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Crystallization and Preliminary X-Ray Diffraction Studies of the Human Major Histocompatibility Antigen HLA-B27

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ABSTRACT **The class I major histocompatibility (MHC) antigen HLA-B27 was purified by immunoaffinity chromatography from the homozygous human B lymphoblastoid cell line LG-2. Detergent-soluble HLA-B27 was cleaved with the protease papain to remove the hydrophobic transmembrane region and the cytoplasmic tail. Crystals of the resulting water-soluble extracellular fragments were obtained in hanging drops by the vapor-diffusion method. The crystals are triclinic, space group *P*1, with unit cell dimensions $a=45.9$ Å, $b=71.0$ Å, $c=83.7$ Å, $\alpha=79.4°$, $\beta=88.5°$, $\gamma=89.9°$, and diffract beyond 2.5 Å resolution.**

Key words: crystallization, X-ray diffraction, immunoaffinity chromatography

INTRODUCTION

HLA-B27 has been strongly associated with susceptibility to several seronegative arthritic diseases, such as ankylosing spondylitis and Reiter's syndrome[1–3] and has been shown to be associated with acute anterior uveitis.[4,5] Although these disease associations have been known since 1973, the mechanism by which HLA-B27 contributes to these diseases is unclear. It is even possible that the association is with another gene that is in linkage disequilibrium with HLA-B27, although no clear evidence for another gene related to these diseases has been obtained.[5–8] Since none of these diseases appears to be associated with a specific subtype of HLA-B27,[3,8] attention has focused on sequences conserved among them. Taurog et al. have suggested that a free cysteine at position 67, predicted to point into the peptide binding cleft by homology with HLA-A2[9,31,32] (Saper, M.A. et al., 1991) and HLA-Aw68,[10] may be covalently modified in ankylosing spondylitis.[11,12] The possibility of designing a covalent blocking agent, perhaps from an antigenic peptide, incorporating a positive charge to complement a pocket in the binding cleft and a thiol to react with cysteine 67 has been suggested.[10]

Several groups have suggested that molecular mimicry may play a role in HLA-B27-associated autoimmune diseases. Peptide sequences corresponding to amino acid residues 72–77 in HLA-B27 have been found in several strains of pathogenic bacteria previously implicated in these diseases.[13–15] In one case,[13] autoantibodies that bind to such a peptide were found in the sera of patients with ankylosing spondylitis and Reiter's syndrome. Thus at present it is unclear whether or not intact HLA-B27 or a peptide fragment from HLA-B27 is involved in the HLA-B27-linked diseases.

Although sequence similarities argue for a common structure among class I molecules,[16] no HLA-B or C allelic products have previously been crystallized. Here we report the purification and crystallization of the extracellular fragment of HLA-B27 from LG-2 cells and the preliminary characterization by X-ray diffraction of crystals of HLA-B27 that diffract to beyond 2.5 Å resolution. HLA-B*2705, the HLA-B27 subtype expressed in LG-2 cells and studied here (see Ref. 17 for review of subtypes), differs from HLA-A2.1 by 38 amino acid residues in the extracellular domains[18,19] compared to only 13 amino acid differences between the extracellular domains of HLA-A2.1 and HLA-Aw68,[20] the two three-dimensional structures already known. Of these 38, however, homology with HLA-A2 and HLA-Aw68 suggests only 11 differences in the peptide binding site, the same number as between HLA-A2 and HLA-Aw68.

MATERIALS AND METHODS

HLA-B27 was purified by immunoaffinity chromatography from the Epstein–Barr virus-transformed human B lymphocyte line LG-2.[21] Briefly, LG-2 cells (approximately 200 g per preparation) were hypotonically lysed, and a crude membrane fraction was prepared by differential centrifugation. The membranes were solubilized with Nonidet P-40, and the soluble fraction was passed through a series of immunoaffinity columns in order to isolate the class I antigens of interest.[22] HLA-B27 was isolated

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using the monoclonal antibody (MAb) B27M1 (specific for HLA-B27 and crossreactive with HLA-B7; Ref. 23) or the MAb W6/32 (class I-specific, Ref. 24) following the removal of HLA-A2 from the LG-2 detergent extract using a column made with the MAb PA2.1.[25] HLA-B27 was eluted from the immunoaffinity columns with 50 mM glycine/0.1% deoxycholate, pH 11.5, immediately neutralized with 2 M glycine, pH 2, and immediately dialyzed against 10 mM TrisOH/0.1% deoxycholate, pH 8. A water-soluble fragment was prepared by a controlled digestion with papain, which served to remove the transmembrane region and cytoplasmic tail of the α-chain of HLA-B27, as follows. Immunoaffinity-purified HLA-B27 (16 mg in 20 ml of 10 mM TrisOH/0.1% deoxycholate, pH 8) was digested with 0.3 mg papain (Sigma, catalog #P-3125) for 10 min at 37°C and then further isolated by anion-exchange chromatography on Whatman DE52, gel filtration on Sephacryl S-200, and a second DE52 column, as for the purification of class I molecules released from the cell membrane with papain.[17] Electrophoresis was performed according to the method of Laemmli.[26] Protein concentration was determined by BCA assay (Pierce).

Crystals were grown by the vapor-diffusion method in 2–8 μl hanging drops on siliconized microscope cover slips inverted over precipitant solutions in 24-well tissue culture plates. Initial protein concentrations were between 4 and 10 mg/ml. Crystallization conditions tested included the use of the "factorial solution" approach suggested by Carter et al.[27] Data-quality crystals were obtained from well solutions containing 24–30% polyethylene glycol (PEG) 3350, 100–400 mM sodium acetate, 100 mM Tris, pH 8.7. Single crystals grew to dimensions of approximately 400 × 200 × 200 μm.

Crystals were harvested into well solution or into a mock well solution supplemented with 25 mM 2-(*N*-morpholino)ethanesulfonate (MES) buffer, pH 6.5, and 0.1% sodium azide. PEG concentrations were monitored by refractive index measurements. Crystals were mounted in 1.0- and 1.5-mm-diameter unwashed glass capillaries. The space group was determined at room temperature on film using a Supper precession camera. Additional data have been collected on a Xentronics multiwire area detector and processed using the BUDDHA[28] and CCP4 (P. Evans, personal communication) programs.

RESULTS AND DISCUSSION

A water-soluble fragment of HLA-B27 was prepared by papain digestion of immunoaffinity-purified HLA-B27 (Fig. 1, lane 2). Papain-solubilized HLA-B27 was further purified by the ion-exchange chromatography method used for separation of HLA-A2 from HLA-B7 or -B12 and HLA-Aw68 from -B40.[17] The HLA-B27 did not appear to be contaminated with HLA-A2, which is also expressed on LG-2 cells (Fig. 1, lane 1).

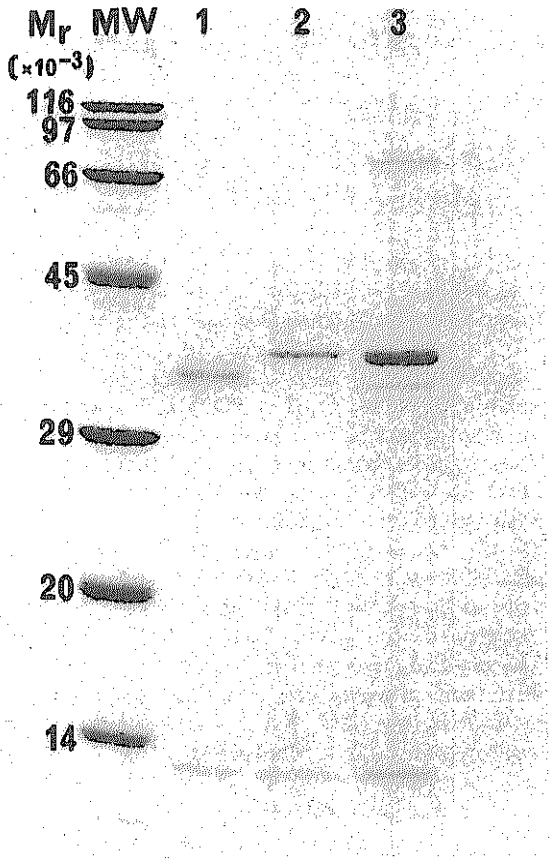

Fig. 1. SDS-PAGE of immunoaffinity-purified, papain-digested HLA-A2 and HLA-B27, and crystallized HLA-B27. Samples were boiled in reducing Laemmli sample buffer, run on a 13% acrylamide gel, and stained with Coomassie blue. **Lanes:** MW, molecular weight standards; 1, papain-solubilized HLA-A2; 2, papain-solubilized HLA-B27; 3, HLA-B27 in washed crystals.

Crystals of papain-solubilized HLA-B27 were obtained under a number of different conditions, including those that are used to crystallize HLA-A2 and HLA-Aw68.[29] The crystals that diffracted to the highest resolution were grown from novel conditions with initial well solutions containing 24–30% PEG 3350/100–400 mM sodium acetate/100 mM Tris, pH 8.7 (Fig. 2). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of washed crystals (Fig. 1, lane 3) showed that they contained both papain-cleaved heavy chain and β_2-microglobulin. Preparations of papain-solubilized HLA-B27 sometimes showed the presence of what appeared to be a product of further proteolytic digestion, as can be seen in HLA-B27 from washed crystals (Fig. 1, lane 3).

The space group was determined to be *P*1 (Fig. 3), with unit cell parameters $a = 45.9$ Å, $b = 71.0$ Å, $c = 83.7$ Å, $\alpha = 79.4°$, $\beta = 88.5°$, $\gamma = 89.9°$. The triclinic crystals are pseudomonoclinic. The β and γ angles are nearly 90° and there is an approximate 2-fold

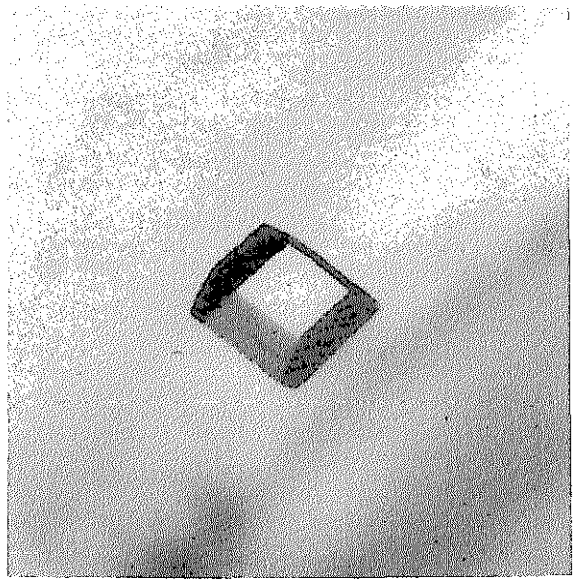

Fig. 2. Crystal of HLA-B27 grown from 26% PEG 3350/250 mM sodium acetate/100 mM Tris, pH 8.7. Crystal dimensions were approximately 300 × 200 × 200 μm.

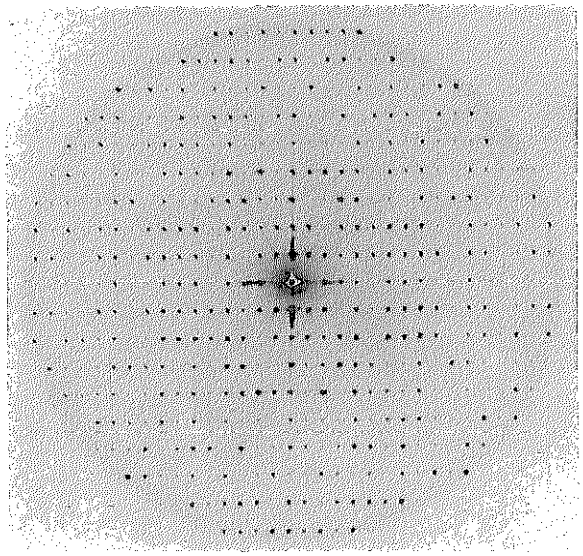

Fig. 3. A 9° precession photograph of a triclinic crystal of HLA-B27. This 010 zone, zero level photograph shows approximate mm symmetry (see text).

symmetry axis along the a axis (see approximate mm symmetry in Fig. 3). Occasional true monoclinic crystals (space group *P*2, poorly diffracting) have been grown with the same unit cell lengths and a true 2-fold symmetry axis along a, strongly suggesting that the triclinic crystals contain two HLA-B27 molecules per unit cell, related by a noncrystallographic 2-fold symmetry axis near the a axis.

The triclinic HLA-B27 crystals diffract beyond 2.5 Å resolution and are stable for many hours in the X-ray beam. It should be possible to determine a high-resolution structure by molecular replacement[30] using HLA-A2 as a starting model.

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