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Immunology

HLA-A2–peptide complexes: Refolding and crystallization of molecules expressed in *Escherichia coli* and complexed with single antigenic peptides

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ABSTRACT **The two subunits of the human class I histocompatibility antigen (HLA)-A2 have been expressed at high levels (20–30 mg/liter) as insoluble aggregates in bacterial cells. The aggregates were dissolved in 8 M urea and then refolded to form an HLA-A2–peptide complex by removal of urea in the presence of an antigenic peptide. Two peptides from the matrix protein and nucleoprotein of influenza virus are known to bind to HLA-A2, and both support the refolding of the recombinant HLA-A2 molecule. An additional peptide, a nonamer from the gp120 envelope protein of human immunodeficiency virus type 1, also supported refolding. Yields of purified recombinant HLA-A2 are 10–15%. In the absence of an HLA-A2-restricted peptide, a stable HLA-A2 complex was not formed. Monoclonal antibodies known to bind to native HLA-A2 also bound to the recombinant HLA-A2–peptide complex. Three purified HLA-A2–peptide complexes refolded from bacterially produced protein aggregates crystallize under the identical conditions as HLA-A2 purified from human lymphoblastoid cells. Crystals of the recombinant HLA-A2 molecule in complex with the influenza matrix nonamer peptide, Mp(58–66), diffract to >1.5-Å resolution.**

Proteins encoded by the major histocompatibility complex present peptide antigens to T lymphocytes. Presentation of peptides by class I major histocompatibility complex molecules on cell surfaces results in the activation of cytotoxic lymphocytes (CTL) and the subsequent lysis of target cells (1). Class I HLA (human leukocyte antigen) molecules are cell-surface glycoproteins that consist of a complex of two noncovalently associated polypeptide chains, a larger or heavy chain and a smaller or light chain, β_2-microglobulin (β_2m) (2, 3). A crystallizable form of HLA-A2, HLA-Aw68, and HLA-B27 can be prepared from human lymphoblastoid cells by papain cleavage of the heavy chain near its transmembrane segment (4–7). X-ray crystallographic analysis indicates that a processed antigen is presented as a peptide bound in a cleft between two α-helices of the heavy chain of the HLA complex (8–12).

HLA-A2 and HLA-Aw68 molecules purified from lymphoblastoid cells appear to contain a heterogeneous set of endogenous peptides (8–11, 13), thereby impeding a clear visualization of the peptide in its binding site. Recently, a model for bound peptide has been proposed based on the crystal structure of HLA-B27 (12) and direct amino acid sequencing of some of the bound peptides (14). The crystallization of HLA-A2 and HLA-Aw68 reconstituted to contain homogeneous bound peptides promises an even more detailed view of the peptide in its binding site (ref. 15; M. L. Silver and D.C.W., unpublished work).

The overexpression of HLA complexes would benefit biochemical and crystallographic studies that have been limited by the small amounts of protein available from homozygous human cell lines. The detectable formation of native HLA-A2–peptide complexes reconstituted from subunits produced in an *Escherichia coli* secretion system has been described by Parker *et al.* (16, 17).

Here we report the high-level expression of the heavy chain of HLA-A2 and the light chain (β_2m) in the cytoplasm of *E. coli* and the efficient refolding of HLA molecules in the presence of antigenic peptides. This expression has yielded an abundant supply of HLA-A2 in complex with either of two peptides from influenza virus or a 9-amino acid peptide from the gp120 envelope protein of human immunodeficiency virus type 1. The gp120 peptide was chosen based on the HLA-A2 "motif" (13) from a 20-amino acid peptide shown to sensitize HLA-A2-bearing target cells to CTL killing (18).

Monoclonal antibodies (mAbs) that recognize folded HLA-A2 from human cells also recognize the refolded recombinant HLA-A2–peptide molecule. The refolded HLA-A2–peptide complexes crystallize under the same conditions (6) used to crystallize HLA-A2 from human cells; the crystals so obtained diffract to high resolution.

MATERIALS AND METHODS

Gene Sources and Bacterial Strains. HLA-A2 heavy chain (amino acids 1–271) and β_2m protein-coding regions were obtained from plasmids p4037 and p714, respectively (16, 19). The expression vector pHN1+ (20) contains a *tac* promoter and the *rrnBT1T2* transcription terminator. The *E. coli* strain XA90 F' lacI^{q1} was used for both DNA manipulation and protein expression.

Construction of Plasmids. The expression cassette for the HLA-A2 heavy chain was constructed by using the PCR with p4037 as the template and the oligonucleotide primers CGCGCGAATTCAGGAGGAATTTAAAATGGGCTCT-CACTCCATG and GCGCAAGCTTTTAGGTGAGGGGCT-TGGG, containing the underlined *Eco*RI and *Hin*dIII restriction sites, respectively. The stop codon TAA at codon 272 was included in the *Hin*dIII oligonucleotide. The expression cassette for β_2m was constructed analogously with p714 as the template and the primers CGCGCCCCGGGAGGAG-GAATTTAAAATGATCCAGCGTACTCCA and GCG-CAAGCTTTTACATGTCTCGATCCCA, containing the underlined *Ava* I and *Hin*dIII restriction enzyme-cleavage sites, respectively. For each subunit the 5' oligonucleotide primer encoded an N-terminal methionine and the first 5 amino acids of the protein. The 5' primer also contained a restriction enzyme-cleavage site, a ribosomal binding site (AGGAGG), and a translational spacer element (20). Both 3' primers contained the C terminus of the subunit, a stop codon, and a restriction enzyme-cleavage site. The amplification mixtures (100 μl) contained dGTP, dATP, dTTP, dCTP (each 200 μM),

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Abbreviations: mAb, monoclonal antibody; β_2m, β_2-microglobulin; CTL, cytotoxic lymphocyte(s).

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oligonucleotide primers (1 μM), template DNA (25 ng), *Thermus aquaticus* (*Taq*) DNA polymerase (Promega) in 50 mM NaCl/10 mM $MgCl_2$/50 mM Tris·HCl, pH 9. The reaction mixtures were subjected to 25 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 2 min, and a final 15 min at 72°C. The amplified DNAs were gel-purified, digested with *Eco*RI/*Hin*dIII or *Ava* I/*Hin*dIII, and ligated into pHN1+ that had been digested with the corresponding enzymes. Difficulties with the β_2m ligation were overcome by using the Klenow fragment to blunt the *Ava* I cohesive ends of both the plasmid and PCR product before ligation. The plasmids were transformed into *E. coli* strain XA90, and clones containing inserts and producing protein upon induction with isopropyl β-D-thiogalactopyranoside were identified. A heavy chain-producing clone (amino acids 1–271) and a β_2m-producing clone were submitted to DNA sequencing to verify their sequence.

A second heavy chain-producing plasmid was engineered to express a heavy chain protein longer by 4 amino acids. The translation termination codon at 272 was moved to codon 276, by amplifying a restriction fragment with an oligonucleotide encoding a *Hin*dIII restriction site and the 4 additional amino acids and an oligonucleotide hybridizing within the coding region located 5′ to the *Sac* I restriction site. Replacement of the shorter *Sac* I–*Hin*dIII fragment by the longer one resulted in a heavy chain-coding region consisting of amino acids 1–275.

Reconstitution by Dialysis. One liter of cells transformed with either the heavy chain (amino acids 1–271) or the β_2m expression plasmid was incubated at 37°C and induced to produce protein by the addition of isopropyl β-D-thiogalactopyranoside (1 mM). The cells were harvested by centrifugation at an OD_{650} of 1.8–2.0. The cell pellets were resuspended in 10 mM Tris·HCl, pH 8 (20 ml), containing lysozyme at 100 μg/ml, phenylmethylsulfonyl fluoride at 50 μg/ml, DNase at 20 μg/ml, RNase at 20 μg/ml, and 1 mM EDTA and incubated at 22°C for 20 min. The cells were lysed by sonication and then centrifuged (10,000 × *g*) for 20 min. The pellet containing recombinant protein was washed with 10 mM Tris·HCl, pH 8 (20 ml), dissolved in 100 mM Tris·HCl, pH 8/8 M urea (10 ml) and centrifuged (150,000 × *g*) for 1 hr at 4°C. The recombinant β_2m in urea was refolded by dialysis against 10 mM Tris·HCl, pH 7, and purified on Q Sepharose (Sigma) in 10 mM Tris·HCl, pH 7, with a linear gradient from 0–100 mM NaCl (19). Fractions containing β_2m were dialyzed against water and concentrated by ultrafiltration with centrifugation (Centriprep, Centricon; Amicon).

Peptides were dissolved in water and refolded, purified β_2m was dissolved in water, and heavy chain (amino acids 1–271) was dissolved in 100 mM Tris/8 M urea as isolated. To begin reconstitution, a solution containing peptide (120 μM), β_2m (6 μM), and heavy chain (3 μM) was made in 25 mM 2-(*N*-morpholino)ethanesulfonic acid, pH 6.5/150 mM NaCl (MBS) containing 6 M NaSCN. After dialysis (500 M_r cutoff) of the mixture overnight at 4°C against MBS without NaSCN, the reconstitution was centrifuged for 10 min at 14,000 rpm in a microcentrifuge at 4°C. The extent of reconstitution was analyzed by gel filtration HPLC in MBS on a 300SW column (Waters), monitoring absorbance at 280 nm. Larger-scale reconstitutions were concentrated in a Centricon-30, (30,000 M_r cutoff). Reconstitution yields were calculated by pooling fractions and measuring A_{280}. A peptide/β_2m/heavy chain molar ratio of 40:2:1 resulted in the highest yield of reconstituted complex formed by dialysis.

Reconstitution by Dilution. One liter of cells harboring either the expression plasmid encoding the heavy chain encoding amino acids 1–275 or the plasmid encoding β_2m was allowed to reach stationary phase and then diluted by adding 100 ml of the stationary culture to 400 ml of LB medium in a 2-liter flask at 37°C. After a further 30-min incubation, 0.5 mM isopropyl β-D-thiogalactopyranoside was added, and the cells were incubated for 4 hr. Cells (from 5 liters) were then collected by centrifugation, and insoluble protein aggregates (inclusion bodies) were isolated essentially as described by Nagai and Thogersen (21), with the modification of a freeze/thaw step after detergent treatment of the cells. The inclusion body pellet was dissolved in 10 ml of 8 M urea/50 mM 2-(*N*-morpholino)ethanesulfonic acid, pH 6.5/0.1 mM EDTA/0.1 mM dithiothreitol, and insoluble material was pelleted by centrifugation at 150,000 × *g*. The protein solution was immediately frozen at −70°C.

Refolding and complex formation was initiated by dilution of the two denatured subunits and peptide into 200 ml of 100 mM Tris·HCl, pH 8/400 mM L-arginine·HCl/2 mM EDTA/5 mM reduced glutathione/0.5 mM oxidized glutathione/0.5 mM phenylmethylsulfonyl fluoride (22). The final concentrations of the heavy chain, β_2m, and the peptide were 31 μg/ml (1 μM), 24 μg/ml (2 μM), and 10 μg/ml (10 μM), respectively. The refolding mixture was incubated at 10°C for 24–36 hr. Extent of refolding was determined by gel filtration HPLC in 20 mM Tris·HCl, pH 7.5/150 mM NaCl. The 200 ml of refolding mixture was concentrated with a Centriprep-10 and a Centricon-10 to a volume of 200 μl. The concentrated protein was subjected to gel filtration HPLC, and the peak at 19 min (42 kDa) was collected in 2 ml. The peak fraction was concentrated to 70 μl, re-diluted to 2 ml with 25 mM 2-(*N*-morpholino)ethanesulfonic acid, pH 6.2/0.1% NaN_3 and re-concentrated to 70 μl. By use of the formula that, for HLA-A2, 1 A_{280} unit is 0.67 mg/ml, the final protein concentration ranges from 10 to 20 mg/ml. The yield of refolding is based on heavy chain A_{280} at the start of the procedure and after purification.

mAb Binding. Samples (1 μl) were pipetted onto Immobilon P poly(vinylidene difluoride) membrane (Millipore) and allowed to adsorb for 2 hr at 22°C. The membrane was blocked with 3% bovine serum albumin in phosphate-buffered saline followed by the respective mAb in 50 mM Tris·HCl, pH 8/100 mM NaCl/0.05% Tween. The blots were developed by using an anti-mouse secondary antibody conjugated to alkaline phosphatase and visualized with nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate.

Other Techniques. The oxidation state of cysteines was determined with Ellman's reagent (23). Protein was quantified, and gels were silver stained with kits from Bio-Rad. Native isoelectric focusing was performed as described (15). Peptides were produced using 9-fluorenylmethoxycarbonyl (Fmoc) chemistry on an Applied Biosystems 431A synthesizer. Peptides were purified with C_{18} reverse-phase HPLC, and their identity was confirmed by mass spectrometry (Harvard University Chemistry Department Mass Spectrometry Facility).

RESULTS

Heavy Chain and β_2m Expression and Purification. Using the PCR, we engineered expression plasmids to produce the two subunits of the soluble human class I HLA-A2 molecule. Coomassie blue-stained SDS gels of induced cell lysates clearly demonstrated the overproduction of each subunit (Fig. 1, lanes 2 and 4). Both subunits appeared from preliminary experiments to be insoluble, which allowed a procedure of washing and centrifuging to achieve a high level of purity of the proteins (Fig. 1, lanes 6–8). The yields were 22 mg/liter and 35 mg/liter for the heavy chain and β_2m, respectively.

Immunoblots of gels containing the recombinant proteins (data not shown) confirmed their identities and revealed no apparent degradation of the proteins. mAb HC-10 (24) was used for the heavy chain and mAb BBM-1 (25) was used for β_2m. N-terminal amino acid sequencing revealed that the

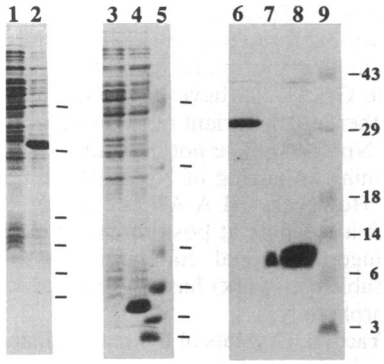

FIG. 1. SDS/PAGE of expression of HLA-A2 heavy chain and β_2m in *E. coli*. Lanes: 1, lysate from cells with plasmid that lacks insert; 2, lysate from cells with plasmid expressing amino acids 1–275 of the heavy chain; 3, lysate from cells with plasmid lacking insert; 4, lysate of cells with plasmid expressing β_2m; 5, standard proteins; 6, heavy chain aggregates (20 μg); 7, β_2m aggregates (4 μg); 8, β_2m aggregates (40 μg); 9, standard proteins sized in kDa at right. Gels were stained with Coomassie blue R-250.

N-terminal methionine had been removed from the heavy chain but was still present on β_2m. An assay of sulfydryl groups of the recombinant proteins (23) confirmed that the cysteines of both proteins were in the reduced state immediately after isolation from lysed cells. The native heavy chain contains four cysteines involved in two intrachain disulfides, and β_2m contains two cysteines linked in a single disulfide bond.

Refolding of HLA-A2–Peptide Molecule. Refolding was initiated by the removal of denaturant by dialysis of a solution of heavy chain (1–271), β_2m, and peptide. The extent of reconstitution of the HLA-A2 complex was analyzed by HPLC gel filtration (Fig. 2). Two characteristic peaks were seen in most reconstitution experiments. The peak at 10 min, the excluded volume of the column, consists of aggregated heavy chain, and the peak at 23 min consists of monomeric β_2m (see below). A peak containing the HLA-A2 complex is expected to appear on the chromatogram between the 10-min and 23-min peaks. No additional peak was observed in the absence of peptide (Fig. 2*a*). In the presence of an irrelevant peptide, hemagglutinin 307–319, known to bind to the human class II molecule HLA-DR1 (26), the HLA-A2 complex was also not formed (Fig. 2*b*). In contrast, with peptides known to be restricted to HLA-A2, complex formation was seen (Fig. 2 *c* and *d*). In the presence of peptide Np85–94 (*c*) or peptide Mp58–68 (*d*), gel filtration revealed another peak eluting from the column at 19 min. Np85–94 and Mp58–68 are, respectively, from the nucleoprotein and matrix protein from influenza virus (15, 27, 28).

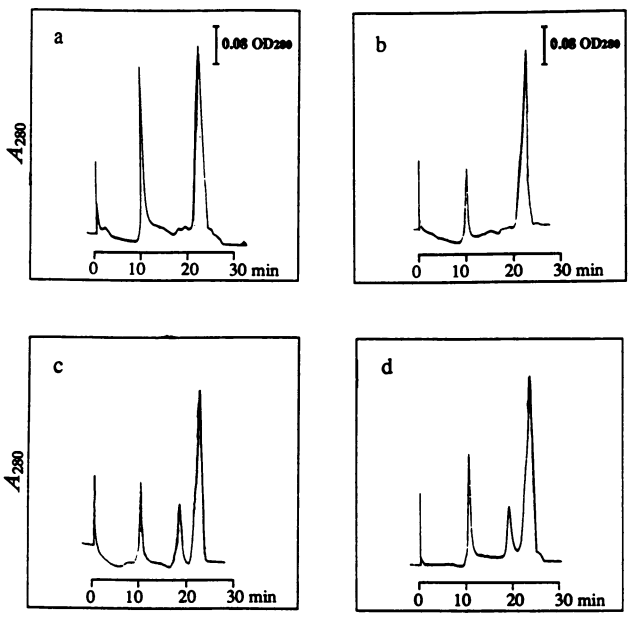

FIG. 2. Gel filtration HPLC profiles of HLA-A2 reconstitution from recombinant heavy chain and β_2m with and without peptides. (*a*) No peptide. (*b*) Hemagglutinin 307–319: PKYVKQNTLKLAT (26). (*c*) Np85–94: KLGEFYNQMM (15, 27). (*d*) Mp58–68: GILGFVFTLTV (28).

The peaks at 10, 19, and 23 min were collected and analyzed by nonreducing SDS/PAGE (Fig. 3). The 10-min peak contained only heavy chain, presumed to be aggregates not pelleted by centrifugation (lane 4). The 19-min peak consisted of both heavy chain and β_2m (lane 5). That the two chains were separated on nonreducing SDS/PAGE implies their noncovalent association. The 23-min peak consisted of β_2m alone (lane 6). Reconstitution yields of 15% with Np85–94 and 13% with Mp58–68 were obtained. Isoelectric focusing under native conditions also demonstrated the formation of HLA-A2 (data not shown).

Several HLA-A2 mAbs bind the refolded HLA-A2–peptide molecule (Table 1). mAb BBM.1 binds to an epitope on native or denatured β_2m and, thus, binds well to native or denatured HLA-A2 (25). mAb HC-10 binds to the heavy chain and recognizes both the native and denatured complex (24). mAb W6/32 recognizes a determinant present on the heavy chain but only when the heavy chain is in complex with β_2m (29, 30). mAb PA2.1 binds only to native HLA-A2 (29, 31), and its binding, together with that of mAb W6/32, suggests that the recombinant HLA-A2 molecule has been correctly refolded.

Refolding of HLA-A2 by Dilution. With the (1–275)-amino acid heavy chain protein, preparative scale refolding by dilution has been developed with any of several peptides. Yields of 10–15% are obtained, and the protein produced by the dilution procedure crystallizes (see below).

Analysis by gel filtration HPLC of a preparative refolding is presented in Fig. 4. The chromatogram is similar to Fig. 2*d* and reveals a heavy chain, aggregate peak (Fig. 4, peak 1) and a β_2m peak (Fig. 4, peak 3). The complex of HLA-A2 with the 9-amino acid peptide from the matrix protein of influenza virus (32, 33) appears at 19 min and at a molecular mass position of 42 kDa compared with standard proteins (Fig. 4, peak 2). The peak height is 1.6 A_{280} units, and the peak contains ≈1 mg of HLA-A2. Peak 2 was collected and concentrated to 7 mg/ml. Aliquots (2 μl) were set up for crystallization, and one such aliquot was loaded on an SDS

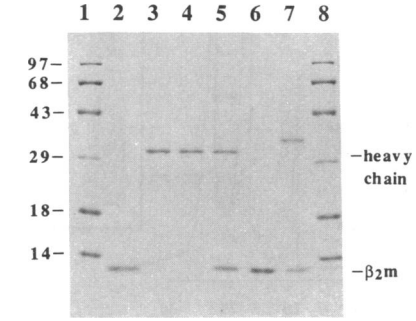

FIG. 3. Nonreducing SDS/PAGE of peak fractions appearing during gel filtration of reconstitution mixture. Lanes: 1, standard proteins sized in kDa at left; 2, β_2m; 3, recombinant HLA-A2 heavy chain; 4, peak at 10 min; 5, peak at 19 min; 6, peak at 23 min; 7, HLA-A2 from JY cells; 8, standard proteins. The HLA-A2 heavy chain from JY cells is glycosylated at Asn-86 and exhibits a larger apparent molecular mass than the recombinant heavy chain. Proteins were detected with Coomassie blue R-250.

Table 1. Recognition of recombinant HLA-A2 by mAbs

mAb	β_2m	Heavy chain	rHLA-A2	JY HLA-A2	LG-2 HLA-B27
BBM.1	+	0	+	+	+
HC-10	0	+	+	+	+
W6/32	0	0	+	+	+
PA2.1	0	0	+	+	0

+, Antibody binding detected; 0, no antibody binding detected; r, recombinant; JY, cell line expressing HLA-A2; LG-2, cell line expressing HLA-B27.

gel (Fig. 4, *Inset*), which revealed protein bands of the expected mobilities for heavy chain and β_2m.

Preparative amounts of HLA-A2 in complex with the 9-amino acid (Np85–93) and 10-amino acid (Np85–94) peptides from the nucleoprotein of influenza virus and the 9-amino acid gp120 peptide (see below) have also been obtained. Refolding in the presence of an irrelevant peptide or in the absence of peptide yielded no stable complexes.

Selection of Peptide from gp120. In a recent report (18), HLA-A2-restricted CTL activity against the gp120 envelope protein of human immunodeficiency virus type 1 was elicited in cells from both human immunodeficiency virus type 1-seropositive and -seronegative donors. Synthetic peptides that were ≈20 amino acids in length were used to localize T-cell epitopes on gp120 (18). One of these peptides—TTSYTLTSCNTSVITQACPK—appears to contain a nonamer peptide with an HLA-A2 "motif" as described by Falk *et al.* (13). The nonamer peptide—TLTSCNTSV (Gp197–205)—was synthesized and used in a dilution refolding experiment. Both gel filtration HPLC and native isoelectric focusing confirmed HLA-A2 refolding in the presence of Gp197–205 (data not shown). The yield of folded complex (15%) was similar to that seen with influenza peptides.

Crystallization of Recombinant HLA-A2 Molecules. Vapor diffusion crystallization experiments using hanging drops were done under identical conditions as has been described

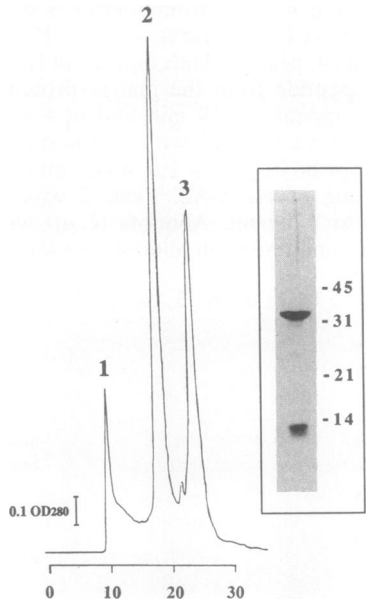

FIG. 4. Purification of the HLA-A2/matrix 9-mer (Mp58–66) molecule for crystallization. The HLA-A2 subunits [(1–275)-amino acid heavy chain] were removed from urea by dilution and allowed to refold and associate. Peaks: 1, aggregates at void volume of column; 2, HLA-A2/matrix 9-mer peptide complex; 3, β_2m. (*Inset*) Peak 2 was collected, concentrated, and a 2-μl (14 μg) sample was applied to a 15% SDS gel and Coomassie-stained. Positions of standard proteins are shown at right.

(6). Thin, overlapping plate crystals formed within 5–7 days, with the occasional formation of single crystals. Crystals of HLA-A2 containing the matrix peptide Mp58–66 and the gp120 peptide Gp197–205 have been obtained. Complexes containing either length variant of the nucleoprotein peptide (Np85–94 or Np85–93) have not crystallized, nor have molecules containing an analog of Np85–94 with an alanine at position 88. However, HLA-A2 containing an analog of Np85–94 with an alanine at position 90 (P. A. Robbins and J. L. Strominger, personal communication; D.N.G. and D.C.W., unpublished work) has crystallized with a similar thin-plate morphology.

X-Ray Diffraction. Crystals of the recombinant HLA-A2 in complex with Mp58–66 were harvested into 20% PEG6000/25 mM 2-(*N*-morpholino)ethanesulfonic acid, pH 6.2/0.1% NaN_3 and transferred to the same buffer containing 20% (vol/vol) glycerol in 3% steps. Each crystal (100 × 200 × 10 μm) in a droplet of glycerol-containing harvest buffer was placed in a loop (2-mm i.d.) of 75-μm wire (34) and frozen in a stream of −170°C nitrogen gas. Diffraction was obtained beyond 1.5-Å resolution by using the synchrotron x-ray source at Cornell (Cornell High Energy Synchrotron Source); reflections at 1.3 Å were observed on a "still" photograph.

DISCUSSION

We have expressed the HLA-A2 heavy chain and human β_2m at high levels in *E. coli*. As has frequently been observed in other overexpression experiments, highly expressed proteins are often found in an insoluble form within the bacterial cell. The insoluble protein aggregates, or inclusion bodies, can usually be dissolved in strong denaturants (here 8 M urea). We have been able to refold and to assemble the denatured chains of HLA-A2 under dilute conditions by the removal of denaturant in the presence of peptides restricted to HLA-A2. The HLA-A2 complexes formed with the peptides used here appear as sharp peaks on gel filtration chromatography at an elution time consistent with the expected molecular weight of the complex. Analysis of the complex peak by SDS/PAGE reveals that it is composed of two polypeptide chains of the expected sizes of the HLA-A2 heavy chain and β_2m. The complex is very stable, and protein stored at 4°C for more than 2 weeks continues to exhibit a single peak on gel filtration and to crystallize.

The Gp197–205 peptide reported here and several other peptides (D.N.G., unpublished work; P. Robbins, personal communication) have been shown to bind to HLA-A2 by supporting the dilution refolding of the complex. Other peptides, identified by specific CTL activity or by a HLA-A2 peptide motif (13), may be tested by these procedures for their participation in the refolding/association of the HLA subunits. It should be possible to produce other class I major histocompatibility complex molecules in bacteria; HLA-Aw68 has been refolded in complex with the nucleoprotein peptide Np91-99 from influenza virus using the procedures reported here (M. Karpusas and D.C.W., unpublished work). Large amounts of specific peptide–HLA complexes will be useful in examining recognition by specific CTLs through the potential activation or competition of cytotoxic function by soluble HLA-A2. It may be possible to isolate specific CTLs by their selective adsorption to plates ("panning") coated with recombinant HLA-A2–peptide complexes (35, 36).

In control refolding experiments performed in the absence of peptide and analyzed by gel filtration, we have observed the formation of short-lived complexes. Such presumed "empty" HLA-A2 molecules appear early in a time course of refolding, but, under the conditions reported here, disappear by ≈24 hr when the peptide—HLA-A2 molecules are collected and concentrated.

mAbs specific for the native HLA-A2 molecule bound to the recombinant complex (Table 1). The complex was recognized by mAb W6/32, which recognizes folded human class I molecules, in general, and by mAb PA2.1, which specifically recognizes the native HLA-A2 molecule (29–31). This recognition by conformation-sensitive mAbs indicates that the recombinant complex contains native epitopes, consistent with the presence of a correctly folded molecular complex.

Bacterially produced HLA-A2 complexed with individual peptides crystallizes, a further indication that it is folded in a native conformation. Thin (10-μm) crystals of HLA-A2–peptide Mp58–66 diffract to beyond 1.5-Å resolution. This is a significant improvement in resolution over crystals of HLA-A2 isolated from human lymphoblastoid cells that also diffract well (2 Å). A number of factors may be responsible for the improved resolution of the observed diffraction. First, the crystals were frozen at −170°C in a thin film of mother liquor (34), which is expected to reduce radiation damage. Second, several differences between HLA proteins from bacteria and human cells contribute to a more homogeneous molecular species: (*i*) a single peptide in the binding site; (*ii*) the absence of glycosylation; (*iii*) the heavy chain C terminus formed by a stop codon, not a potentially "ragged" papain cleavage; (*iv*) the heavy chain C terminus at amino acid 275, completing the β-sheet of the α_3 domain (11). Another difference is the N-terminal methionine that remains on the bacterially produced β_2m.

The overexpression level of 20–30 mg/liter achieved here coupled with a refolding yield of ≈10% provides an abundant source of HLA-A2 and its complexes with antigenic peptides for biochemical analysis. The effect of peptide length on peptide binding can be studied in the complexes of HLA-A2 with nonameric and decameric peptides crystallized here. Crystals that diffract to very high resolution promise a closer look at peptide binding and a greater understanding of the structure of HLA-A2.

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1. Townsend, A. & Bodmer, H. (1989) *Annu. Rev. Immunol.* **7**, 601–624.
2. Cresswell, P., Turner, M. J. & Strominger, J. L. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 1603–1607.
3. Peterson, P. A., Rask, L. & Lindblom, J. B. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 35–39.
4. Springer, T. A. & Strominger, J. L. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2481–2485.
5. Parham, P., Alpert, B. N., Orr, H. T. & Strominger, J. L. (1977) *J. Biol. Chem.* **252**, 7555–7567.
6. Bjorkman, P. J., Strominger, J. L. & Wiley, D. C. (1985) *J. Mol. Biol.* **186**, 205–210.
7. Gorga, J. C., Madden, D. R., Prendergast, J. K., Wiley, D. C. & Strominger, J. L. (1992) *Proteins* **12**, 87–90.
8. Bjorkman, P. J., Saper, M. A., Samraoui, B., Bennett, W. S., Strominger, J. L. & Wiley, D. C. (1987) *Nature (London)* **329**, 506–512.
9. Bjorkman, P. J., Saper, M. A., Samraoui, B., Bennett, W. S., Strominger, J. L. & Wiley, D. C. (1987) *Nature (London)* **329**, 512–518.
10. Garrett, T. J., Saper, M. A., Bjorkman, P. A., Strominger, J. L. & Wiley, D. C. (1989) *Nature (London)* **342**, 692–696.
11. Saper, M. A., Bjorkman, P. J. & Wiley, D. C. (1991) *J. Mol. Biol.* **219**, 277–319.
12. Madden, D. R., Gorga, J. C., Strominger, J. L. & Wiley, D. C. (1991) *Nature (London)* **353**, 321–325.
13. Falk, K., Rotzsche, O., Stevanovic, S., Jung, G. & Rammensee, H.-G. (1991) *Nature (London)* **351**, 290–296.
14. Jardetsky, T. S., Lane, W. S., Robinson, R. A., Madden, D. R. & Wiley, D. C. (1991) *Nature (London)* **353**, 326–329.
15. Silver, M. L., Parker, K. C. & Wiley, D. C. (1991) *Nature (London)* **350**, 619–622.
16. Parker, K. C., Silver, M. L. & Wiley, D. C. (1992) *Mol. Immunol.*, in press.
17. Parker, K. C., Carreno, B. M., Sestak, L., Utz, U., Biddison, W. E. & Coligan, J. E. (1992) *J. Biol. Chem.*, in press.
18. Dadaglio, G., Leroux, A., Langlade-Demoyen, P., Bahraoui, E.-M., Traincard, F., Fisher, R. & Plata, F. (1991) *J. Immunol.* **147**, 2302–2309.
19. Parker, K. C. & Wiley, D. C. (1989) *Gene* **83**, 117–124.
20. MacFerrin, K. D., Terranova, M. P., Schreiber, S. L. & Verdine, G. L. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 1937–1941.
21. Nagai, K. & Thogersen, H. C. (1987) *Methods Enzymol.* **153**, 461–481.
22. Buchner, J. & Rudolph, R. (1991) *Bio/Technology* **9**, 157–162.
23. Habeeb, A. F. (1972) *Methods Enzymol.* **25**, 457–464.
24. Stam, N. J., Spits, H. & Ploegh, H. L. (1986) *J. Immunol.* **137**, 2299–2306.
25. Parham, P., Androlewicz, M. J., Holmes, N. J. & Rothenberg, B. E. (1983) *J. Biol. Chem.* **258**, 6179–6186.
26. Lamb, J., Eckels, D., Lake, P., Woody, J. & Green, M. (1982) *Nature (London)* **300**, 66–69.
27. Robbins, P. A., Lettice, L. A., Rota, P., Santos-Aguado, J., Rothbard, J., McMichael, A. J. & Strominger, J. L. (1989) *J. Immunol.* **143**, 4098–4103.
28. Gotch, F. M., Rothbard, J., Howland, K., Townsend, A. R. & McMichael, A. J. (1987) *Nature (London)* **326**, 881–882.
29. Ways, J. P. & Parham, P. (1983) *J. Immunol.* **131**, 856–863.
30. Barnstable, C. J., Bodmer, W. F., Brown, G., Galfre, G., Milstein, C., Williams, A. F. & Ziegler, A. (1978) *Cell* **14**, 9–20.
31. Parham, P. & Bodmer, W. F. (1978) *Nature (London)* **276**, 397–399.
32. Morrison, J., Elvin, J., Latron, F., Gotch, F., Strominger, J. L. & McMichael, A. J. (1992) *Eur. J. Immunol.*, in press.
33. Bednarek, M. A., Sauma, S. Y., Gammon, M. C., Porter, G., Tamhankar, S., Williamson, A. R. & Zweerink, H. J. (1991) *J. Immunol.* **147**, 4047–4053.
34. Teng, T.-Y. (1990) *J. Appl. Crystallogr.* **23**, 387–391.
35. Elliott, T. J. & Eisen, H. N. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 5213–5217.
36. Elliott, T. J. & Eisen, H. N. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2728–2732.