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An X-Ray Determination of the Molecular Interactions in Hemoglobin C: A Disease Characterized by Intraerythrocytic Crystals

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Summary

The X-ray structure of cyanomet human hemoglobin C has been solved and refined, R ∿27%. The molecular packing can be represented in two dimensions by two sets of parallel strands, one set in the b direction and the other in the c direction. Taken together the two sets of strands interconnect the molecules into square nets or layers where each molecule contacts its four nearest neighbors. Molecules in one layer are displaced in a and b so that they fit into the "holes" of the square arrays of the adjacent layers (normal to a) resulting in a pseudo body-centered cubic packing. This packing can account for the hemoglobin crystallization in and fragility of the erythrocytes. The aberrant β6A3 Lys residue is in a position to influence the crystal formation.

The HbC* molecule is found in ∿2.5% of the black population of the USA and is the second most common variant found in this group. HbC is commonly found in combination with HbA (HbAC) and HbS (HbSC) as well as in the homozygous condition (HbCC). HbAC is generally asymptomatic but HbCC and HbSC both give rise to conditions which are related to intracellular aggregation of Hb molecules. HbSC molecular aggregates occur under deoxy conditions and are thought to be similar to that found in homozygous HbS disease. In contrast, HbCC aggregates appear to form from molecules in the oxy state. Crystalline aggregates of met or oxy HbC molecules have been identified in intact erythrocytes [1-4], blood smears [5], cell free

*HbA = normal hemoglobin, HbS = β6A3 Glu→Val variant
HbC = β6A3 Glu→Lys variant
Hb(DIII) = Virginia White-tailed Deer hemoglobin β chain variant III (odocoileus virginianus)

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solutions [3], and moist preparations [2]. These crystals are thought to decrease the elasticity of the red blood cells, causing damage to the cells as they squeeze through the capillaries in the circulatory system. The clinical symptoms are characterized by mild anemia and spleen enlargement [6]. As part of a study of Hb aggregates, we have examined cyanomet and aquomet HbC under a variety of conditions. We have crystallized a previously unreported form of cyanomet HbC whose structure is likely to be related to that of the intraerythrocytic crystals found with HbC. The X-ray crystal structure of this protein has been solved (R $\sim$ 47%) by means of the molecular replacement method and refined to a residual $\sim$27%. We wish to report the overall molecular packing and subunit contacts in this structure and its relationship to other Hb structures.

Methods and Materials

The hemoglobin C isolation with an A_2 contaminant from lysed erythrocytes, from patients with homozygous C disease, crystallization from phosphate-ammonium sulfate solutions, plate-like crystal morphology, and diffraction pattern have been previously reported [8]. The diffraction pattern reveals the unique space group $P2_12_12_1$ with lattice dimensions: $\underline{a}$ = 157.8A, $\underline{b}$ = 65.5A, $\underline{c}$ = 55.0A. Z was experimentally determined to be 4. During the data collection the lattice repeat in the $\underline{a}$ direction varied as much as 2A from crystal to crystal, and it is not clear whether this is due to the precision of the measurements or a real change in the lattice parameter. Diffractometer data collection and processing were similar to that of deer type III hemoglobin [9] but since the HbC crystals were thin plates, much less data has been collected at this time. The molecular replacement programs of Lattman [10] were used to solve the structure using the deer Hb (Type III) molecule as a model. The rotational angles and translations necessary to move deer type III molecule from the origin of the HbC cell to its proper place are θ_1 = 31.1°, θ_2 = 76.5°, θ_3 = 140.5°, ΔX = 0.13, ΔY = 0.22, ΔZ = 0.04. The residual (R) calculated for this placement is 46.4%, indicative of a proper solution and the restrained least squares refinement is at an R of $\sim$27% [11]. All other crystallographic calculations were done with the XRAY76 program package [12].

Results and Discussion

The molecular packing in this case clearly indicates the reasons for the plate-like crystal morphology, for the crystallization in the red blood cell and for the rigidity of the resulting cell. In fact, the

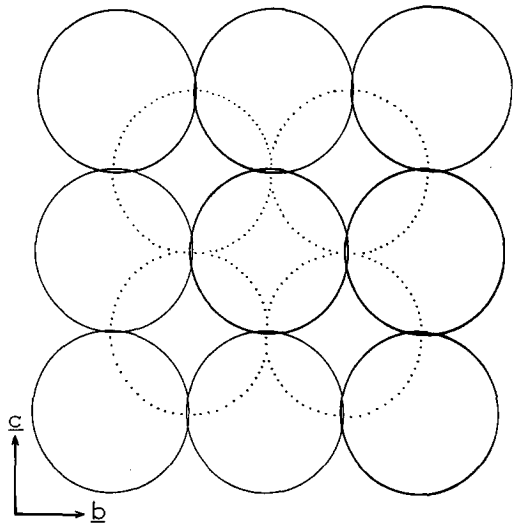

FIGURE. The molecular packing of cyanomet hemoglobin C normal to the a direction. For simplicity the molecules are represented by spheres; the solid circles representing molecules in the plane of the paper and dotted circles representing those displaced ∿ 1/2 molecular diameter above and below this plane. Succeeding layers would alternate between solid and dotted layers. The solid circles are related to each other by simple lattice translations as are the dotted circles, each making up an approximate square array. It is clear that adjacent layers fit into the "holes" of those immediately above and below forming a "body-centered" packing arrangement.

molecular packing of hemoglobin molecules in this case may provide a basic rational for the causes and effects observed in patients with HbCC disease.

The molecular packing within the HbC crystal can be conveniently described in terms of fibrils or strands as is the case for HbS [13] and Hb(DIII) [9]. Each fibril consists of a linear array of hemoglobin molecules. Within a given fibril any one molecule is related to any other by a simple translation along the fibril axis. There are two perpendicular sets of fibrils in the structure of HbC, one extending in the b direction and the other in the c direction. If the two sets of fibrils are combined, the result is a 2-dimensional layer or sheet one molecule thick where each molecule contacts its four nearest neighbors (Fig.). The third dimension of the structure is generated by stack-

ing the layers so that the molecules of one layer are positioned over the "holes" of the layer below. This kind of stacking offsets each layer ∿ one-half of the molecular dimension in the b and c directions relative to its nearest neighbors above and below, resulting in a body centered cubic packaging type of molecular arrangement. From this description and figure, it is clear why the crystals grow as plates with the thin direction (a) perpendicular to sheets of molecules described above.

An important facet of this relatively dense molecular packing is the lack of solvent channels that might be anticipated from the hollow tubular structures seen by White [3] in the electron micrographs of HbC aggregates.

Structural reports on hemoglobin aggregates have emphasized a number of models and structural elements, some of which have counterparts in cyanomet HbC. One of these elements, an antiparallel out of register double stranded moiety, was reported by Wishner [13]. A similar moiety is formed in the present structure if one focuses on two neighboring strands running parallel to b, one from each of two adjacent layers. However, this description fails to predict the formation of plate-like crystals. Among the fiber models reported [13-18] are: six-stranded hollow hexagonal tube [9,15], eight-stranded hollow tube [17], fourteen-stranded rod [18] and seven-stranded rod [14]. This last model comes from an interpretation of the crystal structures of deoxy HbS, deoxy HbC and deoxy HbF [14] and a similar interpretation could be drawn from cyanomet HbC. The fact that the oxy and deoxy form can be crystallized in essentially the same lattice [19] strengthens this correlation. The marked resemblance among the molecular packing schemes could explain the presence of similarly structured intraerythrocytic aggregates under different oxygen tension even though few of the specific contacts found in cyanomet HbC correlate with those found in deoxy HbS or deoxy HbC structures [13].

The specific intermolecular contacts which cause the crystallization of HbC in our case are not yet unambiguous, but certain regions of the molecule have been implicated by short intermolecular contacts calculated from the structure. The interactions between molecules in the strand parallel to b may involve the α_1 - α_2' and α_1 - β_2' subunits; the strand parallel to c, α_1 - β_2' and α_1 - β_2'. Between the layers there appear to be contacts between the β_1 and β_1', α_2 and α_1', β_2 and α_1, β_1 and α_1, β_1 and β_2 subunits. In calculating the subunit contacts the β6A3 Lys residue was found to participate only in the interlayer interactions.

Two pieces of evidence suggest a strong correlation between the X-ray structure reported here and the crystalline aggregate associated with the disease state: 1) The crystals used in the X-ray structural determination exhibit the plate-like crystal morphology as has been reported in intact erythrocytes [4] and 2) The X-ray structure shows the aberrant β6A3 Lys residue, which must cause the disease symptoms, intimately involved in intermolecular contacts.

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