Crystallization and preliminary X-ray crystallographic studies of HLA-A*1101 complexed with an HIV-1 decapeptide

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A major goal of vaccine research for the prevention of AIDS is to determine the immune correlates of protection against HIV-1 infection. In this context, it is of interest to understand how HLA-A*1101, a significantly more prevalent class I allele in a cohort of highly HIV-1-exposed persistently seronegative individuals, functions in relation to protective immunity to HIV-1. Towards this goal, a soluble recombinant HLA-A*1101 molecule has been expressed and used to assemble a complex with β2-microglobulin and a Nef decapeptide. The HLA-A*1101/β2m/Nef complex was crystallized by the hanging-drop vapor-diffusion method. The crystal formed in the monoclinic space group $P2_1$, with unit-cell parameters $a = 77.2$, $b = 88.5$, $c = 64.8$ Å, $\beta = 90.1°$, and contains two molecules in the asymmetric unit. A data set to 2.2 Å resolution was collected and structure determination by molecular replacement is currently in progress. Understanding the three-dimensional structure of the HLA-A*1101/β2m/Nef complex may provide insight into the functional role of this class I allele in relation to protective immunity to HIV-1.

1. Introduction

One of the primary functions of the human immune system is to elicit responses that protect against infections by invading pathogens by controlling their spread and virulence. Central to maintaining protective immunity is the cell-surface presentation of antigenic peptides to CD8^{+} cytotoxic T-lymphocytes (CTLs) by class I major histocompatibility (MHC) complexes. During the course of infection by HIV-1, it has been shown that HIV-1-specific CD8^{+} CTL responses represent an important natural immune defense (Klein *et al.*, 1995; Huynen & Neumann, 1996; Rinaldo *et al.*, 1995; Harrer *et al.*, 1996). Most interestingly, a range of HIV-1-specific CD8^{+} CTL responses have been measured in highly HIV-1-exposed persistently seronegative (HEPS) individuals (Rowland-Jones *et al.*, 1993, 1998; Bryson *et al.*, 1995; De Maria *et al.*, 1994; Rowland-Jones & McMichael, 1995; Beyrer *et al.*, 1999; Sriwanthana *et al.*, 2001). In this context, our epidemiological studies and those of others have identified the class I allele HLA-A*1101 to be statistically more prevalent in cohorts of Northern Thai HEPS donors in comparison to ethnically matched unexposed controls (Beyrer *et al.*, 1999; Sriwanthana *et al.*, 2001; Stephens *et al.*, 1996). In view of these observations, we are using a structural approach to investigate this apparent relationship between HLA-A*1101 and protective immunity to HIV-1 infection. Understanding the biological basis of mechanisms associated with resistance to HIV-1 may lead to significant progress in the development of vaccines or other therapies for HIV-1/AIDS.

We have expressed the first soluble recombinant HLA-A*1101 molecule and used it to assemble a complex with β2-microglobulin (β2m) and the decapeptide QVPLRPMTYK from the Nef region of HIV-1. This HLA-A*1101-restricted peptide was shown to elicit immunodominant CTL responses in both Thai HEPS and HIV-1-infected donors (Sriwanthana *et al.*, 2001; Bond *et al.*, 2001). We have crystallized the HLA-A*1101/β2m/Nef complex and its X-ray crystal structure is currently being determined. Given the importance of CD8^{+} CTL responses to HIV-1 infection, this structure will provide a framework to identify features that may be distinct in the way HLA-A*1101 binds and presents antigenic peptides to T-cell receptors on CTLs. To date, only a few studies linking class I alleles with resistance to HIV-1 infection have been reported (Rowland-Jones *et al.*, 1998; Rowland-Jones & McMichael, 1995; Beyrer *et al.*, 1999; Fowke *et al.*, 1996) which underlines the significance of our efforts.

2. Materials and methods

2.1. Construction of expression plasmids

The cDNA coding for amino acids 1–275 of HLA-A*1101 was generated by the poly-

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merase chain reaction (PCR) using a plasmid pET24a containing the full-length HLA-A*1101 gene (a gift from Drs P. Parham and J. Altman) as a template. The sequence of HLA-A*1101 was amplified

using the synthetic primers 5′-TAGGGC-**GAATTC**TAAGGAGGATATTAAAATG-GGCTCTCACTCCATGAGGTATTTC-3′ (forward) and 5′-GCATGC**AAGCTT**TTA-CTCCCATCTCAGGGTGAGGGGCTT-3′ (reverse) bearing *Eco*RI and *Hin*dIII restrictions sites (bold), respectively. The digested and purified PCR fragments were ligated into the pLM1 vector (Sodeoka *et al.*, 1993) previously treated with *Eco*RI and *Hin*dIII, transformed into DH5α cells (Gibco BRL) and selected on ampicillin-containing LB plates. A plasmid harboring the correct DNA sequence for HLA-A*1101 was transformed into the *Escherichia coli* strain BL21(DE3)pLysS (Gibco BRL) and grown at 310 K in LB medium containing 100 µg ml^{-1} ampicillin and 30 µg ml^{-1} chloramphenicol. Protein expression was induced at mid-log phase with 1 mM IPTG. After 2 h induction, the cells were harvested by centrifugation. The expression plasmid for β2m was a gift from Dr D. C. Wiley (Garboczi *et al.*, 1992). Expression of β2m in LB medium containing 100 µg ml^{-1} ampicillin was induced by the addition of 1 mM IPTG. After 3 h induction, the cells were harvested by centrifugation. Recombinant HLA-A*1101 and β2m were isolated as inclusion bodies from the cell pellets and were washed and solubilized as previously described (Garboczi *et al.*, 1992).

2.2. Complex assembly and purification

Complex assembly was initiated by diluting the solubilized inclusion bodies of HLA-A*1101 (1 µM) and β2m (2 µM) in the presence of an excess of the Nef decapeptide QVPLRPMTYK (10 µM) in a refolding buffer as previously described (Bouvier & Wiley, 1998). The refolding mixture was incubated at 277 K for 48 h, concentrated

Figure 2
Typical crystals of the HLA-A*1101/β2m/Nef complex.

Table 1
X-ray diffraction data for the HLA-A*1101/β2m/Nef complex.

Resolution (Å)	49.3–2.2
Space group	$P2_1$
Unit-cell parameters (Å, °)	$a = 77.2$, $b = 88.5$, $c = 64.8$, $\beta = 90.1$
Total observations	138834
Unique reflections	39698
$I/\sigma(I)$	10.6
Completeness (%)	94.3
R_{sym}† (%)	6.3

† $R_{sym} = \sum |I_{av} - I_i| / \sum I_i$, where I_{av} is the average of all individual observations I_i.

using an ultrafiltration cell (Amicon) and purified on a Biosep Sec-S3000 (Phenomenex) HPLC gel-filtration column in 20 mM Tris pH 7.5, 150 mM NaCl (Fig. 1*a*). The HLA-A*1101/β2m/Nef complex eluted at the expected position (11 min) based on calibration of the column using globular protein standards. The purified HLA-A*1101/β2m/Nef complex was characterized by SDS–PAGE (Fig. 1*b*) and native PAGE (Fig. 1*c*). The presence of bound Nef decapeptide in the purified complex was confirmed by FAB mass-spectrometry analysis (data not shown). Stock solutions (∼40 mg ml^{-1}) in 10 mM MOPS pH 7.4 were kept frozen at 253 K.

2.3. Crystallization

Initial crystallization conditions of the HLA-A*1101/β2m/Nef complex were established using Crystal Screens I and II (Hampton Research) and the hanging-drop vapor-diffusion technique at 293 K. Initial crystals obtained with solution No. 9 of Crystal Screen I were used to produce a stock seeding solution in 16% PEG 6000, 25 mM MES pH 6.5, 0.1% sodium azide. Crystals used for data collection were grown by mixing 1.5 µl of a 12 mg ml^{-1} complex solution with 1.5 µl of 30% PEG 4000, 0.1 M sodium citrate pH 5.6, 0.2 M ammonium acetate and 0.5 µl of seeding solution. Drops were placed over a reservoir solution containing 15% PEG 4000, 0.1 M sodium citrate pH 5.6, 0.2 M ammonium sulfate. Crystals (Fig. 2) appeared after 10 d as plates with maximum dimensions of $0.55 \times 0.35 \times 0.15$ mm. These crystallization conditions are the most acidic reported for the growth of class I MHC complexes.

2.4. Data collection and processing

Immediately prior to data collection, crystals were soaked for several minutes in reservoir solution supplemented with 20% 2-methyl-2,4-pentanediol as a cryoprotectant and then flash-cooled in a nitrogen cold
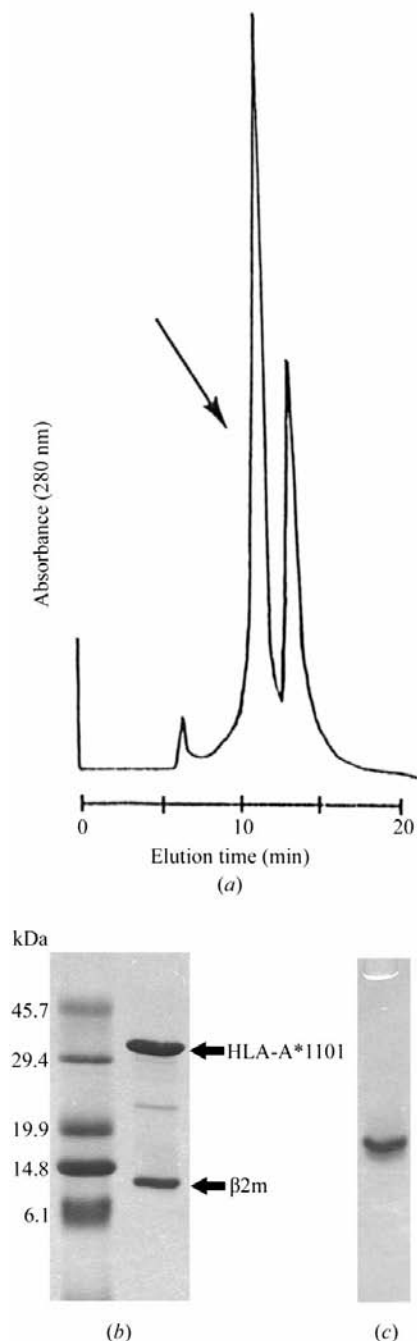
Figure 1
(*a*) Purification of the crude HLA-A*1101/β2m/Nef complex by gel-filtration chromatography. The complex (indicated by an arrow) eluted from a calibrated column at the expected position (11 min) for its molecular mass of 45 kDa. Adjacent peaks are aggregated HLA-A*1101 molecules (7 min) and excess β2m (13 min). (*b*) SDS–PAGE gel (15%) of the purified complex, showing bands for HLA-A*1101 and β2m. (*c*) Native PAGE gel (10%) of the purified complex showing a single compact band.

stream at 100 K (Oxford Cryosystems). Diffraction data for the HLA-A*1101/β2m/Nef complex were collected with a Bruker HISTAR multiwire area detector coupled to a RU-200 rotating-anode generator operating at 60 mA and 40 kV (Cu $K\alpha$, $\lambda = 1.542$ Å) with double-mirror Franks focusing. A total of 1600 frames were collected at a crystal-to-detector distance of 10 cm, with an interval of 0.25° and an exposure time of 120 s per frame. Data to 2.2 Å resolution were reduced and scaled using *XGEN* (Molecular Simulations Inc.) (Table 1).

3. Results and discussion

Crystals of the HLA-A*1101/β2m/Nef complex formed in the monoclinic space group $P2_1$, with unit-cell parameters $a = 77.2$, $b = 88.5$, $c = 64.8$ Å, $\beta = 90.1°$ (Table 1). A total of 138 834 observations were measured and reduced to 39 698 unique reflections, corresponding to a completeness of 94.3% to 2.2 Å resolution. The overall R_{sym} value is 6.3%. The crystal was estimated to contain two molecules (45 kDa each) in the asymmetric unit, which results in a V_M value of 2.4 Å^3 Da^{-1} and a solvent content of 44%.

In reciprocal space, the R_{merge} values of equivalent reflections across planes ($hk0$), ($h0l$) and ($0kl$) were 38, 6 and 38%, respectively, showing that the Laue symmetry is $2/m$. This, in combination with observations of systematic absences for $0k0$ but not for $h00$ and $00l$, indicated that the space group is $P2_1$.

Structure determination by molecular replacement using as a search model the structure of an HLA-A*0201 complex (PDB code 1duz; Khan *et al.*, 2000) is in progress.

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