Enzyme activity is restored to two defective \( \beta \)-galactosidase molecules (M15 protein lacking amino acid residues 11-41 and M112 protein lacking residues 23-31) by incubation with CNBr2 (residues 3-92 of \( \beta \)-galactosidase). M15 and M112 proteins (\( \alpha \)-acceptors) are dimers. Complemented enzyme, like wild type, has a tetrameric structure.

Cleavage of CNBr2 with glutamic acid-specific protease yielded a much smaller \( \alpha \)-donor (3-41 peptide) which was also effective in complementation, indicating that the M15 protein can supply all of the residues from 42-92 for the structure of complemented enzyme. Treatment of M112 protein/3-41 peptide complemented enzyme with trypsin under very mild conditions followed by examination of the products demonstrated that the \( \alpha \)-donor peptide supplies the NH\(_2\)-terminal segment of complemented enzyme. Similar trypsin treatment of M15 protein/CNBr2 indicated that in this complemented enzyme the polypeptide region beyond those residues missing in the \( \alpha \)-acceptor can be provided by either the \( \alpha \)-donor or the \( \alpha \)-acceptor.

Both M15 protein and M112 protein are more susceptible to mild trypsin proteolysis than complemented enzyme, indicating a more open structure. Several anti-peptide antibodies that react with these two proteins do not react with \( \beta \)-galactosidase. M112 protein, like M15 protein, can be activated by anti-\( \beta \)-galactosidase but to a much higher level.

Complementation, the restoration of a biological activity by interaction of two (or more) different proteins or protein fragments, is an intriguing phenomenon. The interaction is noncovalent and appears to have many elements in common with normal folding processes of proteins. Experimentally, the complementing fragments of \( \beta \)-galactosidase were studied in some detail. Under optimal conditions, M15 protein/CNBr2 complemented enzyme is as fully active as the native enzyme (8). Association is complete in about 2 h, and the kinetics favors a model of rapid complex formation followed by slow conformational change. Complemented enzyme is tetrameric and contains 4 molecules of the M15 protein subunit and 4 CNBr2 peptides. Very likely the segment of the polypeptide chain missing from M15 protein is involved in dimer-dimer interaction, because antibodies derived from NH\(_2\)-terminal peptides CNBr2 (residues 3-92) and T8 (60-140) do not bind to native enzyme but do bind to M15 protein (9). Also, this part of the native protein is not available to proteolysis by trypsin under conditions in which denatured \( \beta \)-galactosidase is easily digested (9).

M15 protein/CNBr2 contains two sets of overlapping or redundant sequences/protomer. One, nearest the NH\(_2\) terminus, is the stretch 3-10. The second is on the carboxyl side of the M15 protein deletion and comprises residues 42-92. M112 protein/CNBr2 has analogous overlaps at amino acids 3-22 and 32-92. In enzymatically active complemented enzyme, only one sequence in each overlap must be able to take part in the polypeptide interactions necessary to maintain the conformational integrity of the complex. For each set, which segment, that from M15 (or M112) protein or from CNBr2, is the important one? This paper describes structural studies on the \( \alpha \)-donor, \( \alpha \)-acceptor, and \( \alpha \)-complemented enzyme.

**Experimental Procedures**

**Materials**

Trypsin treated with \( \beta \)-lactoglobulin ketone and soybean trypsin inhibitor were obtained from Worthington and glutamic acid-specific protease of \( \beta \)-Galactosidase was purchased from Calbiochem. Streptococcal protein A-antibody absorbent was prepared as described (10). \( \beta \)-Galactosidase, M15 protein, M112 protein, CNBr2, and anti-peptide antibodies were used.

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isolated or prepared as reported in earlier papers from this laboratory (5–7, 11, 12).

Methods

Analytical Procedures—Assays for α-donor, α-acceptor, and α-complemented enzyme were as described before (5, 7). Incubation periods were for 3 h at 28 °C before β-galactosidase assay except where indicated. A radiomunnoassay was used to measure peptides corresponding to CNBr2 (12). High performance liquid chromatography was performed for analysis of peptide purity using a pH 5 sodium acetate-acetonitrile gradient and an Altex ODS-18 reverse phase column.

Gel Electrophoresis—Electrophoresis on 6% polyacrylamide slab gels was performed in Tris-glycine buffer, pH 8.3, in the absence of denaturant (13). Slab gels containing SDS1 were run using the discontinuous buffer system of Laemmli (14) with 6% separating and 3% stacking gels.

Heat Stabilities—Heat stability measurements of β-galactosidase, M15 and M112 α-acceptor proteins, and α-complemented enzymes were carried out at 53 °C in PM2 buffer (0.1 M sodium phosphate buffer, pH 7.0, 1.0 mM magnesium sulfate, 0.2 mM manganese sulfate, 0.1 M β-mercaptoethanol) containing 1 mg/ml of bovine serum albumin.

Protease Treatments—Proteolytic digestion of CNBr2 with glutamic acid-specific protease was carried out by incubating at 37 °C with a protease-peptide ratio of 1.50 (w/w) in 0.2 M ammonium bicarbonate containing 2 mM urea. Limited trypsic digestion was done at 4 °C in PM2 buffer at 1:50 or 1:100, trypsin:substrate. When necessary, the trypsic digestion was stopped by the addition of soybean trypsin inhibitor. Otherwise, the mixtures were chromatographed immediately on Sephacryl S-300 (1.5 × 90 cm) or subjected to electrophoresis on SDS slab gels.

Antibody Binding—The binding of antipeptide antibodies to M15 and M112 proteins was measured by incubation of 10 μl of the various antisera with 5, 12.5, 25, and 50 pmol of antigen in 150 μl of 0.1 M sodium phosphate, 0.01 M magnesium sulfate, pH 7.0, for 30 min at room temperature. Goat anti-rabbit γ-globulin (50 μg containing 2.5 precipitating units) was then added. After incubating, overnight at 4 °C, the mixture was centrifuged and the supernatant was assayed for α-acceptor activity.

Binding of anti-CNBr2 and anti-T8 to complemented enzyme was done in a similar manner. In a typical experiment, 1, 2.5, 5, or 10 pmol of complemented enzyme were incubated with 5 μl of antisera in a total volume of 300 μl of the same buffer. Antibody plus the antigen-antibody complex was precipitated with staphylococcal protein A-antibody absorbent. β-Galactosidase activity remaining in the supernatant was then measured.

RESULTS

α-Donor

Cleavage—CNBr2 was treated with various proteins in order to determine whether a smaller fragment might have α-donor activity. Hydrolyses contained no activity after trypsin or chymotrypsin treatment, but activity remained after cleavage by glutamic acid-specific protease from S. aureus. The NH2-terminal 92-residue segment of β-galactosidase (Fig. 1) contains glutamic acid residues at 17, 40, 41, 57, 67, 71, 75, and 80, but the first site of cleavage is residue 40 (or 41) because the sequence Glu-Asn-Pro- at positions 17–19 is not hydrolyzed (3).

Two peaks containing α-donor activity were seen after protease treatment of 14C-labeled CM-CNBr2 and fractionation on Sephadex G-50 (Fig. 2A). Neither material was radioactive and therefore cleavage had occurred NH2-terminal to the radioactive label at Cys 76. The larger component was approximately 70 residues and the smaller was estimated to be about 40 residues. This second component was then chromatographed on a sulfopropyl-Sephadex column as shown in Fig. 2B. The purity of the pooled fraction was at least 90% by high performance liquid chromatography analysis, and the NH2-terminal amino acid was found to be isoleucine. The composition is shown in Table I. These results indicate that the fragment corresponds to residues 3-40 and/or 3-41 of β-galactosidase. It is referred to here as 3-41 peptide.

Activity of 3-41 Peptide—A comparison of the α-donor activity of 3-41 peptide with CNBr2 is shown in Fig. 3. It can be seen that the level of activity reached is the same for both peptides, although more 3-41 peptide must be added to reach maximum activity. These results suggest that 3-41 peptide is as effective or nearly as effective an α-donor as CNBr2 but that it may bind more weakly to M15 protein.

α-Acceptor

Susceptibility to Proteolysis—The acceptor in α-complementation, M15 protein, was compared to β-galactosidase for susceptibility to proteolysis by exposure to trypsin under extremely mild conditions. At 4 °C for periods of at least 3 h, β-galactosidase is not touched by trypsin as indicated by examination on SDS gels (Fig. 4A, lanes 1–5) or by assay for enzyme activity (Fig. 4B). By contrast, M15 protein is readily and quickly attacked and is degraded to yield in part a 60,000-dalton product (Fig. 4A, lanes 6–9). Loss in α-acceptor activity correlates with the decrease in quantity of the 113,000-dalton band (Fig. 4A). Similar results (not shown here) were obtained with M112 protein.

Reaction with Antibodies—It had been shown previously that antibodies prepared against CNBr2 (residues 3–92 of β-galactosidase) and T8 (residues 60–140) do not bind to the native protein but do bind to the dimer (M15 protein) (9). These same antipeptide antibodies were tested against the dimeric M112 protein which lacks only 9 amino acids. Both anti-CNBr2 and anti-T8 react with M112 protein to about the same level as with M15 protein (Table II).

M15 protein can regain enzyme activity to some extent upon treatment with anti-β-galactosidase (15). This is also true for M12 protein (Table III). Indeed, the M12 protein can be activated to a level about one-fourth as much as reached with excess CNBr2, which is considerably higher than for M15 protein. The Fab fragment of anti-β-galactosidase is even more effective and can activate M112 protein half as well as CNBr2 (data not shown).

Complemented Enzyme

Heat Stability—Complemented enzymes prepared from M15 protein and the two α-donor peptides CNBr2 (residues 3–40) and 3–41 peptide from M112 protein and the same two peptides were tested for heat stability. It can be seen (Fig. 5) that all of these pairs lose enzyme activity at 55 °C, whereas β-galactosidase itself is completely stable. For comparison, the uncomplemented α-acceptors were also tested under these conditions. M15 protein and M112 protein are both much less stable as measured by their ability to function as α-acceptors after heat treatment.

Reaction with Antibodies—Anti-CNBr2 and anti-T8 were previously found to react with M15 protein/CNBr2 complemented enzyme whereas they do not bind to native β-galactosidase (9). This suggests that anti-CNBr2 and anti-T8 are binding to the overlapping sequences. Evidence to support this suggestion was obtained by testing the binding of the two antibodies to each of the complemented enzymes M15 protein/3–41 peptide, M112 protein/CNBr2, and M112 protein/CNBr2.

1 Staphylococcal protease treatment of the partially purified CNBr2 peptide from β-galactosidase containing tyrosine at position 41 (accompanying paper) yielded an active α-donor peak which eluted at the same position as 3–41 peptide on Sephadex G-50. This peptide was not purified to homogeneity but, on the basis of its elution volume, it corresponds to residues 3–40 of β-galactosidase. This result indicates that 3–40 peptide is also an effective α-donor.

β-Galactosidase α-Complementation

6805
Table 1

<table>
<thead>
<tr>
<th>Amino acid composition of 3-41 peptide</th>
<th>Molar ratio$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptophan</td>
<td>0.6 (2)</td>
</tr>
<tr>
<td>Arginine</td>
<td>3.9 (4)</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.9 (1)</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>4.5 (5)</td>
</tr>
<tr>
<td>Threonine</td>
<td>1.8 (2)</td>
</tr>
<tr>
<td>Serine</td>
<td>2.9 (3)</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>5.2 (5)</td>
</tr>
<tr>
<td>Proline</td>
<td>3.2 (3)</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.5 (1)</td>
</tr>
<tr>
<td>Alanine</td>
<td>4.1 (4)</td>
</tr>
<tr>
<td>Valine</td>
<td>2.4 (3)</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.4 (1)</td>
</tr>
<tr>
<td>Leucine</td>
<td>4.3 (4)</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1.2 (1)</td>
</tr>
<tr>
<td>No. of residues</td>
<td>39</td>
</tr>
<tr>
<td>Yield (%)</td>
<td>27</td>
</tr>
<tr>
<td>NH$_2$-terminal</td>
<td>Isoleucine</td>
</tr>
</tbody>
</table>

$^a$ Numbers in parentheses are number of residues based upon the amino acid sequence of $\beta$-galactosidase extending from residues 3-41 (7).

Fig. 1. The amino acid sequence of CNBr2. CNBr2 (residues 3-92). The bracketed regions indicate those amino acids absent from M15 protein (Δ11-41) and M112 protein (Δ23-31).

Fig. 2. Staphylococcal protease treatment of CNBr2 and purification of cleaved $\alpha$-donor peptide. A, 525 nmoi of CNBr2 were treated at 37 °C for 16 h with glutamic acid-specific staphylococcal protease at an enzyme/peptide ratio of 1:50 (w/w) in 0.2 M NH$_4$HCO$_3$ containing 2 M urea. Peptides were separated by gel filtration on Sephadex G-50 (2.5 X 200 cm) in 30% HAc. This procedure resolved two active $\alpha$-donor peaks, both of which eluted later than uncleaved CNBr2. B, the second peak was pooled and dried in vacuo, and the material was applied to sulfopropyl-Sephadex (1.5 X 20 cm) in 0.01 M ammonium formate, 8 M urea, pH 2.5, and peptides were eluted with a linear salt gradient (0 to 0.5 M NaCl in a total volume of 300 ml). The active $\alpha$-donor peak was pooled and dialyzed and the peptide was chromatographed on Sephadex G-50 (1.5 X 200 cm) in 30% HAc.

3-41 peptide (Table IV). It can be seen that neither antibody binds more than a trace level to M15 protein/3-41 peptide. Because 3-41 peptide does not bind to anti-CNBr2 (data not shown), the antigenic site(s) for anti-CNBr2 must lie at or beyond residue 41. Therefore binding of anti-CNBr2 and anti-T8 to M15 protein/CNBr2 must be to a portion within the redundant segment, residues 42-92.

Similar results were obtained with complemented enzyme containing M112 protein as $\alpha$-acceptor. Both antibodies bind strongly to M112 protein/CNBr2. As expected, anti-T8 binds to only a trace level to M112 protein/3-41 peptide, which contains no overlap corresponding to any of the segment, residues 60-140. However, anti-CNBr2 binds to a low but significant extent to M112 protein/3-41 peptide. The simplest explanation for this binding is that it is due to reaction between the antibody and the overlapping sequence 32-41. However, a conformational change, caused by the 32-41 re-
**β-Galactosidase α-Complementation**

**FIG. 4. Trypsin treatment of M15 protein and β-galactosidase.** A, electrophoresis of trypsin-treated β-galactosidase and M15 protein on 6% SDS-polyacrylamide gels. Lanes 1-5, β-galactosidase treated with trypsin for 0, 0.5, 1.0, 2.0, and 3 h at 4 °C. Lanes 6-9, M15 protein treated with trypsin for 0, 0.5, 1.0, and 3 h. Trypsin treatment of M15 protein for 1.0 and 3.0 h led to gradual destruction of the intact protein band and the higher molecular weight product. B, 2 µg of M15 protein or β-galactosidase were treated at 4 °C for the indicated lengths of time with trypsin at a trypsin/protein ratio of 1:50 (w/w) in PM2 buffer. After trypsin treatment, M15 protein was incubated with soybean trypsin inhibitor for 30 min at 4 °C before addition of excess CNBr2 and assay for β-acceptor activity (○○○). β-Galactosidase was assayed immediately after trypsin treatment (●●●).

**TABLE II**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Anti-CNBr2 (residues 3-92)</th>
<th>Anti-Te (residues 60-140)</th>
<th>nmol antigen bound/ml antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td>M15</td>
<td>4.8</td>
<td>1.0</td>
<td>4.3</td>
</tr>
<tr>
<td>M112</td>
<td>5.1</td>
<td>3.3</td>
<td>1.2</td>
</tr>
<tr>
<td>β-galactosidase</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**FIG. 5. Heat stability of complemented enzyme and M15 and M112 proteins.** β-Galactosidase and complemented enzymes were incubated in the presence of 1 mg/ml of bovine serum albumin at 53 °C for the indicated lengths of time and then assayed for enzymatic activity. M15 and M112 proteins were incubated at 53 °C in the same buffer and then incubated with excess CNBr2 for 3 h at 28 °C before assay for β-galactosidase activity.

**TABLE IV**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Overlapping sequences</th>
<th>Anti-CNBr2 (3-92)</th>
<th>Anti-Te (60-140)</th>
<th>nmol antigen bound/ml antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td>M15 protein/CNBr2</td>
<td>3-10; 42-92</td>
<td>2.6</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>M15 protein/3-41 peptide</td>
<td>3-10</td>
<td>Trace</td>
<td>Trace</td>
<td></td>
</tr>
<tr>
<td>M112 protein/CNBr2</td>
<td>3-22; 32-92</td>
<td>1.8</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>M112 protein/3-41 peptide</td>
<td>3-22; 32-41</td>
<td>0.6</td>
<td>Trace</td>
<td></td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td></td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE III**

Anti-β-galactosidase activation of M112 and M15 proteins

520 α-acceptor units of M15 and M112 proteins (crude extracts) in 100 µl of PM2 buffer were incubated with an equal volume of anti-β-galactosidase for 3 h at 28 °C before assay of enzymatic activity.

<table>
<thead>
<tr>
<th>Serial dilution</th>
<th>M112 protein</th>
<th>M15 protein</th>
<th>% activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:8</td>
<td>7.2</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>1:16</td>
<td>26.7</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>1:32</td>
<td>24.7</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>1:54</td>
<td>22.6</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>1:128</td>
<td>12.0</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>1:256</td>
<td>5.4</td>
<td>0.3</td>
<td></td>
</tr>
</tbody>
</table>

**Cleavage of Overlapping Sequences—The same complemented enzyme, M112 protein/3-41 peptide, was used to determine which chain is the structurally important one at the NH2-terminal side of the overlap. M112 protein has arginine residues beginning at positions 13 and 14 (Fig. 1). After mild trypsin treatment of the complex, an active product was seen on gel electrophoresis in the absence of denaturant (Fig. 6A, lane 2) that had a mobility similar to β-galactosidase (lane 3) and different from that of the untreated complemented enzyme (lane 1).

The cleaved complex was purified on Sephacryl S-300 and treated next with cyanogen bromide, and the digest was passed through Sephadex G-50. Fractions were assayed for α-donor activity (Fig. 6B). The active component migrated identically with authentic 3-41 peptide. Therefore, this peptide (which contains no methionine and could not be cleaved...
Subject to tryptic hydrolysis under the mild conditions used. With trypsin at 4 °C, electrophoresis in SDS (Fig. 6), M112/3-41 peptide was treated with trypsin at 1:50 (w/w) for 2 h at 4 °C. 98% of the initial activity remained. A, a portion of the sample was then applied to polyacrylamide gel electrophoresis under nondenaturing conditions. Lane 1, M112 protein/3-41 peptide; lane 2, M112 protein/3-41 peptide after trypsin treatment; lane 3, β-galactosidase. B, the remaining material was chromatographed on Sephacryl S-300 in 0.1 M NaPO₄, 0.005 M β-mercaptoethanol, 1 mM MgCl₂, pH 7.0, and the active pool was digested with cyanogen bromide before Sephadex G-50 (1.5 × 200 cm) gel filtration chromatography in 30% acetic acid. Fractions were assayed for α-donor activity and cross-reactivity with anti-CNBr2. Arrows mark the elution positions of CNBr2 derived from M112 protein and of 3-41 peptide.

By cyanogen bromide (CNBr2) was not touched by trypsin, even though it contains 3 susceptible arginine residues. Fractions were also examined for cross-reactivity to anti-CNBr2 using inhibition of labeled T8 binding as the assay. This test will not measure 3-41 peptide, but it will detect the peptide segment containing residues 60-92. It can be seen that the cross-reactive component is smaller than that obtained directly from M112 protein (81 residues) and larger than 3-41 peptide (39 residues) (Fig. 6B). Indeed, the peak of the cross-reactive material migrates as expected for a peptide of about 69 or 70 residues, corresponding to cleavage of M112 protein at residues 13 or 14 in the complemented enzyme. Therefore, the NHz-terminal segment of complemented enzyme is provided by the α-donor.

Cleavage experiments were also carried out with complemented enzyme containing M15 protein as α-acceptor. In M15 protein/3-41 peptide, the only redundancy is the segment 3-10. This sequence contains no trypsin-sensitive residue in the overlap (Fig. 1) and therefore the complex should not be subject to tryptic hydrolysis under the mild conditions used. This was in fact the experimental result. After 3 h of treatment with trypsin at 4 °C, electrophoresis in SDS (Fig. 7A, lanes 1-4) indicated that M15 protein was unchanged in size.

M15 protein/CNBr2 complemented enzyme contains overlaps at the segments 3-10 and 42-92. Trypsin-sensitive arginine residues in the overlapping regions are present at positions 43, 52, and 59. In order to determine which component supplies the structural part of the sequence comprising 42-92, the complemented enzyme was treated with trypsin as before. When the product was subjected to electrophoresis in SDS, it could be seen that M15 protein was converted in part to two smaller forms (Fig. 7B, lanes 5-8). This result means that segment 42-92 of complemented enzyme can be supplied either by M15 protein or CNBr2. Examination of the α-donor showed that it was also partially cleaved (Fig. 7B). After purification of trypsin-treated complemented enzyme and digestion with cyanogen bromide, gel filtration on Sephadex G-50 gave two peaks of α-donor activity, one of a size identical with authentic CNBr2 and a second fraction smaller in size. Taken together, these results indicate that the segment 42-92 from either the α-acceptor or the α-donor may act as the structural part of the active enzyme.

**Discussion**

β-Galactosidase α-complementation has some elements in common with the well known association in ribonuclease of the two fragments S-peptide and S-protein produced by sub-
β-Galactosidase α-Complementation

β-Galactosidase α-complementation is a phenomenon where one protein can activate another to a higher level of activity, especially when the two proteins are brought together. This phenomenon has been extensively studied in the context of the bacterial enzyme β-galactosidase, which is a tetrameric enzyme composed of four identical subunits. In most complementation systems, one protein acts as an α-acceptor and the other as an α-donor. The α-acceptor provides a portion of the active site, while the α-donor provides the missing residues for full activity.

Complementation occurs when the α-donor and α-acceptor proteins are brought together. The α-acceptor can be a subunit of the native enzyme or a component derived from a deletion mutant. The α-donor can be a subunit of another enzyme or a component derived from a deletion mutant. In many cases, the α-donor and α-acceptor are complementary in that they can provide the missing residues for each other.

The α-acceptor region of β-galactosidase is in the NH2-terminal fragment of the protein (15). In both systems, enzyme activity is restored by noncovalent binding of an NH2-terminal fragment to a larger fragment comprising the remainder of the protein. Simply on the basis of size, however, α-complementation is more complex. The β-galactosidase polypeptide chain is more than eight times larger than that of ribonuclease, and the latter is a monomer, while β-galactosidase is a tetrameric molecule. Also, α-complemented enzyme contains overlapping or redundant sequences, while the ribonuclease S-peptide:S-protein complex does not.

When complemented enzyme is formed with M15 protein (lacking amino acids 11-41) as the α-acceptor and CNBr2 (residue 3-92) as the α-donor, redundancies exist at residues 3-10 and 42-92. We have now asked which of these overlapping segments are provided by the α-donor and which are provided by the α-acceptor in the three-dimensional structure of complemented enzyme. The results presented here show that 3-41 peptide is a fully active α-donor. Therefore, M15 protein can supply all of the 42-92 region for complemented enzyme. The situation is different when CNBr2 is the α-donor. In this case, either the α-donor or the α-acceptor may provide structural polypeptide for complemented enzyme in the 42-92 region. This conclusion is based upon the fact that both M15 protein and CNBr2 are reduced in size after mild proteolysis of M15 protein/CNBr2 with trypsin. The finding that either component can provide structural polypeptide is not without precedent. A similar equilibrium has been described for staphylococcal nuclease and cytochrome c (2, 17).

The NH2-terminal region of M15 protein has no trypsin-sensitive bonds in the NH2-terminal redundant segment. However, M112 protein, which lacks residues 23-31, contains trypsin-sensitive arginine residues at positions 13 and 14. After proteolysis of M112 protein/3-41 peptide, the 3-41 peptide was intact but M112 protein was slightly smaller. These results indicate that the α-donor is providing this part of the structure and also that residues 1 and 2 of β-galactosidase are not important for structural integrity or enzyme activity.

Antibodies prepared from specific peptides can be useful probes of conformation of a protein. In earlier results, it was shown that anti-CNBr2 does not bind to native β-galactosidase (15). M15 protein/3-41 peptide has only the NH2-terminal overlap to residue 10 and would be expected to react only to a small extent or not at all with anti-CNBr2. On the other hand, M15 protein/CNBr2, with the additional overlap 42-92, could bind strongly with the antibody. These were exactly the results obtained.

For most studies on α-complementation, M15 protein has been used as acceptor. This protein is a dimer but clearly has a similar conformation to that of β-galactosidase itself. Both proteins bind to the same kind of affinity column (5, 18), and M15 protein can be activated to some extent by anti-β-galactosidase. Recently, M112 protein, from another lac2 deletion mutant strain, has been isolated and its structure determined. It is a dimer also but lacks only nine amino acids (6). We now find that M112 protein can be activated by anti-β-galactosidase and in fact is activated to a much higher level than is M15 protein. Activation involves a large increase in molecular weight. Assuming that tetramer formation occurs, it seems plausible that some residues in the segment absent from M15 protein but present in M112 protein also are important in the binding of two dimers to form active tetramer.

No differences in stability or activity were seen among the various complementing partners, but complemented enzymes were less stable than native enzyme and more stable than the uncomplemented dimers, M15 protein and M112 protein. The latter are considerably more sensitive to proteolysis in the uncomplemented than in the complemented state. Under conditions where only overlapping sequences are cleaved by trypsin, both dimers are converted to smaller fragments. This result indicates that M15 protein and M112 protein have more open structures that are made more compact and less available to proteolysis upon complementation. Perhaps the generalization can be made that subunits of an oligomeric enzyme are less stable when free and that this is one evolutionary stimulus toward development of multisubunit proteins. Similar enhanced degradation of isolated subunits relative to their oligomerized counterparts has been observed for the α-chains of hemoglobin (19) and tryptophan synthetase (20) and for the β-chain of RNA polymerase (21).

Whether the α-donor peptide can contain fewer residues than the 3-41 (or 3-40) peptide is unknown. At present, there seems to be no general rule concerning the necessity for overlaps in other characterized complementing systems. In RNase, 1-15 peptide complements the S-protein residues 21-124 (22). In cytochrome c, 1-25H, 28-38, and 56-104 form fully complemented enzyme (23). In thioredoxin and staphylococcal nuclease, fragments which contain no overlapping sequences complement one another (1, 24). In contrast, complementation of glycogen phosphorylase, a tetrameric protein, requires over-lapping segments rather than those that just supply the missing residues (25). Similarly, α-complementation in β-galactosidase requires overlapping segments; CNBr24 complements X90 protein, but T80, containing no overlap, does not (26). This may be due to further complexities involved in intersubunit association. It would clearly be of interest to know the minimum structural requirements for α-complementation.

Acknowledgments—We thank David Dupont and Paulette Osborne for excellent technical assistance.

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