Immunoelectron Microscopic Localization of Glutamyl-/Prolyl-tRNA Synthetase within the Eukaryotic Multisynthetase Complex*

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A high molecular mass complex of aminoacyl-tRNA synthetases is readily isolated from a variety of eukaryotes. Although its composition is well characterized, knowledge of its structure and organization is still quite limited. This study uses antibodies directed against prolyl-tRNA synthetase for immunoelectron microscopic localization of the bifunctional glutamyl-/prolyl-tRNA synthetase. This is the first visualization of a specific site within the multisynthetase complex.

Images of immunocomplexes are presented in the characteristic views of negatively stained multisynthetase complex from rabbit reticulocytes. As described in terms of a three domain working model of the structure, in “front” views of the particle and “intermediate” views, the primary antibody binding site is near the intersection between the “base” and one “arm.” In “side” views, where the particle is rotated about its long axis, the binding site is near the midpoint. “Top” and “bottom” views, which appear as square projections, are also consistent with the central location of the binding site. These data place the glutamyl-/prolyl-tRNA synthetase polypeptide in a defined area of the particle, which encompasses portions of two domains, yet is consistent with the previous structural model.

Aminoacyl-tRNA synthetases are the family of enzymes responsible for correctly linking tRNA molecules with amino acids so that the latter may be subsequently incorporated into growing polypeptide chains. In addition to this fundamental role in protein biosynthesis, these enzymes have additional cellular roles, such as synthesis of signaling dinucleotides. The aminoacyl-tRNA synthetase enzyme family is also widely used in the course of study of such processes as evolution of modular proteins and mechanisms of RNA-protein recognition (reviewed in Refs. 1 and 2). Recently, attention has turned to the aminoacyl-tRNA synthetases as potential targets for new antibiotics (reviewed in Ref. 3). Additional medical interest is due to the observation that the most widely recognized myositis-specific autoantibodies are directed against aminoacyl-tRNA synthetases (reviewed in Ref. 4).

A unique feature of aminoacyl-tRNA synthetases from higher eukaryotes is the existence of a high molecular mass multisynthetase complex. As isolated from a variety of cell types ranging from mammals to insects, this approximately 1 × 10^6-Da particle contains nine aminoacyl-tRNA synthetase activities: the arginyl-tRNA synthetase dimer, the aspartyl-tRNA synthetase dimer, glutaminyl-tRNA synthetase, isoleucyl-tRNA synthetase, leucyl-tRNA synthetase, the lysyl-tRNA synthetase dimer, methionyl-tRNA synthetase, and the glutamyl-/prolyl-tRNA synthetase (GluProRS), which is a bifunctional polypeptide. The multisynthetase complex also contains three additional proteins that are identified by their apparent masses: p43, which contains the complete sequence of the endothelial monocyte-activating polypeptide II cytokine (5); p38, which is of as yet unknown function; and p18, which may mediate association of the multisynthetase complex with elongation factor 1 (6).

In order to fully understand the biological function(s) of this intriguing particle, structural details of the multisynthetase complex are necessary. Thus far, electron microscopy studies have shown that the particle measures approximately 27 nm in diameter and appears to be a cup or elongated “U” shape (7) as suggested by the distinct triangular, rectangular, and square-shaped orientations visible in negatively stained electron micrographs. A general idea of the distribution of polypeptides within the complex has been gained through dissociation of the native particle into subcomplexes (8, 9). Additional evidence in support of interactions between certain of the synthetase polypeptides has been provided through use of the yeast two-hybrid system (10), binding experiments monitored by surface plasmon resonance (11), and chemical cross-linking (12, 13). Within the constraints of these data, a working model has been presented (13), which is composed of three domains arranged into a “Y”-shape made up of two “arms” and a “base.” The composition of each domain as well as likely positions of individual polypeptides within the domains have been suggested, but prior to this study there have been no precise localizations of specific components or sites within the multisynthetase complex.

This article describes the first localization of a particular polypeptide within the particle. Specifically, the bifunctional glutamyl-/prolyl-tRNA synthetase has been visualized by immunoelectron microscopy using an antibody directed against prolyl-tRNA synthetase.

EXPERIMENTAL PROCEDURES

PROTEIN PURIFICATION—The multisynthetase complex was isolated from rabbit reticulocyte lysate (Green Hectares) as described previously (13). Using antisera from two separate rabbits that were immunized with rat liver prolyl-tRNA synthetase (14), immunoglobulin was partially purified by precipitation with 45% ammonium sulfate, desalting with buffer exchange into 10 mM Tris-HCl, pH 7.5, over 10-DG columns (Bio-Rad), then chromatography over DEAE-Trisacryl M (IBF) eluting

1 The abbreviations used are: GluProRS, the bifunctional glutamyl-/prolyl-tRNA synthetase polypeptide; ProRS, prolyl-tRNA synthetase; HPLC, high performance liquid chromatography.

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with a 0–300 mM KCl gradient. As assessed by immunoblot analysis (15), the immunoglobulins specifically reactive with GluProRS were in a peak eluting with approximately 100 mM KCl.

Immunoelectron Microscopy—Samples of approximately 12 μg of multisynthetase complex in storage buffer (50 mM Hepes-NaOH, pH 7.4, 1 mM dithiothreitol, 0.5 mM EDTA, 50% glycerol) were incubated with 5 or 10 μl of one of the two immunoglobulin preparations plus 50 μl of 25 mM Hepes-NaOH, pH 7.2, 25 mM NaCl for 2 h at room temperature, then immunocomplexes were isolated by HPLC gel filtration (GPC 300 column, Alltech) in the same buffer. Three different preparations of multisynthetase complex were used. In order to stabilize the multisynthetase complex for electron microscopy glutaraldehyde was added to 0.3% (7) and then the immunocomplexes negatively stained with 1% aqueous uranyl acetate. Electron micrographs were obtained with a LEO 912AB microscope operated at 80 kV at absolute magnification of 31,500 or 40,000 using the spectrometer slit to increase contrast by removing inelastically scattered electrons.

Image Analysis—Immunocomplexes were identified on micrographs and the orientation of the multisynthetase complex and antibody binding site were recorded. Of 413 immunocomplexes analyzed, only 14 were inconsistent with the primary localization. For preparation of figures, electron micrograph negatives were digitized at an optical resolution of 1000 dpi using an Agfa Duoscan flatbed scanner. Composite figures were prepared using standard graphics programs (ImageWorks, Showcase and Snapshot) on a Silicon Graphics Indigo2 workstation, then converted to tif format.

RESULTS

Specificity of Antibody Binding—The original description and characterization of the antibodies directed against rat liver prolyl-tRNA synthetase (14) demonstrated specific reaction with the GluProRS polypeptide within the multisynthetase complex. That the immunoglobulin fractions prepared for this study retained that specificity was checked by immunoblot analysis (data not shown). Thus, immunoelectron microscopic visualization of the site of antibody binding is a reliable indicator of the position of this polypeptide within the particle.

Localization of GluProRS within the Multisynthetase Complex—Panel A of Fig. 1 shows a gallery of individual particles of negatively stained multisynthetase complex with one bound antibody. Examples of each type of characteristic orientation (7) of the particle are given. In column A, triangular views of the particle are presented. Panels 1–3 show a “Y”-like orientation in which two arms and a base are visible. These likely correspond to the three domains of the working model of the complex (13). The site of antibody binding is clearly seen to be near the division between one arm and the base. In panels 4–6 of column A, the particle appears slightly rotated along the long axis so that the cleft between the arms is no longer visible. These can be termed “intermediate” views. The site of antibody binding is again at the division between the large end of the triangle and the smaller base. This is consistent with the placement based on the first three panels. Column B shows rectangular views of the multisynthetase complex. These have been interpreted to correspond to an approximately 90° rotation around the long axis of the particle from the Y-like view (7). The symmetry of this projection precludes assigning one end or the other as an arm or the base. However, the site of antibody binding is consistently located at or near the midpoint of the long axis. This would correspond to the position seen in the triangular orientations, that is, near the division of the two portions of the particle. Column C shows square-like views of the multisynthetase complex. The images in panels 1–3 have a central “hole” into which stain has penetrated, while those in panels 3–6 do not. These are considered to be views from the “top” and “bottom” of the particle, respectively, given the interpretation that the triangular and rectangular orientations are “front” and “side” views of the “cup”. The site of antibody binding is on the periphery of the multisynthetase particle as suggested by the clear view of bound antibodies. It is also near the midpoint of the particle axis that is orthogonal to the rotational axis relating the triangular and rectangular orientations.

Panel B of Fig. 1 is a gallery of images where two particles of the multisynthetase complex are linked by a single antibody. The dimers shown in column A all contain one particle in which the central cleft between the arms is visible, that is, front views. In each, the site of antibody binding is again at or near the division between an arm and the base. In panels 3 and 5, the other antigen combining site of the antibody is linked to a multisynthetase particle in the rectangular orientation. Again, the binding site is near the center of the long axis of the complex. Panels 1 and 2 of column B clearly show antibodies linking the midpoints of two rectangular views and a rectangular with an intermediate orientation, respectively. The remaining images in column B show linked particles in the top or bottom orientations, which also emphasize the location of the antibody binding site near the midpoint of the complex.

Approximately 3% of immunocomplexes analyzed cannot be interpreted in a manner consistent with the existence of only one antibody binding site near the intersection of two domains of the multisynthetase particle. Examples of these are shown in Fig. 2. In column A are immunocomplexes that appear to have two antibody molecules bound to one multisynthetase complex. In each case, one site is consistent with that described above,
while the other is on the opposite side of the particle. These views suggest the presence of another binding site, but their rare occurrence indicates that it is likely nonspecific binding. In column B, two antibodies are also bound, but their binding sites are very close together. However, both are consistent with localization of the GluProRS polypeptide near the midpoint of the particle. Column C shows dimers that are linked by two antibodies. Panels 1 and 2 each have one multisynthetase particle in the “front” view, which again clearly show that the primary antibody binding site is near the intersection of an arm and the base. The rectangular view of one of the particles in panel 3 emphasizes the location of the epitopes near the midpoint of the long axis.

The above data are summarized in a schematic (Fig. 3) showing the relationship of the different particle orientations. When viewed from the front where the central cleft is visible, the major antibody binding site is near the division between one arm and the base of the particle. Partial rotation of the particle around the long axis gives an intermediate view, while the rectangular orientation can be considered the side view. In both, the primary antibody binding site is still clearly visible near the midpoint of the particle. The top and bottom orientations are obtained by orthogonal rotation, shown here from the intermediate view. Antibody binding is near the midpoint of one side of each square view. The area in the drawings representing the primary antibody binding site encompasses sections of both an arm and the base. This is to account for the variability of antibody location along the long axis as seen in both triangular (Fig. 1, panel A, column A) and rectangular (Fig. 1, panel A, column B) orientations, as well as the examples of closely spaced binding of two antibodies (Fig. 2, columns B and C). As suggested by the examples shown in column A of Fig. 2, a secondary minor binding site is depicted on the opposite side of the particle.

DISCUSSION

The immuno-electron microscopic data presented here provide the first visualization of a specific site within the multisynthetase complex. The GluProRS polypeptide has been localized within a defined area of the particle by mapping the binding of antibody directed against ProRS. The primary area of antibody binding observed in this study overlaps significantly with the placement of GluProRS in the base of the three domain working model, which is based largely on cross-linking data.

Although the primary area of antibody binding observed here is centered around the midpoint of the particle axes, it encompasses sections of both the domain termed the base as well as one arm domain. Not only is this range of locations noticeable in immunocomplexes containing one antibody (Fig. 1, panels A and B), but there are also images where two antibodies are bound in close proximity on the multisynthetase complex (Fig. 2, columns B and C). One explanation of these data is that the ProRS domain of GluProRS is very elongated and bridges the two particle domains. The range of binding site placements would thus be due to antibodies within the polyclonal immunoglobulin preparations that recognize multiple epitopes within the polypeptide. A second possibility is that the two closely bound antibodies are detecting two copies of GluProRS within the complex. A dimer can be envisioned to more easily cover the approximately 10-nm spread of the binding site. A stoichiometry of two copies of the bifunctional synthetase has been reported in complex from sheep liver (16), while others have observed variability from 1.0 to 1.5 in preparations from rat liver, rabbit liver, and rabbit reticulocytes (17). In addition, when mammalian prolyl-tRNA synthetase is isolated in its low molecular mass form, it occurs as a dimer (14, 18). It would thus seem likely that the bifunctional polypeptide form is also a dimer. However, in preparations from this laboratory, the estimated molar ratio of the GluProRS polypeptide has always been close to one (7, 9), but this is a relative value based on densitometric analysis of bands on polyacrylamide gels. The presence of small numbers of complexes with an additional GluProRS polypeptide bound at a separate site within the complex would explain the observation here of the second, but rare, antibody binding site. This would also be consistent with the specificity of binding demonstrated by immunoblot analyses.

If a monoclonal antibody to the GluProRS were available, the appropriate explanation could be readily determined and the binding site likely restricted considerably. As an alternative, tRNAPro and/or tRNAGlu can be used as structural probes for the two active sites within this bifunctional enzyme. Such experiments are currently under way.

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