Production and crystallization of MHC class I B allele single peptide complexes

Scott W. Reid[a,**], Kathrine J. Smith[b,**], Bent K. Jakobsen[b], Chris A. O'Callaghan[b], Hugh Reyburn[b], Karl Harlos[a,c], David I. Stuart[a,c], Andrew J. McMichael[b], John I. Bell[b], E. Yvonne Jones[a,c,*]

[a] *Laboratory of Molecular Biophysics, The Rex Richards Building, South Parks Road, Oxford OX1 3QU, UK*
[b] *Molecular Immunology Group, Nuffield Department of Clinical Medicine, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DU, UK*
[c] *Oxford Centre for Molecular Sciences, New Chemistry Building, South Parks Road, Oxford OX1 3QT, UK*

Received 10 January 1996; revised version received 19 February 1996

Abstract Major histocompatibility complex class I B alleles, HLA B8, B53 and B3501 have been cloned, expressed, refolded and crystallized in specific complexes with a number of different 8-mer and 9-mer peptides. For some of these crystallization was initiated by cross-seeding between different B allele complexes. All crystallize in the space group $P2_12_12_1$, with similar unit cell dimensions of approximately 52 Å × 81 Å × 112 Å, contain one complex per asymmetric unit and diffract to approximately 2.0 Å resolution.

Key words: Protein expression; MHC class I; HLA B allele; X-ray crystallography; Peptide complex

1. Introduction

MHC class I and class II molecules are functionally and structurally distinct proteins encoded within the major histocompatibility complex (MHC) region of chromosome 6 [1,2]. Recognition by the T-cell receptor (TCR) of MHC molecules complexed with peptide antigen is a critical event in the initiation of an immune response. Much of our understanding of peptide binding to class I molecules has come from a complementary combination of analysis of X-ray crystallographic structures and identification of naturally bound peptides [3]. The first class I structure, HLA A2 [4], together with subsequent structural analysis of other human class I alleles [5–11] and murine alleles [12–15] complexed with single peptides, revealed general principles for peptide binding to class I molecules. These structures show that peptides are held in the class I binding groove at the N-and C-termini by a conserved network of hydrogen bonds and by the binding of conserved residues (anchor residues) in pockets lined by polymorphic MHC residues (review [16]).

There are three major loci expressing MHC class I molecules in man: the A, B and C alleles. The majority of extant structural studies have been of A alleles. To broaden our understanding of MHC class I peptide interactions we have embarked on an analysis of B allele single peptide complexes. We have crystallized three different B alleles, HLA B8, B53 and B3501 with various specific peptides; a total of nine different peptide complexes. HLA B8 is unusual among human class I molecules in having sequence motifs indicating anchor residues at positions 3 and 5 of the peptide (in contrast to anchor residues at positions 2 and 9) [17,18]. We have crystallized B8 with an HIV-1 virus p17-3 Gag 9-mer epitope (GGKKKYKLK), 8-mer epitope (GGKKKYKL), and four naturally occurring 9-mer variants (GGKKKYRLK; GGKKKYQLK; GGKKRYKLK; GGRKKYKL). The first peptide (as 8-mer or 9-mer) causes a cytotoxic T lymphocyte (CTL) response whereas the presence of the variant peptides presented by B8 antagonises this same CTL response [19]. HLA B53, a common African allele, which has been shown to be associated with resistance to severe malaria in the Gambia [20], has been crystallized with two 9-mer peptide epitopes, ls6 (KPIVQYDNF) from the malaria parasite *P. falciparum* [21] and TPYDINQML from the Gag protein of HIV-2 [22]. The related allele HLA B3501 has also been crystallized complexed with the 8-mer nef peptide (VPLRPMTY) [23] which comes from a region conserved in the HIV-1 and HIV-2 genomes.

2. Materials and methods

2.1. Expression

Full length B53, B35 and B8 cDNA clones were prepared from Hmy-B53 cell line (a gift from M. Takiguchi), B3501 T2 transfectants (a gift from M. Takiguchi) and an EBV transformed homozygous B8 B cell line, respectively. The regions coding for amino acids 1–276 of human HLA B8 and B3501 heavy chains were amplified, by polymerase chain reaction, using the oligonucleotide primers 5′-GCGCGGATCCCACTCCATGAGGTATTTC-3′ and 5′-CCGCAAGCTTTTATCATGGCTCCCATCTCAGGGT-3′, respectively. HLA B53 heavy chain was amplified with the primers 5′-GCGCGGATCCCACTCCATGAGGTATTTC-3′ and 5′-CCGCAAGCTTTTATCA *ATGGTGGTGATGATGGTG*TGGCTCCCATCTCAGGGT-3′ containing sequence coding for an additional C-terminal tag of six histidines (italics). The resulting fragments were digested with the restriction enzymes *Bam*HI and *Hin*dIII and ligated into the T7 expression plasmid pGMT7 (a pET derivative, [24]). The expression plasmid was transformed into the *E. coli* strain BL21(DE3)pLysS (Novagen) and grown at 37°C in Lurian Bertani medium containing 100 mg/ml of ampicillin (Sigma) and 20 mg/ml of chloromphenicol (Sigma). Protein expression (as insoluble aggregates) was induced at mid-log phase (A_{600} = 0.6–0.9) with 0.4 mM isopropyl β-D-thiogalactopyranoside (IPTG) (Novabiochem) and after 4 h the cells were harvested. The light chain, $ß_2$-microglobulin (ß2m), was provided in a pHN1 plasmid in the *E. coli* strain XA90 (a gift from D. Garboczi) and was similarly overexpressed as insoluble aggregates after induction with 1 mM IPTG. Both heavy and light chain inclusion bodies were isolated from cell pellets by sonication, washed repeatedly in detergent and the resulting protein solubilised in 8 M urea, 50 mM MES pH 6.5, 0.1 mM EDTA and 0.1 mM DTT (buffer A) according

*Corresponding author. Fax: (44) (1865) 275182.

**S.W.R. and K.J.S. contributed equally to this work.

SSDI S0014-5793(96)00226-8

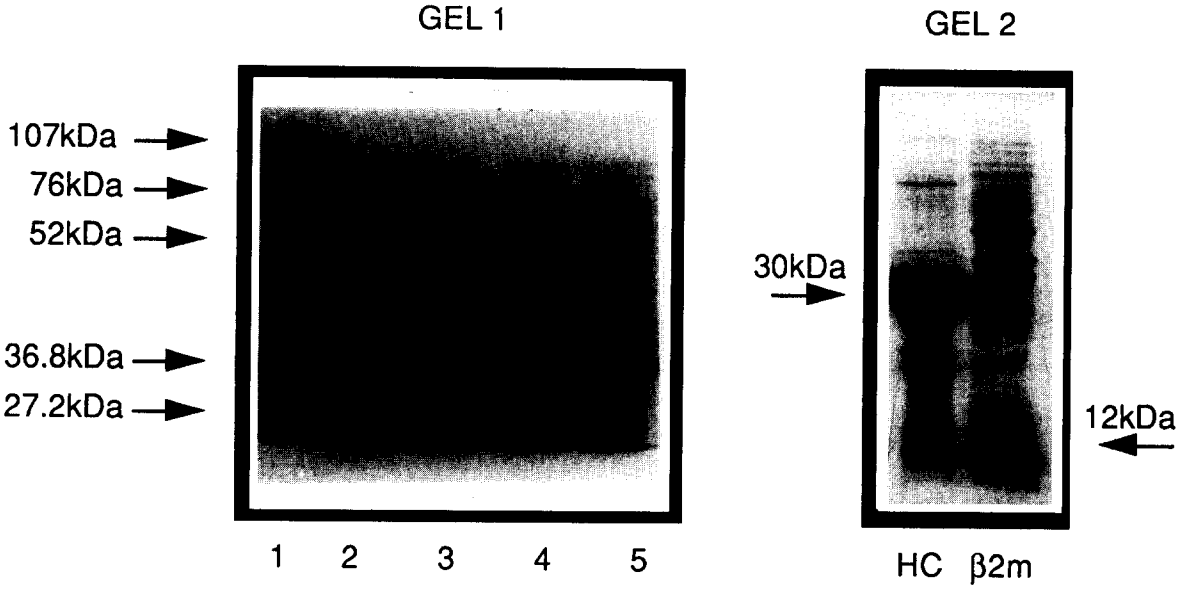

Fig. 1. 15% SDS-polyacrylamide gels showing the expression of HLA B allele heavy chain (gel 1) as a time course after IPTG induction and (gel 2) the relative purities of heavy chain (HC) and ß2m inclusion bodies after the cells have been sonicated and washed with detergent. Lane 1 shows Biorad low range standards with molecular weights documented on the left of the corresponding bands of the gel. Lane 2 shows the total cell protein (1 ml aliquots) before induction with IPTG. Lanes 3, 4 and 5 show the total cell protein at time intervals of 2, 4 and 16 h after induction, respectively.

to the method described in [25]. In addition, the B53 heavy chain was further purified under denaturing conditions by binding onto a Ni^{2+}-NTA agarose column (Qiagen) and eluting with a pH gradient. B allele heavy chain was identified by Western blot analysis with the monoclonal antibody HC10 [26] and verified by N-terminal sequencing (T. Willis, Oxford Centre for Molecular Sciences, OCMS). Purity was assessed by running samples on 15% SDS-polyacrylamide gel electrophoresis in the presence of DTT and concentrations estimated using a BCA protein assay kit (Biorad). Peptides were synthesised on an applied biosystems 430A Automated Peptide Synthesiser, purified by HPLC reverse-phase chromatography, checked for correct molecular weight by electrospray mass spectrometry and stored as a lyophilised powder (S. Shah and M. Pitkeathley, OCMS).

2.2. Refolding and purification

Refolding was performed by dilution of denaturing conditions at 4°C [25]. Firstly, 25 mg of ß2m in buffer A were added to 1 l of 400 mM L-arginine, 50 mM Tris pH 8.0, 0.1 mM EDTA, 0.1 mM PMSF and 0.1 mM sodium azide (refolding buffer). Secondly, 10 mg of peptide was dissolved into 30 mg of heavy chain, solubilised in buffer A, and diluted to 30 ml prior to being added into the same 1 l volume of refolding buffer. The molar ratio of heavy chain:ß2m:peptide used was 1:2:10, respectively. The yield of HLA complex could be increased by starting with 3 mg of refolded ß2m and 'pulsing' the refolding mixture with 4×5 mg additions of heavy chain at 8 h intervals. After 48 h the refolding mixture was concentrated to approx. 100 ml on a stir cell (Amicon) and further concentrated in a 10 kDa cutoff centriprep (Amicon). Refolded HLA complex was purified from the refolding mixture and buffer exchanged into 20 mM Tris pH 8.0 on an FPLC superdex 75 prep grade gel filtration column (Pharmacia). Analysis for correct folding of HLA B53 and HLA B3501 was carried out by capture ELISA using the conformationally dependent antibody, W6/32 [27], and the antibody BBM1 [28]. The antibodies used in the capture ELISA for HLA B8 were rat anti-HLA B and W6/32 [27].

2.3. Crystallisation

Crystallisation conditions for all complexes are listed in Table 1. Crystals were obtained by the sitting drop vapour diffusion method using microbridges [29]. Crystal Screens I and II (Hampton Research) were used to establish crystallisation conditions for the first HLA B8 and B53 complexes (with GGKKKYKLK and KPIVQYDNF peptides, respectively). Typically, 2 µl of a 10 mg/ml protein solution in 20 mM Tris, pH 8.0 (for B8) or 20 mM MES, pH 6.5 (for B53) was mixed in a 1:1 ratio with the crystallisation reservoir solution and the 4 µl drops incubated at 21°C. Once an initial stock of crystals was established, streak seeding, with a dog whisker, between complexes was used to initiate crystal growth [30,31]. Crystals of the B53/ls6 complex were nucleated by cross-seeding with crystals of B8. Likewise, crystals of B3501 complexes were nucleated by cross-seeding with B53. Macroseeding was then used to improve the size of crystals. For data collection, crystals were flash-cooled in reservoir buffer which, in addition, contained 25% glycerol as cryoprotectant.

2.4. Diffraction measurements and data processing

Data for the first complex, B53/ls6, were collected at room temperature, and required four crystals for a complete data set. Subsequent complexes were cryocooled (Table 1) during data collection and required only one crystal for a complete data set. Data for both B53 complexes and three B8 complexes were collected at station 9.6 of the CLRC Synchrotron Radiation Source, Daresbury (λ = 0.87 Å) utilising an MAR-research imaging plate system (30 cm diameter; A. Lentfer and J. Hendrix). B3501 data were collected at beamline BL1 ID13 of the European Synchrotron Radiation Facility (ESRF) also using a 30 cm diameter MAR-research imaging plate (λ = 0.96 Å). The remaining three B8 complexes were collected at BL4 (ESRF) (λ = 0.76 Å) using an X-ray Image Intensifier Charged Couple Device (XRII)/CCD Detector [32]. Data from the XRII/CCD Detector were first corrected for spatial distortion and non-uniformity of response over the detector system [33,34]. All diffraction data were autoindexed, integrated and corrected for Lorentz and polarization effects with the program DENZO [35] followed by scaling and merging in SCALEPACK [35].

3. Results and discussion

HLA B8, B53 and B3501 heavy chains were expressed to high levels (80 mg/l) as inclusion bodies and detergent washed [25] to greater than 90% purity (Fig. 1). HLA B53 heavy chain, with 6 C-terminal histidines, was further purified on a Ni^{2+}-NTA agarose column and this method of affinity binding was very effective at removing all the 'background' cellular proteins, improving the refolding yield for B53.

Since the heavy chain is only soluble in denaturing conditions, and some precipitates during the refolding, yield of

complex was increased by pulsing the refolding mixture with an additional 20 mg of heavy chain. Each refolding produces an excess of the soluble protein, ß2m, which can be recycled and used in subsequent reconstitutions. Refolding by dilution typically allowed HLA B allele complexes to be formed with an estimated efficiency of 10%. Typically, 3 mg of refolded ß2m was used in addition to 30 mg of denatured ß2m and together with heavy chain pulsing increased the yield of refolded complex to 20%. An elution profile from gel filtration reproducibly showed three main peaks (Fig. 2) only one of which contained heavy chain plus ß2m and showed HLA B allele native conformation according to the respective capture ELISA.

Table 1 summarises the crystallisation conditions for the individual peptide complexes. The B8/GGKKKYKLK complex formed numerous long needle-shaped crystals (Fig. 3) which were solid at one end and hollow at the other end. They appeared in the drops within 3–4 days and grew to full size (typically 0.1 mm×0.05 mm×0.3 mm) within 7 days. The drops, on setting up, appeared to form a heavy precipitate which cleared overnight leaving a very fine phase separation. The 3–4 day nucleation period allowed a window for streak-seeding and macroseeding techniques to be investigated to improve crystal quality and size. The B8/GGKKKYRLK complex formed similar crystals to the first B8 complex in identical conditions although these crystals were not as large (typically 0.1 mm×0.05 mm×0.2 mm). Fine screening around this condition produced larger crystals (typically 0.1 mm×0.05 mm×0.3 mm) with 100 mM sodium citrate pH 6.5, 0.03 M ammonium acetate and 30% PEG 4000. The phase separation was less apparent in these drops and growth could only be achieved after cross-seeding with the first B8 complex. The remaining complexes (B8/GGKKKYQLK, B8/GGRKKYKLK, B8/GGKKRYKL and B8/GGKKKYKL) all crystallized at this condition with the same crystal morphology but the two 8-mer complex crystals formed rapidly, often 'showering', without requiring seeding.

The B53/KPIVQYDNF (ls6) complex formed flat, plate-like crystals (typically 0.1 mm×0.1 mm×0.2 mm) within 24 h. Long, fine needle-like crystals with multiple nucleation sites

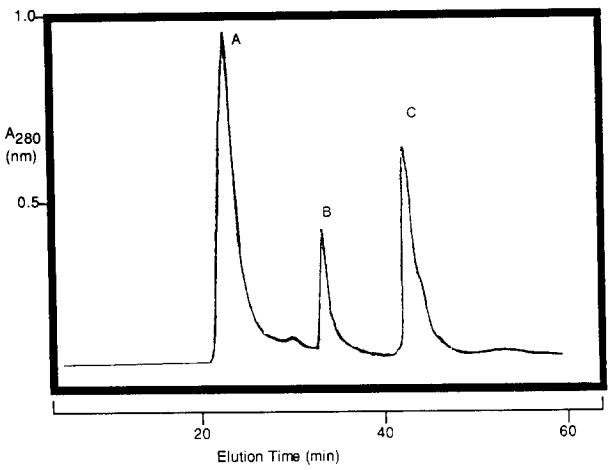

Fig. 2. Chromatogram showing the elution profile of an FPLC superdex 75 prep grade gel filtration column. The flow rate was 5 ml/min, typical loading sample volumes were 5 ml and the elution was monitored at an absorbance of 280 nm. This reproducible profile has a large, sharp peak at approx. 70 kDa (A) containing both heavy chain and ß2m but showing no native conformation; a smaller peak at 44 kDa (B) containing refolded HLA B allele; and a larger peak at 12 kDa containing ß2m only.

were obtained for the B53/TPYDINQML (HIV2) complex. Growth of B53/HIV2 crystals, similar in morphology to the B53/ls6 crystals, could be initiated by cross-seeding with the B53/ls6 or B8 complex crystals. These crystals grew as thick plates (typically 0.1 mm×0.2 mm×0.2 mm) reaching full size within 2 days. The B3501/VPLRPMTY (nef) complex initially produced flimsy thin-plate crystals but cross-seeding with B8 or B53/ls6 crystals, led to large, thick plates. The cross-seeding was equally successful using B8 (21 amino acid differences between heavy chains) and B53 (5 amino acid differences between heavy chains).

Diffraction data were collected using crystals flash-cooled to 100 K, except for the B53/ls6 complex for which data were collected at room temperature. The conditions for cryocooling are detailed in Table 1. All the complexes crystallized in closely related units cells (space group $P2_12_12_1$ with typical unit

Table 1
Summary of individual B allele complexes: conditions which produced crystal growth; cryocooling conditions; resolution; and unit cell dimensions

Allele	Peptide	Crystal growth condition	Cryo-protectant	Resolution (Å)	Unit cell (Å)
B8	GGKKKYKLK	30% PEG 4000, 0.1 M Na citrate, pH 6.5, 0.2 M NH_4 acetate	+15% glycerol	2.05	50.6, 81.4, 110.7
B8	GGKKKYQLK	30% PEG 4000, 0.1 M Na citrate, pH 6.5, 0.03 M NH_4 acetate	+15% glycerol	2.3	50.7, 81.2, 110.6
B8	GGKKKYRLK	30% PEG 4000, 0.1 M Na citrate, pH 6.5, 0.03 M NH_4 acetate	+15% glycerol	2.1	50.4, 80.9, 109.2
B8	GGKKRYKLK	30% PEG 4000, 0.1 M Na citrate, pH 6.5, 0.03 M NH_4 acetate	+15% glycerol	2.2	50.6, 81.3, 110.1
B8	GGRKKYKL	30% PEG 4000, 0.1 M Na citrate, pH 6.5, 0.03 M NH_4 acetate	+15% glycerol	2.1	51.0, 81.6, 111.6
B8	GGKKKYKL	30% PEG 4000, 0.1 M Na citrate, pH 6.5, 0.03 M NH_4 acetate	+15% glycerol	2.3	50.5, 81.1, 110.2
B53 + histidine tag	KPIVQYDNF	18% PEG 8000, 0.2 M Ca acetate, 0.1 M Na cacodylate, pH 6.5	room temperature	2.3	51.3, 83.3, 112.4
B53 + histidine tag	TPYDINQML	18% PEG 8000, 0.2 M Ca acetate, 0.1 M Na cacodylate, pH 6.5	+25% glycerol	2.3	50.9, 82.5, 109.7
B3501	VPLRPMTY	18% PEG 8000, 0.1 M Na cacodylate, pH 6.5	+25% glycerol	2.0	51.3, 82.7, 108.6

For B8, the natural mutations of the peptide are highlighted in bold text.

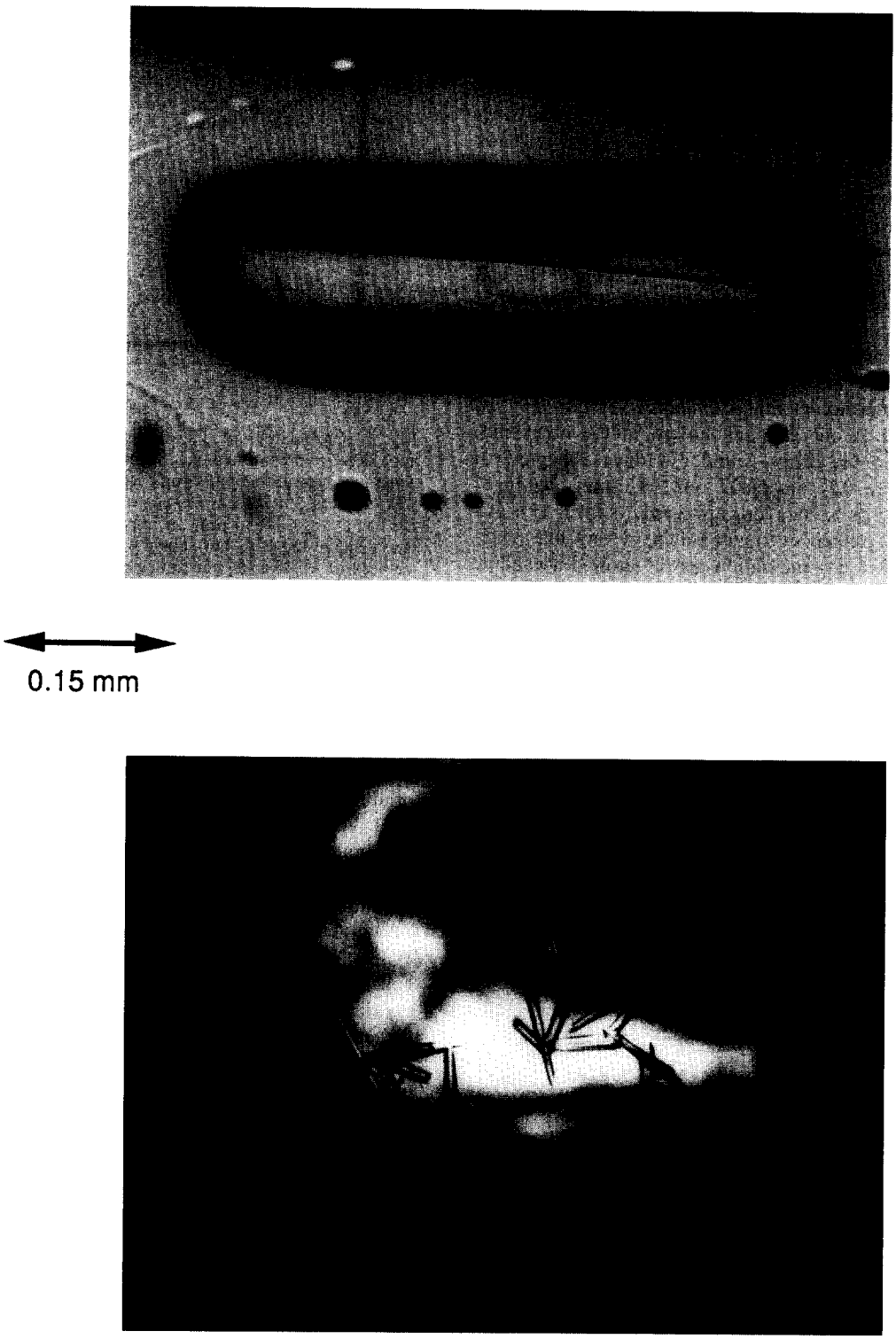

Fig. 3. Photographs of (upper) a typical crystal of HLA B8 (the bar represents 0.15 mm) and (lower) the result of streak-seeding of HLA B53 complexes by B8.

cell dimensions $a = 51$ Å, $b = 82$ Å, $c = 112$ Å, $a = b = c = 90°$) containing one molecule per crystallographic asymmetric unit. Cryocooling resulted in slight variations in unit cell dimensions (Table 1) for each complex. X-ray diffraction data have been collected for all nine complexes to resolutions of at least 2.3 Å using synchrotron radiation. In particular, data collected on station ID13-BL1 of the European Synchrotron Radiation Facility (ESRF, Grenoble, France), from a single, cryocooled, crystal of the B3501/nef complex, indicate that this crystal form is capable of ordered Bragg diffraction to beyond 2.0 Å resolution. Structure determinations of these complexes are currently underway using molecular replacement and will be reported elsewhere (Smith et al. and Reid et al. in preparation).

In conclusion, we have established general protocols for the production and crystallisation of B allele single peptide com-

plexes. The uniformity of crystal form obtained for these complexes contrasts with the diversity reported for a series of A2 single peptide complex crystals [36]. These results suggest that cross-seeding leading to epitaxial growth can be a powerful method for driving crystallisation of closely related molecules into a similar lattice.

Note added in proof

For all HLA B8 complexes in which peptide used for refolding was predominantly a 9-mer, electron density maps indicate that the crystals contain molecules bearing an 8-mer (minus the C-terminal lysine). Sufficient 8-mer peptide has been found in subsequent analysis of original samples by mass spectrometry to be consistent with selective crystallization of 8-mer complexes.

Acknowledgements: We thank E. Garman for help with crystal freezing conditions; P. Frodsham C. Quin, K. Hilyard and G. Gillespie for help with molecular biology techniques and protein production; D. Garboczi for the vector pHN1β2m; M. Pitkeathley and S. Shah for peptide synthesis; T. Willis for protein sequencing; S. Lee for help with the preparation of figures; R. Bryan and R. Esnouf for computing facilities and computer software; the staff of SRS, Daresbury, the ESRF and EMBL, Grenoble for help with X-ray data collection. This work was supported by the MRC and Wellcome Trust. The Oxford Centre for Molecular Sciences is supported by the BBSRC and MRC. E.Y.J. is supported by the Royal Society, D.I.S. and A.McM. are supported by the MRC.

References

[1] Townsend, A., Ohlen, C., Bastin, J., Ljunggren, H.-G., Foster, L. and Karre, K. (1989) Nature 340, 443–448.
[2] Bjorkman, P. and Parham, P. (1990) Annu. Rev. Biochem. 59, 253–88.
[3] Falk, K., Rotzschke, O., Stevanovic, S., Jung, G. and Rammensee, H.-G. (1991) Nature 351, 290–296.
[4] Bjorkman, P.J., Saper, M.A., Samraoui, B., Bennett, W.S., Strominger, J.L. and Wiley, D.C. (1987) Nature 329, 506–512.
[5] Garret, T.P.J., Saper, M.A., Bjorkman, P.J., Strominger, J.L. and Wiley, D.C. (1989) Nature 342, 692–696.
[6] Saper, M.A., Bjorkman, P.J. and Wiley, D.C. (1991) J. Mol. Biol. 219, 277–319.
[7] Guo, H.-C., Jardetzky, T.S., Garrett, T.P.L., Lane, W.S., Strominger, J.L. and Wiley, D.C. (1992) Nature 360, 364–366.
[8] Silver, M.L., Guo, H.-C., Strominger, J.L. and Wiley, D.C. (1992) Nature 360, 367–369.
[9] Madden, D.R., Gorga, J.C., Strominger, J.L. and Wiley, D.C. (1992) Cell 70, 1035–1048.
[10] Collins, E.J., Garboczi, D.N., Karpusas, M.N. and Wiley, D.C. (1995) Proc. Natl. Acad. Sci. USA 92, 1218–21.
[11] Madden, D., Garboczi, D. and Wiley, D. (1993) Cell 75, 693.
[12] Fremont, D.H., Matsumura, M., Stura, E.A., Peterson, P.A. and Wilson, I.A. (1992) Science 257, 919–27.
[13] Zhang, W., Young, A.C., Imarai, M., Nathenson, S.G. and Sacchettini, J.C. (1992) Proc. Natl. Acad. Sci. USA 89, 8403–7.
[14] Young, A.C.M., Zhang, W., Sacchettini, J.C. and Nathenson, S.G. (1994) Cell 76, 39–50.
[15] Fremont, D., Stura, E., Matsumura, M., Peterson, P. and Wilson, I. (1995) Proc. Natl. Acad. Sci. USA 92, 2479–2483.
[16] Madden, D.R. (1995) Annu. Rev. Immunol. 13, 587–622.
[17] Sutton, J., Rowland-Jones, S., Rosenberg, W., Nixon, D., Gotch, F., Gao, X.-M., Murray, N., Spoonas, A., Driscoll, P., Smith, M., Willis, A. and McMichael, A. (1993) Eur. J. Immunol. 23, 447–453.
[18] Malcherek, G., Falk, K., Rotzschke, O., Rammensee, H.-G., Stevanovic, S., Gnau, V., Jung, G. and Melms, A. (1993) Int. Immunol. 5, 1229–1237.
[19] Klenerman, P., Sarah-Rowland-Jones, McAdam, S., Edwards, J., Daenke, S., Lalloo, D., Koppe, B., Rosenberg, W., Boyd, D., Edwards, A., Giangrande, P., Phillips, R.E. and McMichael, A. (1994) Nature 369, 403–407.
[20] Hill, A., Allsopp, C., Kwiatowski, D., Anstey, N., Twumasi, P., Rowe, P., Bennett, S., Brewster, D., McMichael, A. and Greenwood, B. (1991) Nature 352, 595–600.
[21] Hill, A.V.S., Elvin, J., Willis, A.C., Aidoo, M., Allsopp, C.E.M., Gotch, F.M., Gao, X.M., Takiguchi, M., Greenwood, B.M., Townsend, A.R.M., McMichael, A.J. and Whittle, H.C. (1992) Nature 360, 434–439.
[22] Gotch, F., McAdam, S., Allsopp, C., Gallimere, A., Elvin, J., Kieng, M.-P., Hill, A., McMichael, A. and Whittle, H. (1993) J. Immunol. 151, 3361–3369.
[23] Rowland-Jones, S., Sutton, J., Ariyoshi, K., Dong, T., Gotch, F., McAdam, S., Whitby, D., Sabally, S., Gallimore, A., Corrah, T., Takiguchi, M., Schultz, T., McMichael, A. and Whittle, H. (1995) Nat. Med. 1, 59–64.
[24] Studier, F., Rosenberg, A., Dunn, J. and Dubendorff, J. (1990) Methods Enzymol. 185, 60–89.
[25] Garboczi, D., Hung, D. and Wiley, D. (1992) Proc. Natl. Acad. Sci. USA 89, 3429–3433.
[26] Stam, N.J., Spits, H. and Ploegh, H.L. (1986) J. Immunol. 137, 2299–2306.
[27] Parham, P., Barnstable, C.J. and Bodmer, W.F. (1979) J. Immunol. 123, 342-349.
[28] Parham, P., Androlewicz, M.J., Holmes, N.J. and Rothenberg, B.E. (1983) J. Biol. Chem. 258, 6179–86.
[29] Harlos, K. (1992) J. Appl. Crystallogr. 25, 536–538.
[30] Stura, E.A., Matsumura, M., Fremont, D.H., Saito, Y., Peterson, P.A. and Wilson, I.A. (1992) J. Mol. Biol. 228, 975–982.
[31] Stura, E. and Wilson, I. (1992) Crystallization of Nucleic Acids and Proteins; A Practical Approach, Oxford University Press, pp. 99–125.
[32] Moy, J.-P. (1994) Nucl. Instrum. Methods A348, 641–644.
[33] Hammersley, A.P., Svensson, S.O. and Thompson, A. (1994) Nucl. Instrum. Methods A346, 312–321.
[34] Hammersley, A.P., Brown, K., Burmeister, W., Claustre, L., Gonzalez, A., McSweeney, S., Mitchell, E., Moy, J.-P., Svensson, S.O. and Thompson, A., in preparation.
[35] Otwinowski, Z. (1993) Oscillation Data Reduction Program, in Proceedings of the CCP4 Study Weekend: data collection and processing, SERC Daresbury Laboratory, England, 55–62.
[36] Garboczi, D.N., Madden, D.R. and Wiley, D.C. (1994) J. Mol. Biol. 239, 581–587.