subtended by a cone of half-opening angle α_1: $p_1 = 1 - \cos\alpha_1$ or 3.4% with $\alpha_1 = 15°$. For orthogonal directions, the probability for α to be larger than $90° - \alpha_2$ is $p_2 = \sin\alpha_2$ or 8.7% with $\alpha_2 = 3°$. We observe that a large majority (65%) of the 179 local-symmetry axes are parallel to one of the reference directions, another 18% are orthogonal, 17% being randomly oriented. With a more stringent criterion of parallelism ($\alpha_1 = 5°$, $p_1 = 0.38\%$), the parallel axes still represent 32% of the total.

Our sample of eight triclinic crystals contains 15 elements of local symmetry. All but one are proper twofold axes and all but two have special orientations. Preferred directions in triclinic crystals are parallel to cell edges and face diagonals, which, in the absence of crystal symmetry, are equivalent to cell edges except that the translational repeats are slightly longer. There are three triclinic tetrameric proteins. Aldose reductase has a twofold axis approximately parallel to a face diagonal and two axes in general orientations. Lactate dehydrogenase has two axes parallel to reciprocal lattice directions. Glyceraldehyde-3-phosphate dehydrogenase has two axes along the $a \pm c$ face diagonals. Because of the local symmetry, the lattice is centered rectangular in the *ac* plane. Several other glyceraldehyde-3-phosphate dehydrogenases crystallize in space groups of higher symmetry. Most have a tetramer in the asymmetric unit and their lattices are related.

The 33 monoclinic crystals in Table 1 have 49 elements of local symmetry. Three are translations, three screw rotations; the remaining 43 are point-group symmetries. All but five rotations have their axis in a special orientation. The majority (58%) are in the *ac* plane orthogonal to the crystallographic twofold axis. They yield points on the periphery of the stereogram in Fig. 1. In addition to being in the *ac* plane, local-symmetry axes are often parallel to *a* or *c*, or to *a** or *c**. Local twofold axes are sometimes parallel to the crystallographic screw twofold axis *b* in space group $P2_1$, but not to the true twofold in *C*2, in our sample at least. Decameric muconate isomerase has its fivefold axis almost exactly parallel to *a* in $P2_1$, and one of its twofold axes is parallel to *b*. Tetrameric *N*-carbamylsarcosine amidohydrolase has molecular twofold axes parallel to the $a + b$ and *c* directions, which are those of the primitive cell edges in space group *C*2.

Fifty-one orthorhombic crystals contain 69 elements of local symmetry. One is a translation, eight are general screw rotations and 60 are point-group symmetries. Of

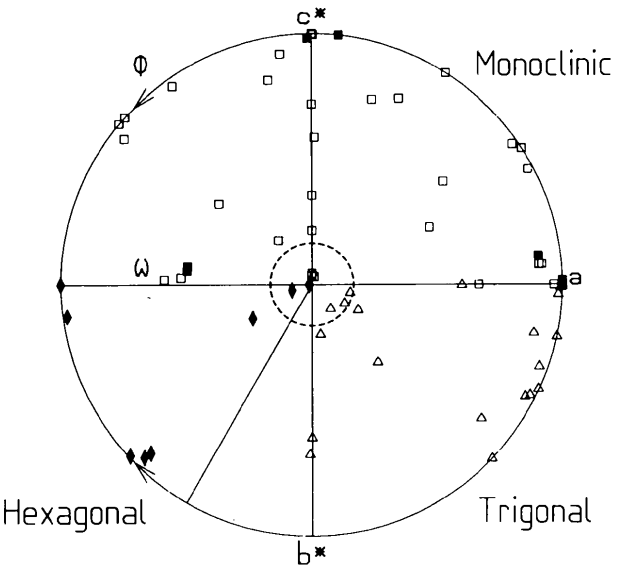

Fig. 1. Local axis orientation in monoclinic, trigonal and hexagonal protein crystals. The local symmetry axis makes an angle $\omega < 90°$ with the unique axis of the crystal, which is *b* in monoclinic and *c* in trigonal and hexagonal space groups; its projection makes an angle φ with *a*. The dashed circle has $\omega = 15°$. Points for monoclinic space groups are above the horizontal axis with φ in the range 0-180°; for convenience the origin of φ has been shifted from *a* to *a** in a few cases (filled symbols). Points for trigonal (△) and hexagonal (♦) space groups are below the horizontal axis. The ranges of φ are 0-120 and 120-180°, respectively.

the 68 rotation axes, 18% have general orientations; 34% are parallel to crystallographic twofold axes along *a*, *b* or *c* and an equivalent number are parallel to face or body diagonals; 13% are orthogonal to one of the reference directions. The 17-fold axis of the tobacco mosaic virus protein disk is parallel to a cell edge, but there it is an obvious consequence of packing large flat disks. In $C222_1$ azurin crystals, a local twofold axis is parallel to the $a + b$ face diagonal, which is an edge of the primitive cell. Five proteins crystallize with a tetramer in an orthorhombic asymmetric unit. Three have twofold axes parallel to a crystal screw axis, two to face diagonals, but six of the 15 twofold axes are general.

Fifteen elements of local symmetry are found in the 12 tetragonal crystals; eight are approximately parallel to reference directions, two are orthogonal, five are general.

Among the 34 elements found in the 24 trigonal and hexagonal crystals, four are screw rotations, four are threefold symmetries and the remainder are twofold symmetries. Six local axes have general orientations. The four screw rotations and ten of the 26 twofold symmetries have their axes orthogonal to *c*. These account for 41% of the rotation axes. Moreover, eight local axes are parallel to *a* (or to the equivalent *b* and $a + b$ directions) and four are parallel to a^*. There are also three cases of a local twofold axis and two of a local threefold axis which is parallel to *c*. These account for 15% of the local axes. In trigonal tumor necrosis factor crystals, the asymmetric unit contains two trimers which are related by a local twofold axis; both have their threefold axis in a plane orthogonal to *a*. In xylose isomerase and in the cro repressor tetramer, one of the twofold axes of the tetramer is orthogonal to *a*; the other two have a general orientation.

Discussion

Our finding that local symmetry axes take non-random orientations in protein crystals is of high statistical significance. The sample is large even though it is not exhaustive. With our convention, the expected frequency of orientations orthogonal to a unique axis is p_2 = 8.7%. The observed frequency is seven times larger in monoclinic crystals and five times larger in trigonal and hexagonal crystals. The expected frequency of orientations parallel to one of three cell edges is $3p_1$ = 10.2% with a 15° cut-off. The observed frequency is 3.5 times larger in orthorhombic, trigonal and hexagonal crystals. The finding of local symmetry axes that are parallel to the face or body diagonals of the crystal cell or to reciprocal cell edges is less significant, as there are 13 such directions, but parallelism is usually much better than 15°: 32% of the local symmetry axes satisfy a 5° cut-off which is unlikely to arise by chance even with 13 reference directions ($13p_1$ = 5%). Moreover, two-thirds of the local symmetry axes that have general orientations occur in tetramers or larger oligomers where another local axis has a special orientation. Thus, the protein molecule itself is also oriented in these cases. No more than 6% of the molecular orientations are truly random (with the 15° cut-off).

When local axes are either parallel or orthogonal to crystal symmetry axes, symmetry elements may combine to approximate those of a more complex space group. Parallel twofold axes can also generate shorter translational repeats. The additional symmetry or the smaller unit cell is sometimes observed at low resolution in the X-ray diffraction pattern. Thus, weak reflections in the diffraction pattern of orthorhombic xylose isomerase crystals simulate space group $I222$, even though the real space group is primitive (Farber, Glasfeld, Tiraby, Ringe & Petsko, 1989). Also, $P2_1$ crystals where glyceraldehyde-3-phosphate dehydrogenase tetramers have a twofold axis parallel to *b*, have a face-centered orthorhombic lattice. The reason why the crystal has monoclinic rather than orthorhombic symmetry could be because of asymmetry in the tetramer, but this is not apparent in the structure of this particular protein (nor in most cases listed here) and it is more likely that the packing is better in $P2_1$.

The selection of particular orientations for the molecules must be related to the mechanisms of protein crystallization. When a dimeric protein crystallizes in space groups like $P1$, $P2_1$ or $P2_12_12_1$, which are very common and lack proper twofold symmetry, the molecular symmetry cannot be used as such, yet the crystal packing takes it into account by orienting the local twofold axis relative to a crystal screw axis. The dipole moment of the protein molecule may be the major orienting factor. While tetramers and oligomers with higher symmetry have no electric dipole, quadrupolar and higher moments could still play a part in selecting orientations.

Many proteins which are monomers in solution obey a point-group local symmetry, usually twofold symmetry, when crystallizing with more than one molecule in the asymmetric unit. An obvious possibility is that they dimerize in the mother liquor and that the species that crystallizes is the dimer. Conditions for crystallization generally also favor protein association to smaller units. This mechanism can also play a part in crystallization of proteins that form fibre-like assemblies, or of protein–DNA complexes where the crystal packing is directed by the DNA helix, but probably not in those few proteins that yield crystals with a local symmetry that is purely translational.

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