ANOMALOUS TEMPERATURE FACTOR BEHAVIOR AND CRYSTAL LATTICE MOBILITY IN CYTOCHROME C'

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Protein structures obtained by x-ray crystallography represent a time and space average of the large ensemble of molecules incorporated in the crystal lattice. The effects of averaging the scattering from molecules that may have slightly differing local conformations or orientations in the crystal lattice, or that undergo thermally induced motions, are reflected in the atomic temperature factors (*B*-values) obtained during crystallographic refinement. Considerable interest attaches to the analysis of *B*-value behavior both because it provides one of the few empirical estimates of protein dynamical behavior that can be correlated with computational simulations (1), and because recent studies suggest that protein surface regions having high flexibility are most effective in eliciting the immune response (2, 3). However, accurate correlations of refined *B*-values with protein dynamical properties in solution necessitate adjustment of the apparent atomic mobilities to account for crystal lattice effects (4).

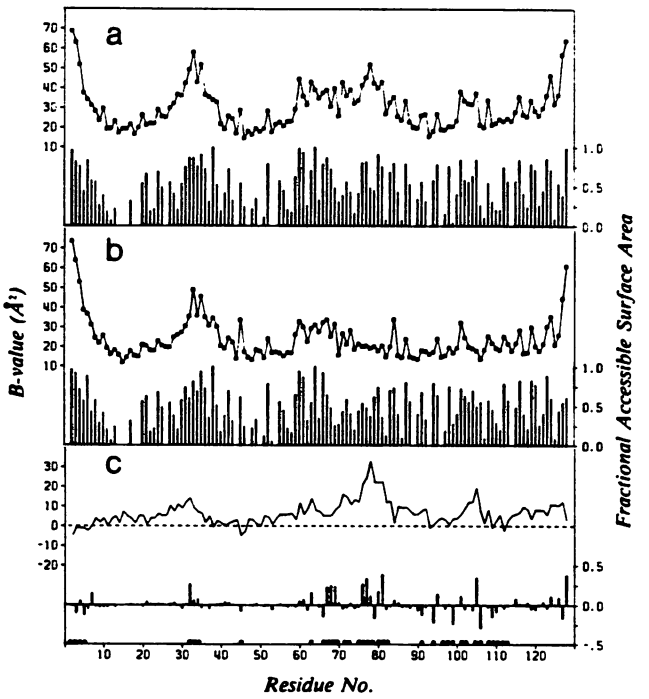

FIGURE 1 Parts *a* and *b* show plots of averaged backbone *B*-values (connected curves) and residue fractional accessible surface areas (bars) in the crystal lattice for cytochrome c' subunits as a function of sequence position. Part *c* shows differences together with positions of residues making different crystal contacts.

We have recently completed the 1.67 Å resolution crystallographic refinement of a dimeric cytochrome c' from the photosynthetic bacterium *Rhodospirillum molischianum* (5). The structure has been refined using constrained least-squares methods to a final crystallographic *R*-factor of 0.19 for 30,055 reflections with $F < 2\sigma F$. Although cytochrome c' is a symmetrical dimer with chemically identical polypeptide chains of 128 residues, the crystal contains a functional dimer in the crystallographic asymmetric unit. As a result, individual monomers are situated in different crystal environments and so provide a means for evaluating lattice effects on apparent *B*-value behavior.

Fig. 1 shows plots of the averaged backbone *B*-value behavior for the independently refined subunits of cytochrome c' and the differences between them. The *B*-value curves have a correlation coefficient of 0.82, which suggests that intrinsic dynamic properties of the monomers are largely unperturbed by crystal lattice effects. Also shown are plots of residue solvent accessible surface area in the lattice (5). These correlate with values of 0.61 and 0.69 with the respective *B*-value behavior of each monomer, and so reflect tendencies for local immobilization of surface groups at crystal contacts. This feature is also evident in Fig. 1 *c* which shows *B*-value and accessible area differences together with designations of residues involved in different crystal contacts in the two monomers. Despite these correlations, there nevertheless remains a significant overall difference in *B*-value of ~6 Å² between the monomers.

Analysis of the crystal lattice packing (Fig. 2) shows that monomers with systematically low *B*-values (cool monomers) form contiguous interactions along the twofold crystallographic screw axes parallel to the *c* axis of the orthorhombic $P2_12_12_1$ crystal cell. In contrast, the (hot) monomers with higher *B*-values make native dimer interactions with the cool monomer helical arrays and crystal contacts with adjacent cool monomer helical arrays, but do not interact with each other. The hot monomers thus appear to organize the lattice by furnishing a series of regular crystal contacts between the strongly interacting cool monomers aligned along the *c*-axis crystallographic screws. This arrangement raises the possibility of concerted disordering or dynamical motion relative to the crystal screw axis.

Fig. 3 plots the difference in average residue *B*-value in the two monomers vs. their difference in radial coordinate relative to the *c*-axis crystal screw. This treatment factors out *B*-value variations that reflect differences in local

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Volume 49 January 1986 73-76

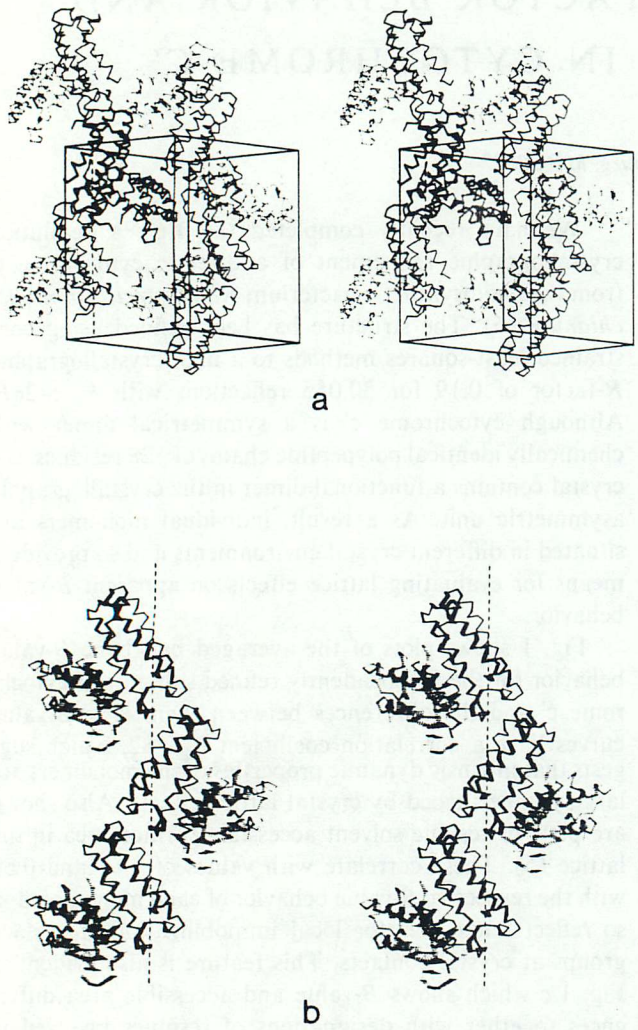

FIGURE 2 A stereoview of the crystal packing in cytochrome c'. (*a*) Cool monomers (solid) align along twofold crystal screw axes parallel to the crystal cell edge. Hot monomers (dashed) connect the cool monomer arrays together to form the crystal lattice. (*b*) Schematically represents the disordering effect of concerted motions about the *c*-axis crystal screw.

secondary structure by comparing corresponding residues in the two monomers. The correlation of ΔB vs. ΔR (0.57) clearly suggests that the higher apparent *B*-value of the hot monomers reflects a systematic static or dynamic disordering of the dimer helical arrays about the *c*-axis crystal screws. This result has interesting implications.

Recent analyses of the comparative *B*-value behavior of proteins whose structures have been determined in different crystal forms has illustrated how the formation of lattice contacts can lead to the immobilization of protein surface groups (4). In these cases the observed *B*-values will suggest apparent solution mobilities that are smaller than actually occur in solution. The apparent relation between efficacy as an antigen and surface group mobility (2, 3) thus motivates development of methods to correct for these lattice immobilization effects (4). However, the present result makes clear that systematic lattice disordering effects may be superimposed on what are predominantly localized ordering effects observed at crystal contacts. Assuming the simplest disordering pattern, where the cytochrome c' dimer helical arrays are rotationally displaced about the *c*-axis crystal screws, these systematic lattice disordering effects can produce *B*-value contributions of up to 12 Å^2 at the hot monomer periphery. This is a substantial effect that would be erronously attributed to local protein flexibility in the general case where the crystal asymmetric unit contains only a single protein molecule. Most importantly, the systematic lattice disordering effect produces both nonuniform and highly anisotropic contributions to the *B*-values that are unlikely to be easily predicted without explicit consideration of the molecules' lattice dynamic properties.

An additional point of interest concerns the question of why an intrinsically symmetrical molecule crystallizes so that equivalent subunits make different lattice interactions. We attempted to analyze the pattern of lattice connectivity by computing the correlation coefficients for *B*-value differences vs. radial distance for hot monomers making crystal contacts with the cool monomers in adjacent *c*-axis helical arrays. As shown in Fig. 2, each hot monomer makes two crystal contacts with adjacent helices of cool monomers. However, the correlation behavior for one contact is 0.16, indicative of a continuous change in *B*-value at the contact, whereas the second is −0.23, indicative of a *B*-value discontinuity at the contact. This difference in apparent lattice connectivity at the two hot monomer crystal contacts is consistent with detailed inspection of the electron density map, which shows more extensive and highly ordered solvent at the crystal contact

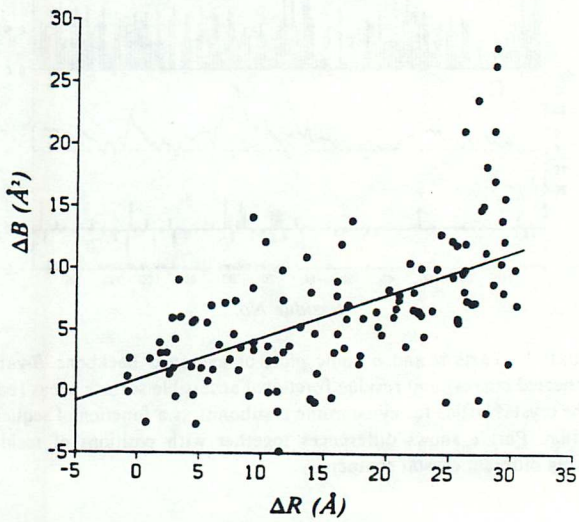

FIGURE 3 A plot of the difference in average backbone *B*-value between corresponding residues of the hot and cool cytochrome c' subunits vs. their radial distance from the crystallographic screw.

with more continuous *B*-value variations. Taken together, these results suggest that the cytochrome c′ dimers form a three-connected lattice with principle interactions occurring between cool monomers along the *c*-axis crystallographic screws, together with interactions between hot monomers and one of the adjacent cool monomer helices. It seems plausible that such an apparently flexible arrangement could reflect coupling between some of the lowest frequency bending vibrational modes of the V-shaped dimer to produce even lower frequency delocalized lattice modes. This would suggest that lattice entropy effects could be important factors in the formation of cytochrome c′, and potentially many other, protein crystals. In any case, these speculations suggest well-defined physical models for protein motion in the lattice that might be tested as improvements over the current crystallographic refinement.

This work was supported by grant GM 33325 and 30393 from the National Institutes of Health.

Received for publication 7 June 1985.

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