brief communication

Flexibility in crystalline insulins

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ABSTRACT Comparisons of atomic models for chemically identical protein molecules solved in differing crystal environments provide information on flexibility in the protein structure. The structures of five T4 lysozyme proteins in differing crystal environments showed large relative displacements of the two domains with conserved backbone conformations that are connected by a flexible hinge (H. R. Faber and B. W. Matthews. 1990. *Nature (Lond.)*. 348:263–266). In contrast, my comparison of the positions of all the atoms in two crystal forms of insulin shows that the structural changes caused by the differing crystal contacts are contained within nearby amino acids and are not propagated through the core of the insulin molecule. Groups of atoms that are most significantly displaced are not shifted in large rigid units but are repacked into new and distinct conformations. The transmission of displacements through the single domain insulin molecule is, like the movements due to thermal vibrations (D. L. D. Caspar, J. Clarage, D. M. Salunke, M. S. Clarage. 1988. *Nature (Lond.)*. 332:659–662), characterized by short-range interactions between small atomic groups.

INTRODUCTION

X-ray diffuse scattering measurements show that most of the thermal fluctuation in the atomic positions in crystalline 2Zn insulin are only correlated over short-range (~6 Å) (1), contradicting recent proposals that such fluctuations in globular proteins could be accounted for by rigid-body movements (2) or long-wavelength elastic vibrations (3). In a crystal structure of T4 phage lysozyme that contains five independent protein molecules per asymmetric unit (4), the relatively large differences between the backbone conformations in the five proteins could be fitted by rigid-body displacements of the two domains about a flexible hinge. To analyze the transmission of force through a compact single domain protein structure, I have compared the positions of all the protein atoms in 2Zn (5) and cubic (6) porcine insulin crystals.

The insulin molecule is composed of a 21 amino acid A-chain and 30 amino acid B-chain. These chains are connected by disulphide bridges A7–B7, A20–B19 and an intrachain disulphide bridge A6–A11. The crystal structure of 2Zn porcine insulin (space group R3), contains two similar but not identical molecules per asymmetric unit (5). Porcine insulin in the cubic crystal (space group $I2_13$) contains only one molecule per asymmetric unit (6). Unlike other insulin crystal structures (5, 7–9), the insulin molecules in the cubic crystal are not associated as hexamers. One crystallographic symmetry operation in the cubic space group generates a dimer similar to the dimer found in the 2Zn insulin crystal asymmetric unit. The other crystal contacts in these two crystal forms are unrelated (Fig. 1).

MATERIALS AND METHODS

Structural comparisons were made between the two molecules in the 2Zn insulin crystal asymmetric unit and the cubic insulin molecule. The two 2Zn insulin monomers were independently superimposed on to the cubic insulin monomer by a least-squares overlap of the C_α atoms contained in structurally similar parts of the insulin molecule. (Figs. 2, and 3, *A* and *B*). To locate these invariant portions of the molecule, C_α atom difference distance matrices (10–13) were computed between each pair of structures. The C_α atom for which the rms value of the corresponding set of matrix elements was largest was removed, and the rms difference over all remaining off-diagonal elements in the matrix calculated. This procedure was repeated until the rms difference for the surviving parts of the matrix was <0.2 Å. In the comparison of molecule 1 of 2Zn insulin with the cubic insulin molecule 18 C_α atoms were eliminated and the rms positional difference over C_α atoms used in the overlap was 0.26 Å. For the comparison with molecule 2 of 2Zn insulin 29 C_α atoms were eliminated and the rms difference was 0.27 Å. As would be expected, the overall effect of this procedure is to "lock in" on invariant structure near the dimer interface for making the molecular superpositions.

RESULTS

The molecular superpositions show that the cubic insulin molecule is most greatly displaced from the structures of both molecules 1 and 2 of 2Zn insulin in the region B1–B4 and markedly displaced over A11–A15 and B20 (Figs. 2, and 3, *A* and *B*). The helical region A1–A9 is appreciably shifted in the comparison of molecule 2 of 2Zn insulin with the cubic insulin monomer (and differs in similar fashion from molecule 1 of 2Zn insulin [1, 14]). The structural displacements are

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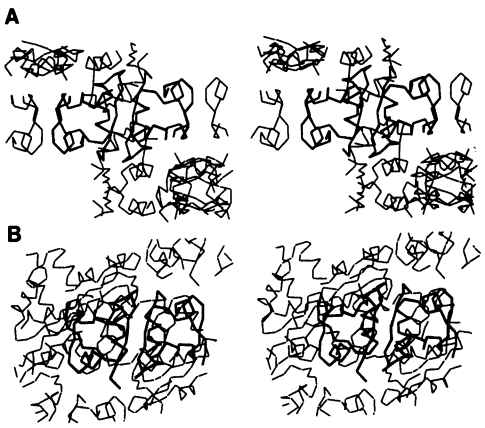

FIGURE 1 Stereo views of portions of (*A*) the cubic insulin crystal lattice and (*B*) the 2Zn crystal lattice showing the differing packing arrangements. In the cubic insulin crystal the protein molecules are arranged in rows running in three perpendicular directions. In the 2Zn insulin crystal, the insulin dimers are associated into roughly spherical hexamers. For clarity only the C_α atoms are shown. One crystallographic dimer in the cubic crystal and one corresponding noncrystallographic dimer in the 2Zn crystal asymmetric unit are marked in heavier lines.

confined to exterior portions of the insulin molecule and do not propagate through the core.

Examination of the displacement vectors for the centroids of the main and side chain atomic groups (Fig. 3, *C–F*) does not reveal any recognizable pattern of large-scale concerted (rigid-body) movement. Portions of the insulin structures which differ the most in the molecular superpositions are repacked into new conformations. Examination of Figs. 3*A–3B* (or the difference-distance matrix elements) shows that it is not possible to superimpose the C_α atom chain fragments B1–B4 and A11–A13 of the cubic insulin molecule on to the 2Zn insulin molecules. The helical segment A1–A9 which is shifted in molecule 2 of 2Zn insulin becomes distorted in the altered conformation. In particular, the orientations of the external side chains are strongly influenced by the particular crystal packing environment. After omitting the five glycine and alanine amino acids (for which no χ_1 angle is defined) and eight residues which are buried in monomers of the three insulin models, eight out of 39 side chains are reoriented (χ_1 changes by >90 degrees) in the comparison between 2Zn insulin molecule 1 with the cubic insulin monomer and 11 out of 39 side chains are reoriented in the comparison between 2Zn insulin molecule 2 and the cubic insulin monomer. Because most side chains in proteins are oriented near two or three common rotomer orientations about χ_1 (15, 16) the particular atomic contacts between molecules are critical in determining the side chain orientation.

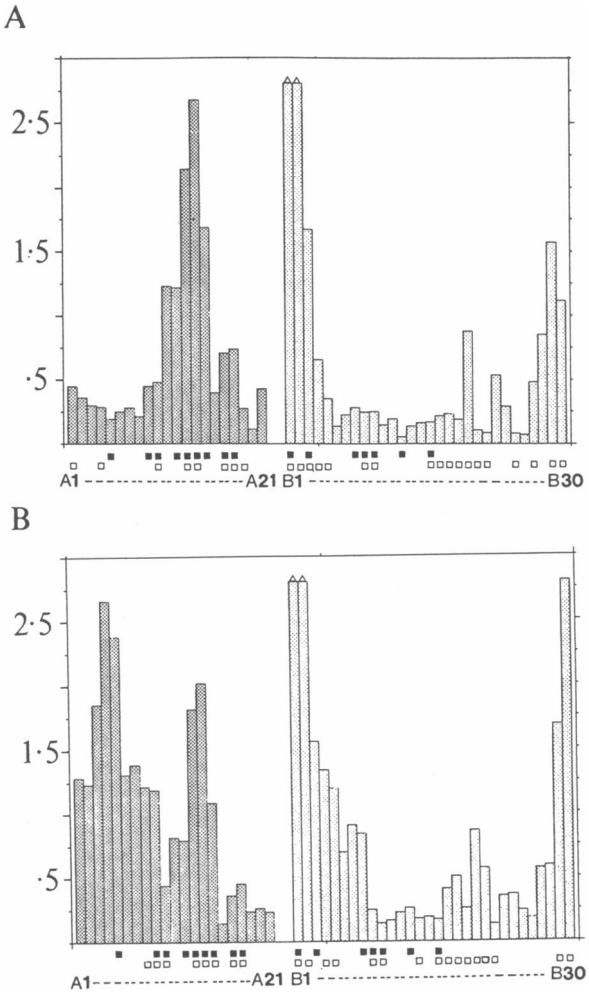

FIGURE 2 Distance between equivalent C_α atoms atoms after molecular superpositions for (*A*) cubic insulin and molecule 1 of 2Zn insulin, and (*B*) cubic insulin and molecule 2 of 2Zn insulin. Amino acids labeled with a filled square make crystal contacts in the cubic crystal. Amino acids labeled with an open square are involved in intermolecular contacts in the 2Zn insulin crystal. The definition of a crystal contact is that two atoms from different molecules approach each other by <4 Å. Contacts between the two independent molecules in the 2Zn insulin asymmetric unit and the crystallographic molecular dimer in cubic insulin are not listed. The C_α atom displacements for amino acids B1 and B2 greatly exceed the scale of the graphs. In (*A*), the values are 6.2 Å and 5.9 Å and in (*B*) the values are 6.4 Å and 5.9 Å, respectively.

DISCUSSION

Comparisons between these different crystal structures demonstrate that the insulin molecule readily adopts very dissimilar conformations to optimize particular crystal packing interactions. Although crystallographically refined model coordinates are quite accurately determined, with errors often estimated at ~0.2 Å

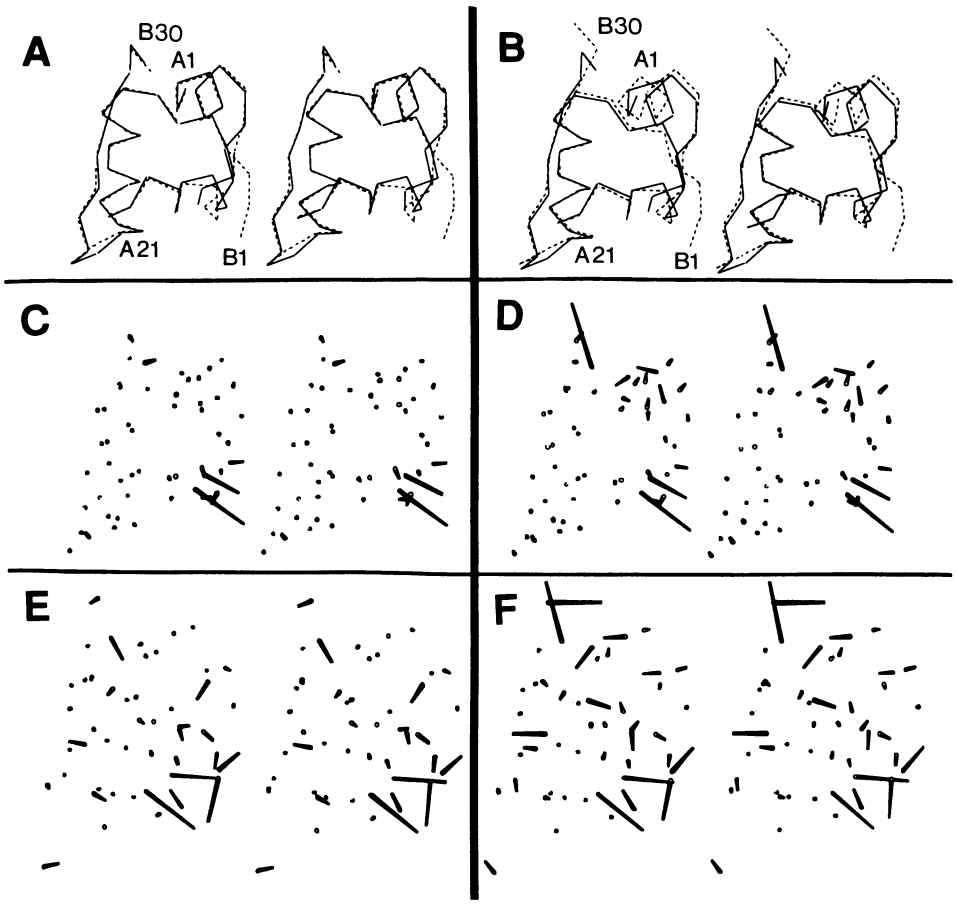

FIGURE 3 Stereo views of (*A–B*) superpositions of the C_α atoms of 2Zn insulin molecules 1 and 2, respectively, on to the cubic insulin molecule. The cubic insulin C_α atom trace is marked in continuous lines. (*C–D*) Displacement vectors per residue for main chain centroids corresponding to the superpositions (*A–B*). (*E–F*) Displacement vectors per residue for side chain centroids corresponding to the superpositions (*A–B*). In (*C–F*) the small open circles, 0.2 Å in radius, correspond to the cubic insulin centroid positions.

in well-ordered parts of the protein, crystal lattice interactions may introduce much larger differences from biologically relevent structures. In the case of insulin, measurements of CD (17) and NMR (18) spectra show that the structure of the monomer in solution does differ somewhat from the models obtained by x-ray crystallographic analysis. The portions of the molecule altered by crystal packing interactions represent flexible segments that could easily be displaced upon insulin receptor binding.

Atoms displaced by relatively large distances (>1 Å) between the differing insulin crystal structures are contained in protein main and side chain segments that are greatly altered in local conformation. When deformed beyond this elastic limit (14), the protein relaxes into new and distinct conformations. The protein deformations caused by external crystal packing interactions, perhaps analogous to the "induced-fit" model for docking between molecules, involve short-range interactions between small atomic groups. This description of conformational differences between insulin crystals in terms of locally coupled atomic displacements is similar to observations of local "liquid-like" coupling in the thermal movements in 2Zn insulin crystals (1). Thus, most of the flexibility in the insulin molecule may be due to repacking adjustments between atomic groups of dimensions ~5–10 Å.

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