Tabelle 5. *Übersicht über die Wasserstoffbrücken Abstände* (Å) (*Standardabweichungen in Klammern*)

X—H···Y	X···Y	X—H	H···Y	Symmetrie-operation für Y
O(3)—H(30)···O(10)	2,749 (7)	0,79 (6)	2,09 (7)	$-x, -y, 2-z$
N(5)—H(5)···O(10)	3,068 (7)	0,92 (6)	2,17 (6)	$1+x, y, z$
N—H(1N)···O(4)	2,81 (1)	1,07 (9)	1,76 (8)	$x, 1+y, z$
N—H(2N)···O(1)	2,88 (1)	0,85 (6)	2,04 (6)	x, y, z
N—H(3N)···O(1)	2,83 (1)	1,04 (7)	1,82 (7)	$-x, 1-y, 2-z$
N—H(4N)···O(10)	2,84 (1)	0,90 (5)	1,94 (5)	$1+x, y, z$

Zwischen benachbarten Kofgruppen liegt das Ammoniumkation, dessen H-Atome als Donor von vier Wasserstoffbrücken wirken (Tabelle 5).

Mit der vorliegenden Kristallstruktur wurde erstmals die Packung in den elektronenmikroskopisch sichtbaren Plättchen racemischer Doppelschichtmembranen aufgeklärt.

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Solvent Model for Protein Crystals: on Occupancy Parameters for Discrete Solvent Sites and the Solvent Continuum

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Abstract

On the basis of test calculations, Kundrot & Richards [*Acta Cryst.* (1987), B**43**, 544–547] suggest that for models of protein crystal structures based on limited-resolution X-ray data, it is not appropriate to vary both the occupancy (Q) and thermal (B) parameters for the solvent molecules during refinement. There are compelling reasons, however, to adjust both parameters. If the data are sufficiently extensive to include a B parameter for each solvent site, then an adjustable Q parameter should also be included if the model is to represent physical reality. To fix Q at some arbitrary value as suggested, means that differences in occupancy will be absorbed in the 'thermal' parameters, leading to the errors corresponding to the electron density plots of Kundrot & Richards. Although the errors appear to be relatively small, they will be accentuated in Fourier maps of complex structures based on real data, causing error peaks, both positive and negative, that may greatly exceed the random errors. The current practice of using the scattering factor of the O atom, f_O, for the water molecule neglects the scattering from the H atoms. A modified scattering factor based on f_O would approximate the scattering from water mol-

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ecules better. Finally, the solvent continuum should be included in the model, and the low-order data included in calculating the Fourier maps from which the Q parameters are estimated.

Introduction

The aqueous solution in typical protein crystals is observed in electron density maps as a structureless continuum, except in the region near the surface of the protein. In this region discrete solvent peaks appear (Watenpaugh, Sieker, Herriott & Jensen, 1972), with densities generally decreasing with increasing distance from the protein, and merging into a structureless continuum beyond 5–6 Å from the protein surface (Watenpaugh, Margulis, Sieker & Jensen, 1978). Most peaks in the aqueous solution are assumed to represent water molecules. As such, each peak can be simply modeled as an O atom with an occupancy factor, Q, often assumed to be proportional to the peak height, and an isotropic thermal parameter, B, to account for the electron distribution in the assumed water molecule. Although the occupancy factor and the temperature factor are different functions of $\sin\theta/\lambda$, they are rather highly correlated for the limited range of X-ray diffraction data observed for most protein crystals (Watenpaugh *et al.*, 1978).

In view of this correlation, Kundrot & Richards (1987) have raised a question concerning the differences in the calculated electron densities for a model having both Q and B parameters for each water site compared to one having only B parameters, keeping Q fixed at some arbitrary value for all water sites. On the basis of test calculations for an isolated O atom with B values of 0 and 50 Å^2, occupancies in the range 1·0–0·25 and error-free data in the ranges 10–2 and 10–1 Å, Kundrot & Richards suggest that the use of a single 'thermal' parameter B_m to replace Q and B would not lead to significant peaks in a ΔF map in most physically reasonable cases.

Bhat (1989) reaches a different conclusion by comparing the differences between the scattering factor for the O atom, f_O, modified by Q and by $\exp(-B\sin^2\theta/\lambda^2)$ summed at $\sin\theta/\lambda$ values of 0·05, 0·10, 0·15, and 0·20 (d spacings of 10, 5, 3·3, and 2·5 Å). Plots for various Q values of the residual R, defined by the equation,

$$R = \sum_{d_{\min}}^{d_{\max}} |Qf_O - f_O\exp(-B\sin^2\theta/\lambda^2)| \Big/ \sum_{d_{\min}}^{d_{\max}} Qf_O$$

as a function of B in the range 0–34 Å^2 show rather serious deviations. In contrast, the plots of Kundrot & Richards' (1987) test calculations show that for 10–2 Å ideal data the differences in electron density between the one and the two parameter models appear to be relatively small, exceeding the 2σ estimate of error only for cases with unrealistically low B values and certain occupancy differences in the range 0·75–0·25.

In defence of Q

Despite the apparently small differences in most of Kundrot & Richards' plots of electron density and difference densities [see Figs. 1 and 2 in Kundrot & Richards (1987)], these differences are by no means negligible in practice. Although Q and B are rather highly correlated, the parameters represent different, but equally significant features of the water structure. Therefore, if the model is to represent physical reality, it must include Q for the water sites as well as B. In fact, if the data are regarded as sufficient in extent to warrant individual atom B parameters (preferably data extending to at least 2 Å resolution), then it is reasonable to include Q parameters as well. To fix Q in the refinement as suggested by Kundrot & Richards means that occupancy differences will be absorbed in the B_m parameters, resulting in values that are no longer simply comparable to the B values of the protein atoms.

Substituting a single parameter for Q and B introduces *model errors that are systematic*, and when these are added to experimental errors, they will complicate the interpretation of Fourier maps, particularly difference maps. In the case of large complex structures such as proteins, small errors from many sites will add and may reach troublesome magnitudes. Furthermore, such model errors can only add to the inevitable series-termination errors that result from the use of incomplete data sets, which are often encountered in practice.

An additional reason to include adjustable occupancy parameters is the need, in various crystallographic calculations, to know the number of electrons associated with each atomic site. Some calculations, such as those for Wilson plots (1942), require this information before the structure is solved, and for proteins only a very approximate estimate can be made at that point for the ordered water sites. If occupancy factors have been determined for the water model, however, the information will be available for any subsequent need that may arise.

Estimating and refining Q

The justification for variable occupancies of the ordered water in protein crystals is based directly on high resolution (1·5 and 1·2 Å) electron density and difference density maps (Watenpaugh, Sieker, Herriott & Jensen, 1973; Watenpaugh *et al.*, 1978). The most prominent water peaks in such maps are discrete and well shaped. Their peak densities will depend on both *B* and *Q*, but *Q* is dominant in the sense that its effects extend equally across the whole range of X-ray data. Accordingly, for an initial approximation we may take the *Q*'s as proportional to peak heights, calibrating them against the highest water peaks as fully occupied or against representative carbonyl O atoms.

The lower water peaks may also be well shaped, but more often they are irregular or elongated and may comprise two or more sites fused into a single peak or plateau. Adjacent sites may be separated by distances that are less, sometimes much less, than twice the van der Waals radius of the O atom (1·40 Å; Pauling, 1960), if the sum of their occupancies does not exceed unity. Thus, the structure represented by the low occupancy peaks may be complex and dominated by disorder (Smith, Hendrickson, Honzatko & Sheriff, 1986). Nevertheless, even low-density irregular or extended peaks can be modeled approximately by a judicious choice of partially occupied, multiple water sites (Jensen & Watenpaugh, 1986).

In practice, the correlation between the *B* and *Q* parameters for water precludes refining them simultaneously, even at high resolution (Watenpaugh *et al.*, 1978). It is feasible, however, to alternate cycles in which *Q*'s are fixed and *B*'s refined with cycles in which *Q*'s are refined and *B*'s fixed (Sielecki, Hendrickson, Broughton, Delbaere, Brayer & James, 1979; Hendrickson, 1985). Even proceeding in this way, refinements may still be ill-behaved, leading to excessive parameter shifts for atoms in poorly determined regions of the structure such as low-occupancy water sites or atoms with high *B* values. If this behavior is encountered, it will be necessary to reduce the shifts by a damping factor or to restrain the shifts (Hendrickson, 1985).

Improving the solvent model

Present practice is to use the scattering factor for the O atom, $f_{\rm O}$, to model the ordered water molecules, but to do so accounts for only eight of the ten electrons in the molecule. While the scattering of H atoms is small, the two H atoms in each water molecule account for 18–25% of the ordered O-atom scattering to 2 Å resolution. The scattering of any inferred H-atom positions can be calculated directly, *e.g.*, the position of the H atom in a hydrogen bond, but the rest of the H atoms can be allowed for only approximately by modifying the scattering factors, $f_{\rm O}$, used for the water O atoms. We may take the difference between $f_{\rm O^-}$ and $f_{\rm O}$ as an approximation of the H-atom scattering. Accordingly,

$$f_{\rm HO} = (f_{\rm O^-} - f_{\rm O}) + f_{\rm O} = f_{\rm O^-}$$

if only a single H atom associated with a water site is to be allowed for, and

$$f_{\rm H_2O} = 2(f_{\rm O^-} - f_{\rm O}) + f_{\rm O} = 2f_{\rm O^-} - f_{\rm O}$$

if both H atoms are to be allowed for (*International Tables for X-ray Crystallography*, 1960).

As noted at the outset, most of the aqueous solution present in typical protein crystals is observed as a structureless continuum beyond 5–6 Å from the surface of the protein molecules. Because this continuum lacks structural detail and occupies much of the inter-protein space throughout the unit cell, it does not contribute appreciably to the higher-resolution data, *i.e.*, reflections with *d* less than ~10 Å (Watenpaugh *et al.*, 1973). The usual practice therefore, has been to delete the low-resolution reflections from the refinement, effectively eliminating the continuum in the derived maps. It would be preferable, however, to include it (Phillips, 1980), not only to complete the solvent model but also, because the continuum extends into the ordered solvent region (see Fig. 4 and discussion in Watenpaugh *et al.*, 1978), to account for its effect on the occupancies of the discrete water sites. The computer program *XPLOR* (Brünger, 1988) has an option to include the continuum in the model, and as programed, it addresses the problem of piecing the continuum into the ordered solvent. The need now is to accumulate experience in completing the solvent models of typical proteins in order to test the proposed model improvements and the programs used in the calculations.

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Conformational Polymorphism of Dimethyl 3,6-Dichloro-2,5-dihydroxyterephthalate. II. Structural, Thermodynamic, Kinetic and Mechanistic Aspects of Phase Transformations among the Three Crystal Forms

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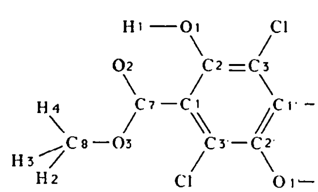
Abstract

The molecules in three polymorphic forms of the title compound – Y (yellow), LY (light yellow) and W (white) – are known to be conformational isomers with the ester groups approximately in the plane of the benzene ring (Y), nearly perpendicular to it (W), and in an intermediate orientation (LY) [Byrn, Curtin & Paul (1972). *J. Am. Chem. Soc.* **94**, 890–898; Yang, Richardson & Dunitz (1989). *Acta Cryst.* **B45**, 312–323, Part I]. Phase transformations among these crystal forms have been studied by simultaneous differential scanning calorimetry measurements and optical microscopy observations recorded with a video-camera. At room temperature and below, the order of thermodynamic stability is Y > LY > W; above 360 K, it is W > Y > LY. On heating, crystals of LY transform to W but sometimes first to Y; crystals of Y transform to W, often through the transient intermediacy of LY; crystals of W melt at 453 K to yield a yellow liquid that freezes (on supercooling) to give crystals of all three forms but mostly Y. The solid–solid transformations occur over a wide temperature range. They seem to be controlled by the presence of certain types of defect and to proceed by a nucleation and growth type of mechanism. These results are discussed in terms of the known crystal structures at several temperatures with particular reference to the atomic displacement parameters and their temperature dependence.

Introduction

The work to be described here is the second part of a contribution from our laboratory dealing with the remarkable conformational polymorphism of dimethyl 3,6-dichloro-2,5-dihydroxyterephthalate [(I), see structural formula for atom labelling]. This compound exists in three differently coloured crystal forms, designated as Y-(I) (yellow), W-(I) (white) and LY-(I) (light yellow). The study of the first two forms goes back to Hantzsch (1915), who observed that Y-(I) transforms to W-(I) on warming to about 410 K and suggested that the two forms are tautomers. Much later, from X-ray diffraction studies (Byrn, Curtin & Paul, 1972; BCP), it became clear that the molecules in these two forms are actually conformational isomers of (I). In Y-(I) the molecules are almost coplanar, with intramolecular hydrogen bonds to carbonyl O atoms; in W-(I) the methoxycarbonyl groups are rotated nearly perpendicular to the plane of the other atoms, with hydrogen bonds to carbonyl O atoms of different molecules. BCP also made detailed observations (mainly by optical microscopy and differential scanning calorimetry) on the solid-state transformational of Y-(I) to W-(I).



(I)

In Part I of this work (Yang, Richardson & Dunitz, 1989) we reported the existence of the third form [LY-(I)], which had apparently been overlooked by the earlier investigators; in this form the ester groups are neither nearly coplanar with the benzene ring, as in Y-(I) (torsion angle about 5°), nor nearly perpendicular to it, as in W-(I) (torsion angles

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