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Studies of Monoclinic Hen Egg White Lysozyme. II. The Refinement at 2·5 Å Resolution – Conformational Variability between the Two Independent Molecules

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Abstract. Monoclinic hen egg white lysozyme crystals (*M*1 form) [Hogle, Rao, Mallikarjunan, Beddell, McMullan & Sundaralingam (1981). *Acta Cryst.* B**37**, 591–597] containing two independent molecules in the asymmetric unit have been refined to an *R* value of 0·26 for 6045 reflections in the resolution range $5{\cdot}0 > d > 2{\cdot}5$ Å. The electron density is strong and clearly defined for the entire backbone and side chains of both molecules, except for the residues 62–63 and 125. The α-carbon atoms of the two independent molecules have an r.m.s. deviation of 0·70 Å. With the SEWFA (systematic elimination of the worst-fitting atoms) procedure, the conserved part of the structure is shown to consist of the helical residues as well as residues in the active site. A comparison of this structural variability with the average thermal paramaters shows that all regions of large thermal factors occur in the variable part of the structure, except for residues 61–65; these apparently have internal motions.

Introduction. A question of paramount importance in protein structure analysis is the degree of variability a protein undergoes in a crystal field. Such information can be gleaned by studying the structure of a protein which crystallizes in different crystalline forms or, even better, if multiple copies of the protein occur in the asymmetric unit of the crystal. The former would give some idea of the degree of deformation experienced by the protein molecule in different crystalline environments, but usually suffers from the difficulty that the data collection and the refinement procedures may not all be carried out under the same conditions and some of the observed variability may arise from such effects. If there are two or more independent molecules in the crystal, these problems are circumvented.

Hen egg white lysozyme (HEWL) is an ideal candidate for such studies since it crystallizes in many polymorphic forms (*e.g.* tetragonal, triclinic, monoclinic and orthorhombic) that diffract to high resolution (Steinrauf, 1959). Furthermore, the monoclinic form has the additional advantage that it contains two independent molecules. In part I, we reported the 4 Å MIR structure determination of this form (Hogle, Rao, Mallikarjunan, Beddell, McMullan & Sundaralingam, 1981) and a 6 Å study has also been recently reported by Artymiuk, Blake, Rice & Wilson (1982). We have now refined the structure at 2·5 Å resolution using the constrained least-squares procedure (Konnert, 1976; Hendrickson & Konnert, 1980*a*,*b*). The atomic thermal parameters were allowed to vary with tight constraints. In this paper, we discuss the variations in the positions and thermal factors of the residues in the two independent molecules.

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Experimental. We collected the 2·5 Å data sets containing two equivalents $(h,\pm k,\pm l)$ from two native crystals using an Enraf–Nonius CAD-4 diffractometer with Ni-filtered Cu radiation. The θ–2θ scan mode was used with scan speeds of 1–2° min^{-1}. 100 scaling reflections were also measured on each crystal. The data reduction was carried out using the standard procedures as described earlier (Hogle *et al.*, 1981). About 93% of the data in the 2·5 Å resolution set were observed with $I > 2\sigma(I)$. The scaling reflections had an R value of 0·02; the equivalent reflections also gave an R value of 0·02 ($R = \sum\sum|\,|F_i| - |\bar{F}_i|\,|/\sum\sum|\bar{F}_i|$).

Difference Fourier refinement. The approximate atomic coordinates of the two independent molecules from the 4 Å analysis (Hogle *et al.*, 1981) formed the starting point for the work at 2·5 Å. $2|F_o| - |F_c|$ maps were calculated by sequentially omitting about 10 residues at a time from the phasing calculation and the omitted residues were adjusted into these maps. Three complete rounds of fitting were performed over all the residues of both the molecules using the computer-graphics system at Texas A & M University (see Swanson, 1979). These coordinates were subjected to 20 cycles of difference synthesis refinement at Madison. After every four cycles, the coordinates were repaired to restore proper geometry using an idealization procedure developed by Dodson, Isaacs & Rollett (1976). This brought the conventional R value down to 0·31 (Hogle, 1978).

Constrained least-squares refinement. Using the coordinates from the difference synthesis analysis, we refined the structure using the constrained least-squares method (Konnert, 1976; Hendrickson & Konnert, 1980*a*, *b*) on a VAX-780 computer with 6045 reflections in the resolution range $5{\cdot}0 > d > 2{\cdot}5$ Å. The data with $d > 5$ Å were omitted to reduce the influence of solvent densities on the refinement of the molecular model (Hendrickson, 1981). The overall thermal factor was set at 14 Å^2.

Initially, 16 cycles of refinement were performed varying the positional parameters of all the 2002 atoms along with an overall scale and an overall thermal factor. Some of the error levels targeted for the geometrical parameters are: bond length (1–2 neighbor) 0·05 Å; bond-angle distance (1–3 neighbor) 0·10 Å; planarity 0·05 Å; chiral volume 0·15 Å^3; *thermal factors*: 1–2 *neighbor* 1 Å^2, 1–3 neighbor 2 Å^2.* The R value dropped to 0·29 with a concomitant improvement in the geometry and the r.m.s. discrepancies in all the geometrical parameters were equal to or less than the specified errors. This was followed by an additional 13 cycles of refinement in which both the positional and isotropic thermal factors of the atoms were varied; the thermal factors of neighboring atoms were restrained to similar values with relatively tight constraints. The R value dropped to 0·26.

The $2|F_o| - |F_c|$ electron density maps calculated using the refined coordinates clearly show the backbone and side chains of both molecules. The only exceptions are weak densities for the residues 62, 63 and 125 of both molecules. Several potential sites of solvation (water, nitrate ions) are seen in the difference maps, but have not yet been incorporated into our analysis.

The r.m.s. movements in the α-carbon atoms from our earlier 2·5 Å difference Fourier studies are 1·12 Å for molecule *A* and 1·08 Å for molecule *B*.*

Discussion. *Conformational variability in the two independent molecules.* When the 129 α-carbon atoms of one molecule are superposed on the corresponding α-carbon atoms of the second molecule (Rao & Rossmann, 1973), the r.m.s. deviation is 0·70 Å (Figs. 1 and 2*a*). In order to identify the regions in the two molecules which are most conserved, we adopted a procedure where we systematically eliminated the worst-fitting atoms (SEWFA) iteratively to convergence. The cut-off distance was set arbitrarily at 1·5 times the r.m.s. deviation between corresponding atoms in each step. Starting with all the 129 α-carbon-atom pairs, 15 α-carbon atoms having a deviation of 1·05 Å or more were eliminated in the first round. The remaining 114 atom pairs when equivalenced held an r.m.s. deviation of 0·59 Å. In the next few rounds, 10–15 atom pairs were eliminated per cycle and the procedure converged after seven rounds, leaving 62 atom pairs (about half the starting number) with an r.m.s. deviation of 0·39 Å. In Fig. 2(*b*) the residue numbers are plotted against the cycle number in which they were eliminated; the 62 atom pairs that survived this procedure are those in the plateau regions of the plot. These residues of the conserved part of the structure are in the helical regions and the active site. Thus, the active-site cleft, which is the functional part of the molecule, is as conserved as the helical regions of the molecule.

Comparison of the average B values in the two independent molecules. The thermal factors (B values) averaged over the backbone atoms for each residue are

* A table listing all the targeted values and actual errors in the model along with a figure depicting the progress during the refinement cycles have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 38117 (3 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

* Atomic coordinates and structure factors have been deposited with the Protein Data Bank, Brookhaven National Laboratory (Reference: 1LYM and R1LYMSF), and are available in machine-readable form from the Protein Data Bank at Brookhaven or one of the affiliated centers at Cambridge, Melbourne or Osaka. The data have also been deposited with the British Library Lending Division as Supplementary Publication No. SUP 37007 (2 microfiche). Free copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

plotted against the residue number in Fig. 2(*c*). Molecule *A* is shown in solid lines while molecule *B* is shown in dashed lines. Even though it is somewhat

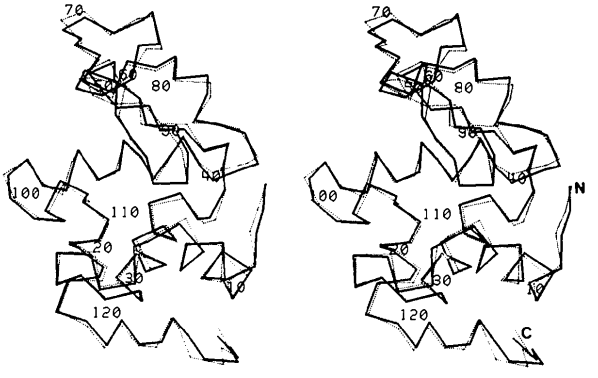

Fig. 1. Stereodiagram of the α-carbon-atom skeleton of molecule *A* (solid) superposed on molecule *B* (dashed). The r.m.s. deviation between the corresponding α-carbon atoms is 0·70 Å.

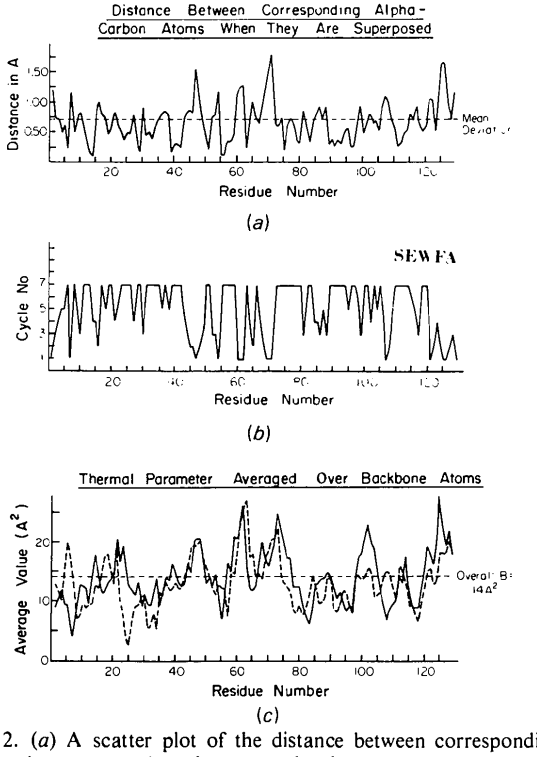

Fig. 2. (*a*) A scatter plot of the distance between corresponding α-carbon atoms when the two molecules are superposed *versus* the residue number. The r.m.s. value of 0·70 Å is shown as a horizontal dashed line. (*b*) The cycle number in which a particular α-carbon atom is eliminated in the SEWFA procedure (see text) *versus* the residue number. The plateau regions represent the residues that are most conserved. (*c*) Plot of the average *B* values of the backbone atoms *versus* residue number for molecule *A* (solid lines) and molecule *B* (dashed lines).

difficult to assess the significance of the *B* factors at 2·5 Å resolution, the trends in the *B* values are generally very similar for the two independent molecules. The regions of large *B* values, interestingly, are also generally the regions where the structural variability is also large (Fig. 2*a*). In an earlier work, Artymiuk, Blake, Grace, Oatley, Phillips & Sternberg (1979) showed that for tetragonal HEWL and human lysozymes, the trends in the average *B* values are quite similar. Ramanadham, Sieker, Jensen & Birkens (1981) found similar trends in their studies on the triclinic HEWL. The pattern of *B* values found in the monoclinic HEWL for both the molecules bears a very close resemblance to the human, tetragonal and triclinic lysozyme structures, supporting the observation of Artymiuk *et al.* (1979) that it probably reflects some intrinsic property of the molecular fold.

Correlation of intra-/intermolecular contacts to the structural variability. It is found that the structural variability is correlated to the intramolecular contacts, *i.e.* the regions with a smaller number of intramolecular contacts are also generally the regions of large variability and large average *B* values. However, unlike intramolecular contacts, the intermolecular contacts, have, if any, very little correlation with the variability or the *B* values. It appears, then, that the intramolecular forces have a greater influence on the variability of the protein molecule than the intermolecular interactions or crystal forces.

B values and molecular motion. The atomic thermal factors from an X-ray analysis have the effects of both spatial (static) disorder and molecular motion (dynamic disorder) combined in them. To evaluate the molecular motion, its relative contribution to the observed *B* values should be estimated. Frauenfelder, Petsko & Tsernoglou (1979) showed that this can be done by studying the protein structure at different temperatures. As mentioned before, the trends in the *B* values for different crystal forms of HEWL are very similar. It is difficult to identify the contribution from structural variability from a comparison of the lysozyme structures in different crystal forms, since these studies were made with different modes of data collection and methods of refinement. Part of the observed structural variability may be due to these differences. The monoclinic HEWL, containing two independent molecules, does not suffer from these problems. A comparison of Figs. 2(*a*) and (*c*) shows that, in general, regions with large *B* values are also the regions with large variability. These regions probably exhibit microconformational states in the crystal. However, the region around 61–65 appears to be an exception; it is a conformationally conserved region and yet displays large *B* values and can be tentatively identified as having a high degree of inherent motion. This, however, is restricted to only a short stretch of the polypeptide chain; we will watch this region, with particular interest, in our higher-resolution studies.

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Structure of 3-(2-Pyridyl)-2-(2-tolyl)-1,3-thiazolidin-4-one, $C_{15}H_{14}N_2OS$

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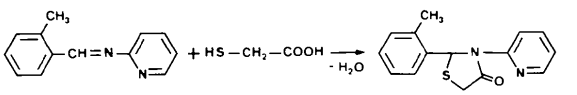
Abstract. $M_r = 270{\cdot}36$, space group $P\bar{1}$, $a = 10{\cdot}304$ (1), $b = 13{\cdot}845$ (2), $c = 19{\cdot}852$ (3) Å, $\alpha = 80{\cdot}87$ (1), $\beta = 78{\cdot}19$ (1), $\gamma = 89{\cdot}25$ (1)°, $Z = 8$ (four molecules in the asymmetric unit), $V = 2736{\cdot}4$ Å^3, $D_m = 1{\cdot}30$ (1), $D_x = 1{\cdot}313$ Mg m^{-3}, $F(000) = 1136$, and $\mu(\text{Cu } K\alpha) = 2{\cdot}0$ mm^{-1}. The structure of this antibacterial drug was solved by direct methods and refined by least-squares calculations to $R = 0{\cdot}066$ for 10713 observed reflections. Two different conformations appeared, differing mainly in torsional angles, the four molecules being related in pairs through a pseudoglide plane. In one conformation the thiazolidine ring was found to be in an envelope conformation, whereas in the other it was found to be significantly more twisted.

Introduction. The synthesis of the thiazolidinone investigated has been described elsewhere (Fenech, 1960). It is obtained from the reaction of thioglycolic acid with the corresponding Schiff base:



This molecule is a member of the substituted 2-aryl-1,3-thiazolidin-4-one family, which have great and diverse therapeutical interest (some have antibacterial, antifungal, anti-tubercular, myorelaxant, or antiviral properties) (Newkome & Nayak, 1979; Fenech, 1972–73). The title compound shows antibacterial activity (Fenech, 1962).

Furthermore, from a theoretical point of view, very few reports have appeared concerning the 1,3-thiazolidin-4-one conformation (Anantha-Murthy & Murthy, 1975).

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