effect on the starting point of crystallization, which occurred 40 times faster. In addition, the mutation may have increased the crystal growth rate. This point was difficult to establish, however, because the growing surfaces of the numerous smaller mutant crystals were larger than those of the single wild-type crystals, which may well explain the observed rate difference of a factor of about three in this series of experiments (Figs. 3*a*,*b*).

The gas-diffusion progress in the crystallization drops (Fig. 3*c*) indicated that the mutant enzyme succeeds in nuclei formation shortly after the PEG-10000 concentration crosses the solubility and crystal stability limits of about 5.3%, requiring almost no supersaturation. In contrast, the wild-type enzyme requires a high and enduring supersaturation for nucleation. Wild-type nuclei form 60 h after the drop crosses the solubility and crystal stability limit, which is 25 h after the drop has equilibrated at a high level of supersaturation (Fig. 3*c*). Most surprising is the uniform timing in all eight successful crystallization trials of the series (Figs. 3*a*,*b*).

In conclusion, we find that the addition of contact *C'* accelerated the crystal nucleation process, shortening the crystallization time by a factor of 40. The engineered contact may have increased the crystal growth rate. It did not affect protein solubility, crystal stability and crystalline order. The experiments demonstrate that contact engineering can have a strong influence on crystallization, nurturing the hope that it can be used for improving the crystalline order in the future.

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Crystallization and preliminary investigation of xylose isomerase from *Bacillus coagulans*.

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Abstract

Xylose isomerase from *Bacillus coagulans* has been crystallized in two different crystal forms. One crystal form is in space group $P2_12_12$, cell dimensions $a = 462$, $b = 165$, $c = 82$ Å. The other is in space group $I422$, cell dimensions $a = b = 113$, $c = 153$ Å.

Introduction

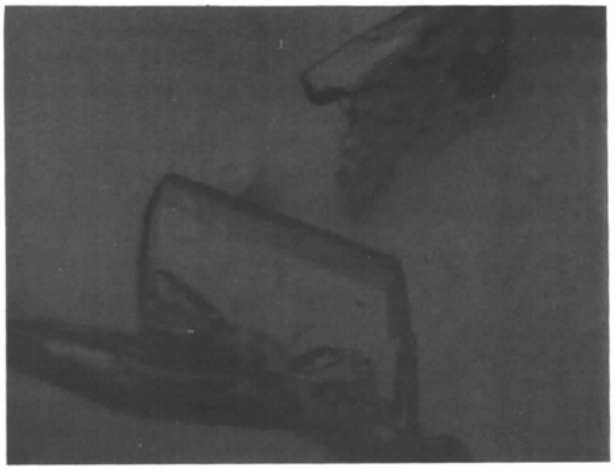
Xylose isomerase, XI (E.C. 5.3.1.5) catalyzes *in vivo* the isomerization of D-xylose to D-xylolose (Hochster & Watson, 1953). This activity is known to involve two divalent ions (Mg^{2+}, Mn^{2+}, Co^{2+}) per molecule (Kasumi, Hayashi & Tsumura, 1982).

This paper describes the crystallization and preliminary investigation of xylose isomerase from *Bacillus coagulans* (BXI).

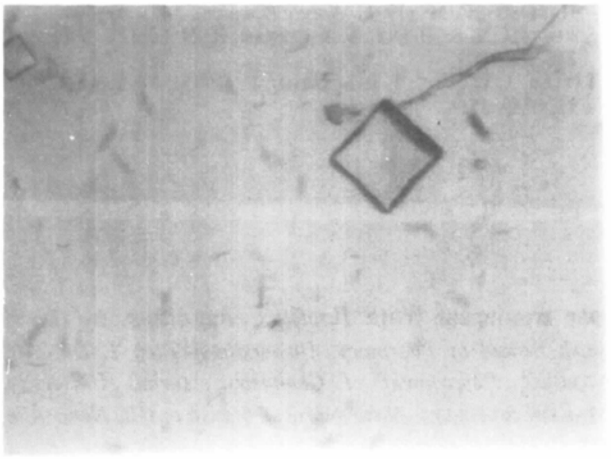
The crystallographically determined structure of XI from a number of other species is known: XI, *Actinoplanes missouriensis*, 2.8 Å resolution (Rey *et al.*, 1988); XI, *Streptomyces olivochromogenes*, 3.0 Å resolution (Farber, Glasfeld, Tiraby, Ringe & Petsko, 1989); XI, *Arthrobacter*, 2.5 Å resolution (Henrick, Collyer, Blow, 1989); XI, *Streptomyces rubiginosis*, 1.9 Å resolution (Carrell *et al.*, 1989; Collyer, Henrick & Blow, 1990); XI, *Streptomyces albus*, 1.65 Å resolution (Dauter, Terry, Witzel & Wilson, 1990); XI, *Streptomyces rubiginosus*, 1.6 Å resolution (Whitlow *et al.*, 1991).

The sequence of BXI differs remarkably from the other known species, as does the optimum activity and stability. While the activity of *Streptomyces* species increases with increasing pH without reaching optimum activity, BXI has an activity optimum at pH 8.2. A similar phenomenon is seen for activity as a function of temperature between 313 and 263 K. No temperature optimum is found for *Streptomyces* species while BXI has its optimum at 358 K (personal communication, Novo Nordisk A/S). An alignment of the sequence of BXI to the sequences of the other species using the program *FASTP* (Lipman & Pearson, 1985) of the package *PIR* (*Protein Identification Resource*; National Biomedical Research Foundation, 1991) only gives about 25% sequence identity.

BXI contains 442 residues per monomer giving a calculated molecular weight of 50 180 Da.



(*a*)



(*b*)

Fig. 1. (*a*) Crystal form *A*, the longest dimension of the crystal is 1.0 mm. (*b*) Crystal form *B*, the longest dimension of the crystal is 0.2 mm.

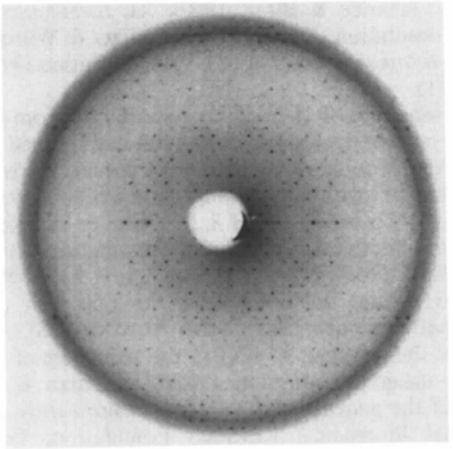


Fig. 2. Precession photograph of crystal form *B*, 0*kl* zone (precession angle: 10°, crystal-to-film distance: 75 mm, exposure time: 40 h, crystal size: 0.2 × 0.2 × 0.2 mm).

Experimental

XI from *Bacillus coagulans* was isolated and purified by Novo Nordisk A/S, Copenhagen, Denmark, to a purity of more than 99% shown by sodium dodecyl sulfate (SDS) gel electrophoresis and isoelectric focusing. Crystals were grown by the sitting-drop vapor-diffusion method at room temperature. Two crystal forms were obtained (Fig. 1).

Crystal form *A* was grown from 30 mg ml^{-1} XI solution in 50 m*M* $MgSO_4$, 50 m*M* TRIS buffer pH 8.5 and 6% PEG 4000 over 50 m*M* $MgSO_4$, 50 m*M* TRIS buffer pH 8.5 and 15% PEG 4000.

Crystal form *B* was grown from 30 mg ml^{-1} XI solution in 50 m*M* $MgSO_4$, 50 m*M* TRIS buffer pH 8.5, 1% dioxane and 0.4 *M* $(NH_4)_2SO_4$ over 50 m*M* $MgSO_4$, 50 m*M* TRIS buffer pH 8.5, 1% dioxane and 1.6 *M* $(NH_4)_2SO_4$.

For preliminary characterization crystals were mounted in glass capillaries with a small amount of mother liquor. Photographs were produced using a rotating Cu anode as X-ray source (λ = 1.5412 Å, 36 kV, 40 mA).

Results and discussion

The crystals of form *A* grow very rapidly, reaching a maximum size of 2.0 × 1.0 × 0.3 mm in a week. This fast growth often resulted in a crystal with hole at one end. The crystals are fragile and physically very unstable when handled.

From precession photographs the space group of this crystal form was determined to be orthorhombic $P2_12_12$ with unit-cell dimensions a = 462, b = 165, c = 82 Å. The very long a axis makes it difficult to resolve the reciprocal diffraction pattern. Furthermore, the crystal only diffracts to 4 Å resolution and is sensitive to X-ray exposure. Crystal form *A* is, therefore, unsuitable for X-ray analysis.

Crystals of form *B* have been much more difficult to obtain; however, they are much more suitable for X-ray analysis. After 2 weeks of growth the maximum crystal size is 0.2 × 0.2 × 0.2 mm.

The space group of crystal form *B* has been determined to be the tetragonal *I*422 with unit-cell dimensions $a = b$ = 113, c = 153 Å. This gives a volume $V = 1.95 \times 10^6$ Å^3. If two molecules per asymmetric unit are assumed, each molecule having a molecular weight of 50 180 Da, then the volume-to-mass ratio $V_m = 2.43$ Å^3 Da^{-1}. This number is close to the average for proteins (Matthews, 1968). The crystal diffracts to at least 2.8 Å resolution on a rotating Cu-anode generator (Fig. 2).

Efforts have to be made to grow larger crystals of crystal form *B* in order to obtain a data set of good resolution.

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Crystallization and preliminary X-ray diffraction analysis of tetra-heme cytochrome c_3 from sulfate- and nitrate-reducing *Desulfovibrio desulfuricans* ATCC 27774.

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Abstract

Crystals of the tetra-heme cytochrome c_3 (M_r = 13 kDa, 107 residues, four heme groups) from sulfate- and nitrate-reducing *Desulfovibrio desulfuricans* ATCC 27774 have been obtained and crystallographically characterized. They belong to space group $P6_122$ with cell dimensions $a = b = 61.84\,(4)$ and $c = 109.7\,(2)$ Å, and $Z = 12$. Intensity data were initially collected on a FAST system with a rotating-anode X-ray source leading to a total of 22 592 observations, from which only 4930 were unique, in the resolution range 20.0–2.4 Å with an $R_{merge}(I)$ of 7.0%. Higher resolution data were measured on a FAST system at station 9.6 of the SRS (Daresbury, England), leading to 19 328 intensities, of which 11 179 were unique, in the resolution range 20.0–1.75 Å and an $R_{merge}(I)$ of 5.5%. Cross-rotation and translation functions were performed with *ALMN* and *TFSGEN* programs from the *CCP*4 suite. The packing of the molecules in the unit cell was checked with *TOM/FRODO*. Rigid-body refinement of the model and subsequent refinement using molecular dynamics were performed with *X-PLOR*, leading to a current R factor of 25.9%, for data up to 2.3 Å.

Introduction

Desulfovibrio desulfuricans ATCC 27774 is a bacterial strain belonging to the sulfate-reducing *Desulfovibrionaceae* family, with the unique capability of growing either in sulfate or in nitrate. These anions can be used as terminal acceptors of the electron-transport chain, while organic compounds or hydrogen are the initial electron donors for this obligate anaerobic family of bacteria (Postgate & Campbell, 1966; Peck, 1984). The metabolism of hydrogen is regulated by a reversible hydrogenase, which appears to be reduced or oxidized by the low-potential cytochrome c_3 (LeGall, Moura, Peck & Xavier, 1982; LeGall & Fauque, 1988). This is a *c*-type cytochrome which belongs to class III (Ambler, 1982; Moore & Pettigrew, 1990), which includes tri-heme, tetra-heme, octa-heme and also hexadeca-heme cytochromes. These are characterized by bishistidinyl iron coordination with 20–40 residues per heme. The characteristically very low redox potentials of these cytochromes, in the range −110 to −400 mV, differentiates them from class I (0 to +470 mV) or class II (−10 to +200 mV) cytochromes (Ambler, 1991; Moura, Costa, Liu, Moura & LeGall, 1991).

Three multi-heme *c*-type cytochromes were isolated from the soluble fraction of *D. desulfuricans* ATCC 27774 grown under nitrate- or sulfate-reducing conditions: *split-soret* cytochrome *c*, dodeca-heme cytochrome *c* and the tetra-heme cytochrome c_3. However, only the latter is similar to c_3 cytochromes found in other sulfate-reducing bacteria (Liu *et al.*, 1988).

The tetra-heme c_3 cytochromes have been intensively studied as they constitute very attractive models for intra- or inter-electron transfer molecules. Sequence homology for these four heme cytochromes (Meyer & Kamen, 1982, and references therein) can be as low as 30%, with conserved residues accounting mainly for the heme attachment. There are always 16 conserved residues of the 110–120 amino acids of the peptide chain, responsible for the covalent linkage to the hemes plus the histidinyl groups coordinating the four Fe atoms. Although the heme-heme arrangement found in the structures determined already seems to remain highly conserved in its architectural geometry, the wide range of redox potentials observed must be related to differences to be found in the three-dimensional molecular structures of the various c_3 cytochromes. Both X-ray diffraction analysis (Haser, Pierrot *et al.*, 1979; Higuchi, Kusunoki, Matsuura, Yasuoka & Kakudo, 1984; Kissinger, 1989; Morimoto, Tani, Okumura, Higuchi & Yasuoka, 1991; Matias, Frazão, Morais, Coll & Carrondo, 1993) and two-dimensional NMR (Coutinho, Turner, LeGall & Xavier, 1992; Turner, Salgueiro, LeGall & Xavier, 1992) techniques have been used to study the three-dimensional structures of these molecules. These structures were essential for the interpretation of abundant physicochemical data from Mössbauer spectroscopy, circular dichroism (CD), electron paramagnetic resonance (EPR), nuclear magnetic resonance (NMR) and from electrochemical methods (Moura *et al.*,

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