Structure Determination of a Monoclonal Fab Fragment Specific for Histidine-containing Protein of the Phosphoenolpyruvate: Sugar Phosphotransferase System of Escherichia coli*

Lata Prasad†, Margaret Vandonselaar, Jeremy S. Lee, and Louis T. J. Debaere§
From the Department of Biochemistry, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0

(Received for publication, June 3, 1987)

Jel 42 is a monoclonal antibody specific for histidine-containing protein, a small phosphocarrier protein required for sugar transport in Escherichia coli. Fab fragments prepared from this antibody by papain digestion consisted of three major isoelectric forms which were separated on a chromatofocusing column. Two of these forms produced large crystals in space group P2₁, and unit cell dimensions a = 117.48 Å, b = 66.58 Å, c = 67.31 Å, and β = 118.7°, with two Fab fragments per asymmetric unit. Data were collected to 3.5-Å resolution. The structure of Fab Jel 42 was solved by the Molecular Replacement method (least-squares refined to R = 0.282) using the known structure of Fab HED 10 (12) as the search model; the amino acid residues of the hypervariable and elbow regions of Fab HED 10 were omitted from the starting model. A Fourier map calculated at this stage revealed electron density which corresponded to the hypervariable loops forming the antigen-binding crevice and the elbow region of Fab Jel 42. The elbow angles for the two independent Fab molecules are 159 and 187 degrees, similar to that of the Fab HED 10 search model which has an elbow angle of 162 degrees. There is no local noncrystallographic axis of symmetry relating the two molecules in the asymmetric unit.

Most antibodies recognize only one antigen, yet the diversity of the immune system is enormous. A major goal of current molecular immunology is to understand how specificity and diversity can be combined within the structural framework of antibody molecules. One approach to studying this problem involves x-ray diffraction studies of the Fab fragments of immunoglobulins (reviewed in Refs. 1 and 2). Recently the structure of a lysozyme-Fab complex was reported (3). The antigen/Fab interface is tightly packed with 16 amino acids on the lysozyme interacting with 17 residues on the Fab fragment. These authors detected no structural change in either the Fab fragment or the lysozyme molecule as a consequence of complex formation. More recently, however, the structure of a Fab fragment in a complex with influenza virus neuraminidase was reported (4). In contrast, these latter workers found that conformational changes occurred in both the Fab fragment and the enzyme as a consequence of the binding together of these two macromolecules. Thus some aspects of the specificity of antigen/antibody interactions could be understood from these studies.

Our approach to the problem of antibody specificity and diversity involves the study of Fab fragments which bind to HPr, a small phosphocarrier protein required for sugar transport in Escherichia coli (5). This system has several advantages: (a) HPr is a small protein (Mr = 9120) whose structure has been solved at neutral pH by NMR techniques (6) and at pH 3.7 by x-ray diffraction (7); (b) many mutants of HPr are available. Some of these mutants have been produced by site-directed mutagenesis and contain single amino acid substitutions at putative antibody binding sites. (c) HPr and mutants thereof are immunogenic and four monoclonal IgGs have been produced which bind to mutant and/or wild-type proteins; (d) With the aid of competitive solid-phase radioimmunoassay relative binding constants for the interaction between antibodies and various mutant HPrs can be rapidly assessed.

In this paper we describe the structure of Fab Jel 42 at 3.5-Å resolution. This antibody was raised against wild-type HPr (8); the binding constant to a mutant containing Lys-4 in place of Gln-4 is about 10-fold less. Moreover, the antibody does not inhibit phosphotransfer by HPr so that the surface of HPr to which Fab Jel 42 binds can be mapped with some precision.

MATERIALS AND METHODS

The preparation of antibody Jel 42 IgG has been published (9). However the procedure for separating the different species of Fab fragments has been modified from the preliminary report (10). Papain digestion of the intact IgG produced three major isoelectric forms of anti-HPr Fab fragments as shown in Fig. 1 (lane 4). These forms were separated by use of a chromatofocusing column and the individual species are shown in lanes 1-3 of Fig. 1. The forms b and c produced the largest crystals; the photomicrograph in Fig. 2 illustrates a crystal that was produced from form b (pH 8.6) in a solution of 1% Fab, 14% polyethylene glycol 6000, 0.2 M sodium chloride, 50 mM phosphate buffer at pH 8.0. The crystals are typically 0.7 × 0.7 × 0.5 mm in size with space group P2₁, with unit cell dimensions (that differ somewhat from the initial values (9)) a = 117.48 Å, b = 66.58 Å, c = 67.31 Å, β = 118.7°, and Z = 4. The crystals exhibit the forms [100], [011], and [011] and the solvent content is 47%.

Quantitative x-ray diffraction data were collected on a single native crystal to 3.5-Å spacings on an Enraf-Nonius CAD4 diffractometer. An ω scan of 0.5° was employed and 9358 reflections out of a total of 11,633 reflections collected over a period of 16 days had net I /σ(I) > 3. The merging Rmerge = 0.047 for 432 reflections that were merged; Rmerge = Σ|I - I(average)|/Σ|I|. The maximum applied absorption correction (10) was 1.35 and the maximum applied decay factor was 1.94. Four different heavy-atom derivative crystals were prepared but a subsequent electron density map at 4-Å resolution was not readily interpretable. The abbreviation used is: HPr, histidine-containing protein.

† To whom correspondence should be sent.
‡ Recipient of a Saskatchewan Health Research Board Fellowship.
§ E. B. Waygood, unpublished experiments.

1. S. Lee and E. B. Waygood, unpublished experiments.

* This research was supported by funds from the Medical Research Council of Canada (to J. S. L. and L. T. J. D.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1794 solely to indicate this fact.

E. B. Waygood, unpublished experiments.
exhibits the 8.4 of Jel 42 Fab fragments. The pH gradient is 8-10.5 (acidic at the bottom).

RESULTS AND DISCUSSION

Structure Solution—Coordinates from the HED 10 Fab fragment were kindly provided by Drs. W. F. Anderson and M. Cygler of the University of Alberta (12). It was hoped that these different Fab fragments would have sufficient structural homology to allow Molecular Replacement to succeed; the resulting structure of the anti-HPr Fab fragment shows that this was indeed the case. The starting model had the amino acid residues of the elbow and the hypervariable regions deleted from the HED 10 Fab fragment coordinates since these areas were expected to differ from the Jel 42 Fab fragment. Rotation functions were calculated using the Crowther algorithm (13), and the aforementioned method. Several combinations of resolution shell, radius of integration, and number of reflections were used. Two peaks appeared consistently in all of the maps. Elbow angles have been found to vary between 130° and 180° in Fab fragment crystal structures. Since the elbow angle of the model and the Jel 42 Fab fragment was not expected to be necessarily the same, four peaks were likely to occur in the rotation map, i.e. one peak each for the V and C domain pairs for the two molecules in the asymmetric unit as occurred previously for Cygler et al. (12). Therefore rotation functions were also calculated using only the V or the C domain pair for the starting model. However, the same two peaks appeared in both of these maps. It was then concluded that the elbow angle of the HED 10 model and the anti-HPr Fab fragment must be very similar and that the two peaks which were consistently obtained from the rotation maps were indeed the orientations of the two molecules in the asymmetric unit. The data from 10–5-Å d spacings were used in the final rotation and subsequent translation searches.

The next step was to determine the correct translations for the two crystallographically independent molecules in the unit cell. This was achieved by the method of Crowther and Blow (14). The two Fab fragment molecules were rotated into the orientations indicated by the rotation function peaks and a map of intermolecular vectors was calculated. From the vectors on this map the amounts by which the molecules had to be translated from their initial positions was calculated. Space group P21, has the y axis as a polar axis. Therefore the y translation of molecule 1 was set equal to 0 and the y translation of molecule 2 was determined relative to this origin. The rotational and translational parameters thus obtained were refined by varying these parameters somewhat to find a local minimum in the R value (15). The final parameters are the following:

<table>
<thead>
<tr>
<th>Molecule</th>
<th>α</th>
<th>β</th>
<th>γ</th>
<th>Δx</th>
<th>Δy</th>
<th>Δz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecule 1</td>
<td>4.61°</td>
<td>142.23°</td>
<td>236.49°</td>
<td>0.175</td>
<td>0</td>
<td>0.067</td>
</tr>
<tr>
<td>Molecule 2</td>
<td>59.93°</td>
<td>57.13°</td>
<td>87.97°</td>
<td>0.011</td>
<td>0.258</td>
<td>0.787</td>
</tr>
</tbody>
</table>

where α, β, and γ are the rotation Euler angles and Δx, Δy, and Δz are the translation fractional coordinates in order to obtain the correct placement of the two molecules in the unit cell. The above calculations were performed by using the MERLOT program package (16).

Refinement—The initial R value was 0.538 for the rotated and translated molecules. There were a total of 5268 nonhydrogen atoms included for both molecules out of a total of 5644 atoms which would be present in two HED 10 Fab fragment molecules. The 5268 atoms were used in subsequent calculations for the anti-HPr Fab fragments. Ten cycles of restrained least-squares refinement (17) produced an R factor of 0.282. The first four cycles had relatively loose restraints and were carried out on the diffraction data between 10 and 5-Å d spacings. The last six cycles had more stringent restraints and employed the data of 10-3.5-Å d spacings. At this stage a 3.5-Å electron density map was calculated by using coefficients that suppress model bias (18). The map was contoured, transferred to plexiglass sheets, and the α-carbon backbone was traced. The electron density corresponding to the amino acid residues which were omitted from the model,
FIG. 3. Stereoscopic view of molecule 1 of the Jel 42 Fab fragment. The distal portions of the complementarity determining regions are labeled as 1L, 2L, and 3L for CDR1, CDR2, and CDR3, respectively, of the light chain; the distal portions of the complementarity determining regions are labeled 1H, 2H, and 3H for CDR1, CDR2, and CDR3, respectively, of the heavy chain.

FIG. 4. Perspective view of molecules 1 and 2 showing their relative orientations.

e.g. those of the elbow and hypervariable regions, were evident on this Fourier map. In addition, the Cys-214 residue of the light chain which was missing from the HED 10 model was found for both molecules in the Jel 42 map; this residue forms a disulfide bridge with Cys-128 of the heavy chain and links the C terminus of the light chain with the heavy chain of the Fab fragment. The four intradomain (V_, VH, CH, CL) disulfide bridges were present in the model and were also evident in this map. The electron density map was transferred to an IRIS 3030 Computer Graphics System and displayed by using the FRODO software (19) which was converted and updated for the IRIS System (20). Glycine residues were inserted in the electron density which corresponded to the missing residues of the initial model since the amino acid sequence of the anti-HPr Fab fragment is presently being determined.4

A stereo drawing of the α-carbon atoms of molecule 1 of the Fab fragment is illustrated in Fig. 3. The distal portions of each complementarity determining region are labeled as 1L, 2L, and 3L for CDR1, CDR2, and CDR3, respectively, of the light chain and 1H, 2H, and 3H for CDR1, CDR2, and CDR3, respectively, of the heavy chain. At this stage of the structure determination, the antigen-binding region appears

* H. W. Duckworth, unpublished experiments.
to be that of a crevice formed by 1L, 2L, 1H, and 2H with 3H closing one side of the crevice. These hypervariable loops have a different conformation from those occurring in the HED 10 Fab fragment (12). As the amino acid sequence becomes available it will be substituted into the model and the refinement extended to higher resolution. The current elbow angle is 159° for molecule 1 and 167° for molecule 2. These are very similar to the 162° elbow angle of the HED 10 Fab fragment (12).

Fig. 4 shows the relative orientation of molecules 1 and 2 in the crystal. There is no local noncrystallographic axis of symmetry relating the two molecules. The contents of a unit cell are shown in Fig. 5. Molecules 1 and 2 alternate in a zigzag manner and form layers parallel to the 011. Molecule 1 in one layer interacts with molecule 2 in the adjacent layer in a head to tail fashion; the V domain of one molecule packs against the C domain of the neighboring molecule forming infinite chains along the x direction. Two adjacent layers are related by the 2-fold screw axis of symmetry. Molecule 1 and molecule 2 have similar elbow angles and similar packing arrangements. The detailed intermolecular interactions should be available after the amino acid sequence of Fab Jel 42 is completed.

Small crystals of the complex of E. coli HPr with anti-HPr Fab fragment have been obtained. It will be interesting to discover if any conformational changes occur upon complex formation and to compare this structure with the other published structures of complexes (3, 4).

Acknowledgments—We thank W. F. Anderson and M. Cygler for providing coordinates of the Fab HED 10 fragment, P. M. D. Fitzgerald for the MERLOT program package, and C. Balion for running the chromatofocusing column.

REFERENCES