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Crystallization and X-ray Diffraction Studies on the Histocompatibility Antigens HLA-A2 and HLA-A28 from Human Cell Membranes

The human histocompatibility antigens HLA-A and HLA-B are polymorphic cell surface glycoproteins encoded by the major histocompatibility complex. These molecules are the major targets for the immune response during tissue transplantation. They are recognized by cytolytic T-lymphocytes during the immune response against virally infected cells, and have been linked to variations in susceptibility to human autoaggressive and neoplastic diseases. To permit a description of the sites of interaction with alloantisera and T-cell receptors, we have begun a three-dimensional structure determination of HLA-A. We report the isomorphous crystallization of two antigenic specificities of papain-solubilized HLA-A, A2 and A28. Isoelectric focusing indicates that the well-ordered crystals incorporate the sialic acid microheterogeneity of the oligosaccharides. Crystallographic evidence indicates that the HLA-A molecule has an approximate 2-fold rotational symmetry axis which, combined with biochemical data, suggests that the domains of the molecule are paired α_1 to α_2 and α_3 to β_2-microglobulin. This domain organization is similar to the arrangement of domains in the Fab and Fc fragments of immunoglobulins.

HLA, a class I transplantation antigen, is an integral membrane protein composed of two polypeptide chains (Cresswell *et al.*, 1973): the HLA heavy chain, a glycoprotein coded in the major histocompatibility complex, and β_2-microglobulin (Grey *et al.*, 1973; Peterson *et al.*, 1974; Berggard & Bearn, 1968). Amino acid sequences of the HLA heavy chain indicate that 34,000 M_r and one carbohydrate moiety exist as an N-terminal extracellular region, 24 uncharged hydrophobic amino acid residues span the membrane bilayer, and 30 residues comprise a C-terminal cytoplasmic domain (Strominger *et al.*, 1979). β_2-Microglobulin (12,000 M_r), associated with the extracellular portion of HLA, shows sequence homology to the C_H3 domain of immunoglobulins (Peterson *et al.*, 1972; Smithies & Poulik, 1972). Primary sequence data for the extracellular portion of the HLA heavy chain suggest a three-part structure: an N-terminal glycosylated region (α_1, 91 amino acid residues), followed by two regions (α_2 and α_3, 91 and 92 amino acid residues) each containing large disulfide loops reminiscent of those in immunoglobulin domains (Terhorst *et al.*, 1977). In the human genome, the three regions α_1, α_2 and α_3 are encoded by separate exons, suggesting that they may have arisen from a common ancestral gene (Malissen *et al.*, 1982). The second disulfide loop region, α_3, exhibits amino acid sequence homology to immunoglobulin constant domains at the same level of significance as different immunoglobulin constant domains show to each other and to β_2-microglobulin (Orr *et al.*, 1979, Tragardh *et al.*, 1979). Sequence homology has been observed between the regions α_1 and α_2 but, with exception of the suggestive disulfide loop in α_2, no significant sequence homology links α_1 or α_2 to α_3 or β_2-microglobulin. Based on these homologies and circular dichroism studies indicating a predominance of β-pleated sheet in HLA (Lancet *et al.*, 1979), it has been suggested that the α_3 and β_2-microglobulin regions of HLA may fold into immunoglobulin-like domains (Orr *et al.*, 1979). Upon removal of a carbohydrate moiety, proteolysis of the H-2 antigen (the murine analog of HLA) releases the α3 domain bound to β-microglobulin, suggesting that these regions are paired in the intact molecule (Yokoyama & Nathenson, 1983).

(a) *Crystallization*

The hydrophilic extracellular portion of the HLA molecule can be released from the membrane by papain (first shown by Nathenson & Shimada (1968) for mouse H-2 antigens), as a soluble glycoprotein with full antigenic activity. Because this extracellular moiety exists as a stable structure independent of the membrane segment, we have crystallized the papain-released portion of HLA in order to circumvent aggregation problems. HLA-A2 and A28 were purified from the cell membranes of homozygous human lymphoblastoid cell lines (Turner *et al.*, 1975; Parham *et al.*, 1977). Crystals of both were grown from protein solutions (15 mg/ml in 25 mM-2-(*N*-morpholino)ethanesulfonic acid or 100 mM-imidazole, pH 6·2 to 6·5) in two-microliter drops with 15% (w/v) polyethylene glycol 6000 by vapor diffusion. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis of washed crystals demonstrated that they contain both β_2-microglobulin and the papain-released heavy chain. Isoelectric focusing was used to examine the HLA incorporated into crystals, because the HLA-A2

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glycoportein is known to be heterogeneous in charge due to variable numbers of terminal sialic acid residues on its oligosaccharide (Parham *et al.*, 1974). Both dissolved crystals and pure protein contain multiple sialic acid-containing subpopulations of molecules (data not shown). Contaminating amounts of HLA-B7 seen in purified HLA-A2 protein are not, however, incorporated into the A2 crystals.

(b) *HLA-A2 crystals*

HLA-A2 crystallizes in two space groups: monoclinic and orthorhombic. Precession photographs of the monoclinic crystal show reciprocal lattice symmetry C_2 with systematic absences along b such that only reflections for which $k = 2n$ are present. This establishes the space group as $P2_1$. The unit cell dimensions are $a = 60{\cdot}4$ Å, $b = 80{\cdot}4$ Å, $c = 56{\cdot}5$ Å, and $\beta = 120{\cdot}4°$. The asymmetric unit of the crystal is estimated to contain one molecule based on average volume to mass ratios (V_m) of protein crystals (Mathews, 1968). The orthorhombic crystal has reciprocal lattice symmetry D_2 with odd reflections absent along a, b and c. This establishes the space group as $P2_12_12_1$. The unit cell dimensions are $a = 60{\cdot}2$ Å, $b = 80{\cdot}4$ Å, $c = 112{\cdot}2$ Å and one molecule per asymmetric unit as estimated above.

(c) *HLA-A28 crystals*

HLA-A28, an antigenically distinct but closely related HLA allotype (Joyson & Wolf, 1978), forms

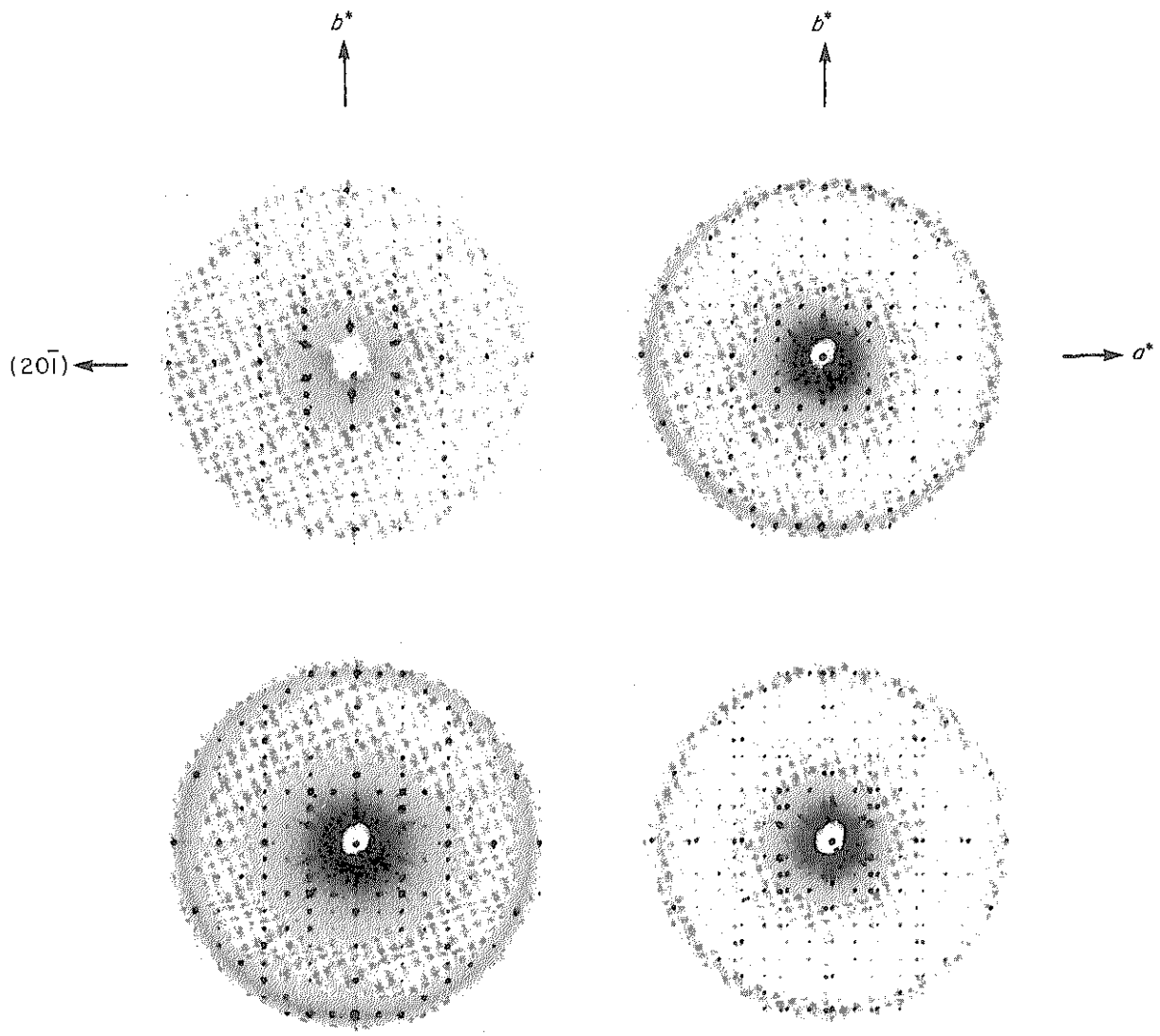

Figure 1. Comparison of $P2_1$ and $P2_12_12_1$ transforms. Upper left: A 6° screened precession photograph taken from a $P2_1$ crystal with the X-ray beam parallel to the monoclinic [102] axis. Upper right: A 6° screened precession photograph taken from a $P2_12_12_1$ crystal with the X-ray beam parallel to the orthorhombic c axis ($hk0$). Lower left: The 2 photographs are superimposed to demonstrate that the reciprocal lattices are sampled in identical positions. Lower right: The 2 photographs are superimposed, but offset slightly, to demonstrate that the 2 reciprocal lattices have similar intensity distributions.

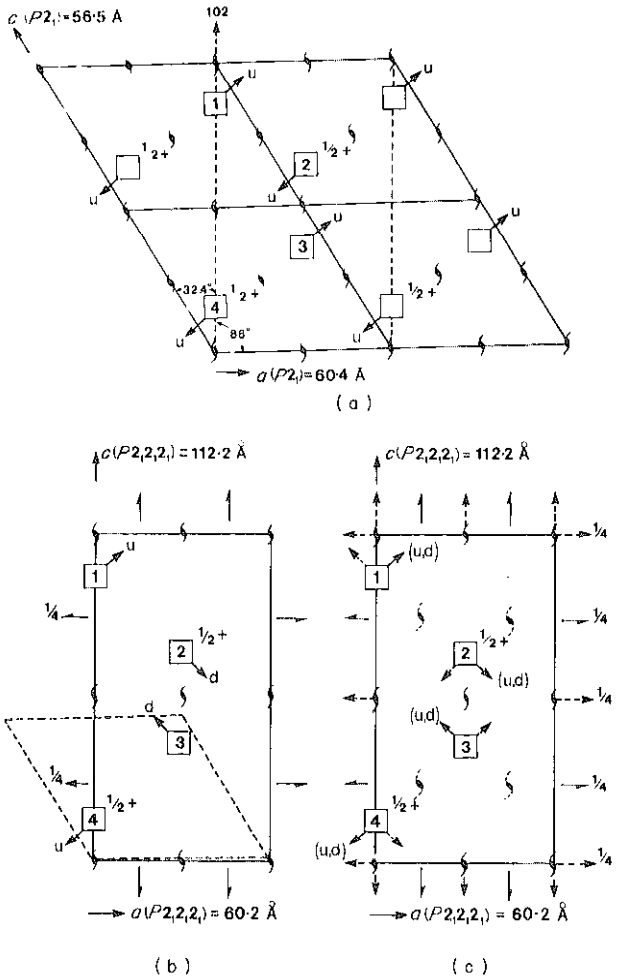

Figure 2. Proposed packing scheme for monoclinic and orthorhombic lattices. Unit cells are drawn to scale. Objects marked u are facing up with respect to the plane of the paper, while objects marked d are rotated by 180° and are facing down with respect to the plane of the paper. (a) $P2_1$ packing arrangement viewed down the b axis. In this $P2_1$ lattice, 4 molecules from 2 unit cells occupy a nearly orthogonal cell formed by the a axis and the [102] axis (broken lines). (b) $P2_12_12_1$ packing arrangement viewed down the b axis. To simplify the real space comparison to the $P2_1$ unit cell (broken lines), the origin of the $P2_12_12_1$ lattice has been drawn at the 2-fold screw axis along b. Molecules in the $P2_12_12_1$ lattice are drawn to occupy specific positions ($y = 0$, $z = 0$). In these positions, the objects labeled 1 to 4 are arranged similarly to the objects labeled 1 to 4 in the monoclinic lattice. However, objects 2 and 3 are rotated by 180° with respect to objects 1 and 4 in the orthorhombic lattice, whereas all objects in the monoclinic lattice are oriented in the same direction with respect to the plane of the paper. This difference is due to the presence of 2-fold screw axes perpendicular to the b axis in the orthorhombic space group that are absent in the monoclinic space group. We propose that the HLA molecule is nearly invariant to the 2-fold rotation operation that differentiates molecules 1 and 2 in the monoclinic cell from molecules 1 and 2 in the orthorhombic cell. To achieve this, the molecule would have an approximate dyad axis perpendicular to the crystallographic b axis. (c) $P2_12_12_1$ packing arrangement viewed down the b axis including symmetry of the $B22_12$ space group. Symmetry operators present only in the $B22_12$ space group are drawn with broken lines. Other symmetry operators are common to both space groups. Objects are drawn in the positions $y = 0$, $z = 0$, which are positions that four 2-fold symmetric objects can occupy in the space group $B22_12$. An exactly 2-fold symmetric object would be indistinguishable facing up with respect to the plane of the paper or facing down (thus objects are labeled (u, d)). The presence of pseudo-centering in the HLA-A2 $P2_12_12_1$ data towards the space group $B22_12$ suggests that molecules in the $P2_12_12_1$ lattice are arranged as shown in this Figure, and that each molecule contains an approximate dyad axis. This packing arrangement is identical to the packing arrangement in (b), which is independently derived from the similarity between the monoclinic and orthorhombic lattices. This Figure does not represent the only packing arrangement of the molecules in each unit cell that would explain the similarity in the space groups and the pseudo face-centering observed in the $P2_12_12_1$ data. For example, the diagrams could be redrawn to position molecules at $x = 0$, $y = 1/4$. This arrangement would predict that the approximate HLA molecular dyad would be parallel to the a axis. There are no data at present to favor either the a or the c axis as the direction of the approximate molecular 2-fold axis.

orthorhombic crystals isomorphous with those of HLA-A2. Small differences in the intensities have been observed on 6° precession photographs of the $h0l$ zones. Over 90% of the primary sequences of both HLA-A2 and A28 have been determined. The available data indicate an overall homology of approximately 95% (Lopez de Castro *et al.*, 1982).

All three crystals grow as thin plates (typical size 0·5 mm × 0·4 mm × 0·02 mm) with the a and b axes in the plane of the plate. The crystals diffract to at least 2·5 Å resolution.

(d) *A packing model*

An examination of the relationship of the two crystal forms of HLA-A2 suggests that a common packing scheme is utilized to generate the two space groups. The packing model also suggests that the HLA molecule has an approximate 2-fold rotational symmetry axis.

The two crystal forms of HLA-A2 are closely related. The a and b axes of both lattices are nearly identical in orientation and length. In one orientation, transforms can be compared directly because they are sampled in identical positions. Figure 1 shows a comparison of the $hk0$ $P2_12_12_1$ photograph and the comparable photograph taken from a $P2_1$ crystal (the X-ray beam parallel to the real space [102] axis). The lattices are superimposable, and the intensity distributions are similar to low resolution.

Crystals of both space groups are morphologically identical, and crystals of one form will grow when crushed seed crystals of the other form are added to the crystallization solution. These observations, in addition to the similarity of the lattices (both in dimensions and transform), imply that the packing of molecules is similar in both space groups. If the

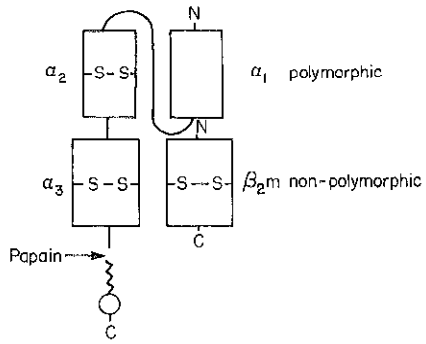

Figure 3. Proposed model for the domain organization of HLA-A. Crystallographic evidence suggests that the molecule is approximately 2-fold symmetric, implying that the homologous domains of HLA are paired α1 to α2, and α3 to β_2-microglobulin (β_2m). This pairing of domains has been suggested, based on the similarities between class I and class II antigens (see e.g. Kaufman *et al.*, 1984).

molecules in the orthorhombic unit cell are placed in specific positions ($y = 0$ or 1/4, either x or $z = 0$), then molecules in both lattices can be packed in a similar arrangement. Figure 2(a) and (b) illustrate how four molecules in one orthorhombic unit cell can be arranged in a similar manner to four molecules in a nearly orthogonal cell formed in the $P2_1$ lattice by the a, b and [102] axes. In the orthorhombic unit cell, two of the four molecules are rotated by 180° due to the presence of the 2-fold screw axes perpendicular to the b axis, while the analogous molecules in these positions in the $P2_1$ lattice are related only by a translation. That is, molecules 1 and 2 in Figure 2(a) are related by a 2-fold screw axis parallel to the b axis in the monoclinic cell, but molecules 1 and 2 in the orthorhombic cell (their positional counterparts) are related by a 2-fold screw axis parallel to the a axis. Thus, if molecule 2 is considered to be facing up in the monoclinic cell, its counterpart will be facing down in the orthorhombic cell. Similarly, molecules 1 and 3 are related by a translation in the monoclinic cell, but by a 2-fold screw axis parallel to c in the orthorhombic cell.

The common packing arrangement of the monoclinic and orthorhombic HLA crystals, differing only (except for the cell constant difference between space groups in the orthorhombic c direction) in the orientation of molecules up or down with respect to the b axis, suggests that the shape of the molecule involved in packing is nearly invariant to a 180° rotation; i.e. the molecule contains an approximate 2-fold rotational symmetry axis (perpendicular to the b axis of either lattice). The similarity, to low resolution, of the intensity distribution (in projection: Fig. 1) of the transform of the $P2_1$ lattice (in which all molecules are aligned in the same direction with respect to the b axis) to the transform of the $P2_12_12_1$ lattice (in which one half of the molecules are rotated by 180° with respect to the b axis) is another indication that

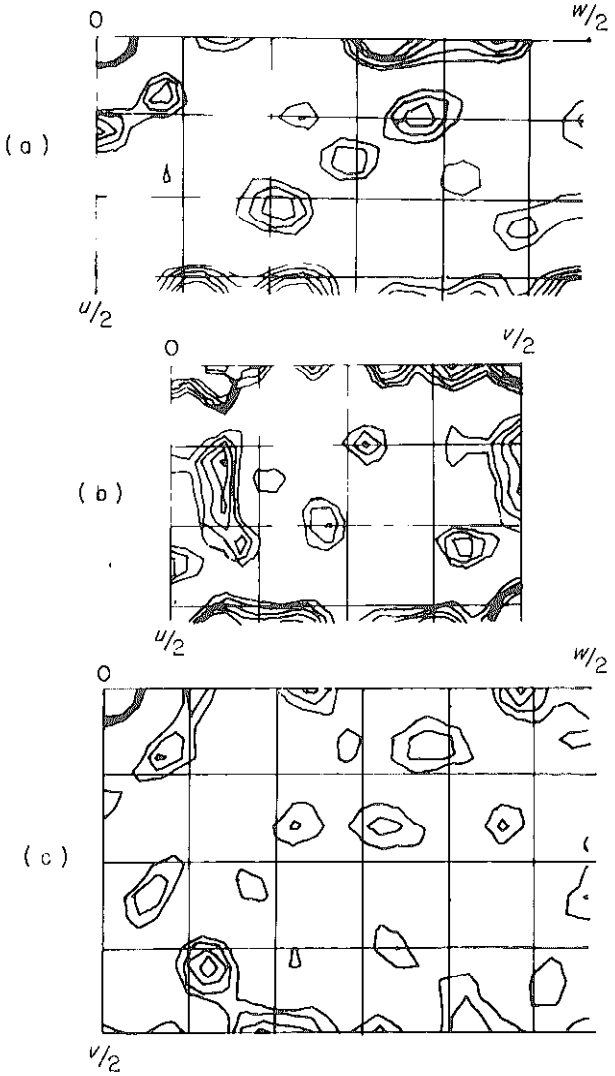

Figure 4. HLA-A2 $P2_12_12_1$ native Patterson projections at 7·4 Å resolution. (a) $h0l$ projection Patterson showing an approximate center of symmetry at $u = 1/4$, $w = 1/4$. (b) $hk0$ Patterson projection showing approximate mirror symmetry planes at $u = 1/4$, $v = 1/4$. (c) The $0kl$ Patterson projection shows no approximate symmetry. This observation, in conjunction with the observations that in the $hk0$ projection (b) the $u = 1/4$ plane is more nearly a mirror than the $v = 1/4$ plane, suggests that the approximate 2-fold molecular symmetry axis, while nearly parallel to a or c, has a significant component along the b direction.

the molecule is 2-fold symmetric to low resolution. The presence of approximate molecular dyad symmetry suggests that the regions implied in the amino acid sequence are structural domains arranged in homologous pairs α1 with α2, and α3 with β_2-microglobulin (see Fig. 3).

(e) *Pseudo face-centering of the HLA orthorhombic data*

An independent indication of the existence of both the approximate molecular 2-fold axis and the

preceding scheme for the packing of the HLA crystals comes from the observed pseudo-centering at low resolution in the $P2_12_12_1$ data toward the space group $B22_12$†. Three-dimensional data to 7·4 Å resolution have been collected on the HLA-A2 orthorhombic crystal by automatic diffractometry (857 reflections, $R = 1{\cdot}99\%$). The $h+l = 2n+1$ reflections are absent or weak to 20·0 Å resolution ($I_{h+l=\text{even}}/I_{h+l=\text{odd}} = 2{\cdot}7$) consistent with the scape group $B22_12$, in which these reflections would be absent. An approximate inversion center at $u = 1/4$, $w = 1/4$ in the $h0l$ native Patterson projection and approximate mirror planes at $u = 1/4$ and $v = 1/4$ in the $hk0$ projection (Fig. 4) are consistent with the molecules being packed in an approximately centered relationship. Since there are only four molecules in the unit cell, these observations of pseudo-centering also suggest the existence of an approximate molecular 2-fold rotational symmetry axis (see Fig. 2(c)).

(f) *Conclusions*

These experimental results indicate the papain-released HLA glycoproteins form crystals suitable for high resolution X-ray crystallographic structure determination. The isomorphous pair of crystals from HLA-A2 and HLA-A28, two related but antigenically distinct allotypes, offer the possibility of determining the structural difference recognized by alloantisera and T-cell receptors by difference Fourier analysis, once one structure is solved. The formation of isomorphous crystals by the allotypes A2 and A28 is independent evidence that these molecules have very nearly the same three-dimensional structure.

Analyses of the packing in two related crystal forms and pseudo symmetries of HLA-A2 in the low resolution (7·4 Å) X-ray data indicate that the HLA molecule itself exhibits approximate 2-fold rotational symmetry. This symmetry suggests that the regions implied in the amino acid sequence are structural domains arranged in homologous pairs α_1 with α_2, and α_3 with β_2-microglobulin (see Fig. 3), implying that the quaternary organization of HLA is similar to an Fab or Fc fragment of an antibody.

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† We note that crystals of the human Fc fragment also show pseudo-centering of the space group $P2_12_12_1$ ($a = 80{\cdot}4$ Å, $b = 146{\cdot}9$ Å, $c = 50{\cdot}3$ Å) towards $A2_122$ (Goldstein *et al.*, 1968; Colman *et al.*, 1974). In this case, the molecular 2-fold and the centering are perpendicular to an 80·4 Å axis. This is analogous to the pseudo-centering of the HLA data and the hypothesized molecular dyad, which are also perpendicular to an 80·4 Å axis.

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