Localization of Lipid-Protein and Protein-Protein Interactions within the Murine Retrovirus gag Precursor by a Novel Peptide-mapping Technique*

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In HTG2 hamster cells infected with the replication-defective Gazdar murine sarcoma virus only immature virus particles are formed, with the uncleaved gag precursor Pr65 as the only major protein in the virion. We have investigated the structure of these particles by using in situ cross-linking followed by chemical and enzymatic cleavages of Pr65 to localize sites of lipid-protein and protein-protein interactions. Lipid-protein cross-links were localized within a 10-kDa fragment in the p15 region of Pr65. Homotypic protein-protein cross-links between Pr65 units were localized within the p15 regions and also within the p10 regions of Pr65. Similar data for processed gag proteins in Rauscher murine leukemia virus, a prototype of a mature C-type virus, suggest that these interactions of the gag precursor are not altered during maturation. To identify the sites of cross-linking within Pr65, we have developed a two-dimensional peptide mapping technique that is based on nearest neighbor analysis of fragments released by cyanogen bromide treatment of partial cleavage products in gel slices. In conjunction with cross-linking, the peptide mapping technique is a powerful means for localizing specific interactions on a polypeptide backbone.

Infectious retroviruses package three classes of virus-encoded proteins that are derived from the gag, pol, and env genes. These proteins are cleaved proteolytically from large precursor polyproteins during assembly (1). The gag proteins account for about 80% of the protein in the virus, forming the internal structure that is visible in thin sections by electron microscopy. The env or surface glycoproteins make up 20% of viral protein, forming complexes that protrude outward from the virion. Reverse transcriptase (pol) accounts for only a few per cent of the total protein. Although all these proteins play important roles in infection and in replication of the virus, studies with viral mutants suggest that only the gag proteins are required for assembly of the virion (2). Thus, an understanding of the interactions among the gag proteins is essential to understanding assembly.

In murine retroviruses, the gag precursor Pr65 is cleaved into four internal structural proteins which are ordered NH2-p15-p12-p30-p10-COOH on the precursor. Of these proteins, p10 is associated with the viral RNA (3), p30 is associated with the core shell (4, 5), and p15 is associated with the viral membrane (6, 7). The location of p12 is uncertain although a minor amount interacts specifically with viral RNA (8). The homotypic interactions of p30 (9), p15 (Ref. 7; see below), and p10 (see below), presumably reflecting the structural organization of the virus, are consistent with models in which different viral proteins are arranged in concentric shells (4) surrounding the ribonucleoprotein core. Immature A-type viruses, which contain unprocessed precursor, must have a different arrangement of the gag proteins in the virion since they have a distinctive morphology, but the molecular nature of these differences has not been investigated. The small fraction of immature particles in most preparations of virus makes them difficult to study. Preparations enriched in immature viruses have been obtained with mutants that are temperature sensitive for assembly (10, 11). With these mutants, a population of immature particles can be produced synchronously after temperature shift, which then can undergo normal processing. In this manner, proteolytic cleavage of the precursors has been correlated directly with the changes in morphology and in infectivity that are observed during maturation (10, 11). However, like wild type virus, the temperature-sensitive mutants are difficult to analyze structurally since immature particles exist only transiently.

A second type of assembly mutant, represented by Gazdar MSV,1 in the hamster cell line HTG2, produces immature A-type particles in which Pr65 is not processed. Unlike other mutants, these virions are permanently fixed in the immature state. Pr65 from Gazdar MSV is immunologically (12, 13, 14) and structurally (14, 15) similar to other MuLV Pr65 polyproteins, suggesting that the defect in its processing is in the virus-associated protease (16) or in modification enzymes such as kinases (17) that also may play a role in assembly. Since the virions are morphologically similar to the transient A-type particles of wild type virus, and since Pr65 is the only major protein in Gazdar MSV (14), this virus provides a unique opportunity to study interactions of the gag precursor that exist in the immature particles. Here we have combined a new peptide-mapping technique and bifunctional cross-linking to localize protein-protein and lipid-protein interactions in Gazdar MSV. These studies suggest that many of the interactions that exist in the mature virion occur prior to cleavage of the gag precursor.

MATERIALS AND METHODS

Viruses—The cell lines HTG2, producing Gazdar MSV (13, 18), and JLSV-9, producing Rauscher MuLV, were grown at 36 °C as monolayers in Dulbecco's modified Eagle's medium containing 10% fetal calf serum and antibiotics (penicillin, 50 units/ml; streptomycin, etc.)

1 The abbreviations used are: MSV, murine sarcoma virus; DMS, dimethyl suberimidate; MuLV, murine leukemia virus; PDM, phenyl reine dimaleimide; DFDNB, difluorodinitrobenzene; SDS, sodium dodecyl sulfate; CNBr, cyanogen bromide.

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50 µg/ml). Procedures for metabolic labeling with [14C]glycine and with [14C]ethanolamine HCl and for virus purification on isopycnic sucrose gradients were as described (27). Yields for labeled Gazdar MSV were about 5 times lower than those described for other viruses.

Cross-linking of Virus—Gazdar MSV (100 µg/ml) in virus suspension buffer (5% sucrose, 50 mM triethanolamine HCl, pH 8.3, 100 mM NaCl, 1 mM EDTA), either [14C]phosphatidylethanolamine-labeled for lipid-protein cross-linking or unlabeled for protein-protein cross-linking, was incubated with 0.5 mg/ml DMS (Pierce Chemicals) for 2 h at 23°C. Virus was then diluted 10-fold in virus suspension buffer, centrifuged to form a pellet at 4°C in a 50 Ti rotor (1 h, 33,000 rpm), and immediately disrupted in electrophoresis sample buffer (2% SDS, 50 mM Tris-HCl, pH 6.8, 12.5% glycerol, 2% 2-mercaptoethanol). Samples were then electrophoresed in SDS-polyacrylamide gels (15% acrylamide, 0.06% N-methylene bisacrylamide) according to the procedure of Swank and Munkres (19). Stacking gels contained 7.6% acrylamide and 0.21% bisacrylamide. Unlabeled proteins were visualized by staining with Coomassie brilliant blue R. Radioactive protein component of Gazdar murine sarcoma virus consists of one major protein, the gag precursor Pr65. When gel slices containing Pr65 are treated with cyanogen bromide, nine fragments are generated. The resolution of these fragments after SDS-urea-polyacrylamide gel electrophoresis is shown in lanes a–c of Fig. 1. The relative intensities of the nine cleavage fragments vary as a function of CNBr concentration and reaction time, but no additional fragments are generated. The effect of concentration on the cleavage profile of Pr65 is evident from a comparison of lanes b and c in Fig. 1. After a 1-h incubation with CNBr, 25% of the Pr65 remained intact at 30 mg/ml CNBr (lane b), and only about 2% at 100 mg/ml CNBr (lane c). Varying incubation time from 30 min to 1 h had the same effect as varying CNBr concentration by a factor of 2 (not shown). However, reaction times were kept under 1 h both to prevent leaching of polypeptides from the gel slices and to prevent acid-induced cleavage reactions.

Complete cleavage products can be distinguished from partial products by their resistance to further cleavage in a subsequent treatment with CNBr. We developed the following procedure to perform such an analysis rapidly. Samples first are treated as described in Fig. 1. The entire longitudinal gel strip is excised, incubated with CNBr, and the resulting products electrophoresed orthogonally out of the strip into an SDS-urea slab gel. Complete cleavage products, which resist cleavage in this second CNBr treatment, yield only a single fragment. Partial cleavage products yield two or more fragments. A silver-stained pattern from such a gel for analysis of Pr65 is shown in Fig. 2. At the top is a parallel first dimension strip, silver stained directly, that displays the nine original cleavage products (denoted b–j). Of the nine fragments, four (tracks f, g, i, and j) are resistant to the second cleavage, and thus define the four complete products (1, 2, 3, and 4) that are referred to throughout the text. The split of fragment 4 into two closely migrating species is frequently

RESULTS

Ordering of the Cyanogen Bromide Fragments of Pr65—The protein component of Gazdar murine sarcoma virus consists of one major protein, the gag precursor Pr65. When gel slices

FIG. 1. Localization of phospholipid cross-link to a 26-kDa cyanogen bromide fragment at the NH2 terminus of Pr65. [14C]Glycine-labeled Gazdar MSV and [14C]ethanolamine-labeled virus that was cross-linked with DMS were disrupted in electrophoresis sample buffer and subjected to SDS-polyacrylamide gel electrophoresis. The Pr65 regions were excised with a razor blade and stored at −20°C. The gel slices were incubated with CNBr for 1 h at 23°C and washed, and cleavage products were electrophoresed into an SDS-urea polyacrylamide gel. Radioactive spots were visualized by fluorography. Lanes a–c are from [14C]glycine-labeled Pr65. Lanes d–f are from Pr65 [14C]phosphatidylethanolamine complexes. Lanes a and d, 0.5 mg/ml CNBr; lanes b and e, 30 mg/ml CNBr; lanes c and f, 100 mg/ml CNBr. 1, 2, 3, and 4 correspond to the complete cleavage products defined in Fig. 2.
complete cleavage products. At the five other products of Pr65 were electrophoresed into an SDS-urea-polyacrylamide gel. As the appropriate partials, partials derived from Pr65 by a two-dimensional electrophoretic analysis. Gel slices containing 10 pg of Pr65 from Gazdar MSV were treated with 100 mg/ml CNBr for 1 h at 23 °C and washed, and the cleavage fragments were electrophoresed into an SDS-urea-polyacrylamide gel. The entire strip was then treated with 200 mg/ml CNBr and fragments were electrophoresed into an SDS-urea slab gel. A silver stain of this gel is shown. 1-4 at the left correspond to the complete cleavage products. At the top is a one-dimensional gel profile of cleavage products from 1 µg of Pr65 that was silver stained directly. Tracks b-j mark the nine cleavage products observed and does not represent a different cleavage product. The five other products (tracks b, c, d, e, and h) are cleaved, each releasing fragments that form a subset of the nine original fragments.

The four complete cleavage fragments can be ordered 1-2-3-4 by nearest neighbor analysis using information derived from the partial products. Of the five partial fragments, three release two of the final products (tracks c, e, and h) and two (tracks b and d) release three of the complete products as well as the appropriate partials. Partial c, e, and h, which release fragments 1-2, 2-3, and 3-4, respectively, define the order of the fragments as 1-2-3-4. The other partials, 1-2-3 (track b) and 2-3-4 (track d), are consistent with this analysis.

The mapping information for Pr65 is summarized schematically in Fig. 3. A shows the positions of methionines in Pr65, as inferred from the nucleic acid sequence of the Rauscher and Moloney MuLV genomes (2). The three methionines, at positions 193, 394, and 473 out of 533 amino acid residues, divide Pr65 from NH2 to COOH terminus into two large followed by two small fragments. Since the CNBr treatment also generates two large and two small cleavage products, we infer that fragment 1 contains the NH2 terminus. We have confirmed the identity of all four fragments by immunoblotting analysis (30), using antisera directed against each of the mature viral proteins to probe the CNBr cleavage fragments after electrophoretic transfer to nitrocellulose (not shown). Fig. 3B summarizes the CNBr cleavage data in a schematic gel profile. The fragmentary composition of the different partial products is indicated at the right of the diagram. This electrophoretic profile serves as a fingerprint of Pr65 in the experiments that follow.

Localization of the Site in Pr65 Associated with the Membrane—We have shown previously (7) that the association of certain proteins with membrane, in particular p15 of Rauscher MuLV, can be identified by lipid-protein cross-linking. This result was obtained by cross-linking [3H]phosphatidylethanolamine-labeled Rauscher MuLV with DMS and then identifying lipid-protein complexes from fluorographs of SDS-polyacrylamide gels. When Gazdar MSV is analyzed similarly, Pr65 also is cross-linked to radioactive lipid. This result is shown in Fig. 4, lane f. No label is detected in Pr65 without cross-linker (lane e) or when virus is cross-linked in the presence of 1% SDS (not shown). Quantitation of the cross-linked fraction as described previously (7) suggests that a majority of the Pr65 molecules reacts with the radioactive lipid under these conditions. Radioactive lipid is also detected in DMS-induced dimers (Pr65), trimer (Pr65), and high molecular weight complexes that do not enter the gel.

The site of lipid-protein cross-linking within Pr65 can be localized by CNBr mapping. As shown in lanes d-f of Fig. 1, a series of three radioactive spots is generated when gel slices containing the [3H]phosphatidylethanolamine-Pr65 complexes are incubated with CNBr and then subjected to electrophoretic analysis. These spots correspond to fragment 1 and the two fragment 1-containing partials, which localizes the interaction with membrane within the p15-p12 third of Pr65.

The lipid cross-link in fragment 1 can be localized further by limited proteolysis with S. aureus V8 protease. The effect of varying the concentration of V8 protease on cleavage data. The distribution of methionines in Pr65 based on a combination of data from nucleic acid sequencing (29) and protein sequencing (33) is shown schematically in A. The three methionines divide Pr65 into four complete fragments: CNBr-1 (26 kDa), containing p15 plus most of p12; CNBr-2 (22 kDa), containing the remainder of p12 plus the NH2 two-thirds of p30; CNBr-3 (9 kDa), containing the COOH third of p30 minus the last four amino acids; and CNBr-4 (7 kDa), containing the remainder of p30 plus all of p10. In B, these data are summarized with respect to the electrophoretic display of cleavage fragments. Lane a, gel profile; lane b, schematic profile. Numbers correspond to the complete fragments indicated in A.
profiles of [14C]glycine-labeled Pr65 is shown in lanes c–e of Fig. 5. About 70% of the Pr65 remained intact after incubation with 50 μg/ml V8 protease (lane d), while less than 10% of the Pr65 remained intact after a similar incubation with 150 μg/ml V8 protease (lane e). When lipid cross-linked Pr65 is incubated with V8 protease over the same range of protease concentrations, only one major cleavage product is generated, a 10-kDa fragment. This result is shown in lanes h–j of Fig. 5. Minor intermediate products at 25, 40, and 55-kDa are also detected, but reduction of the amounts of these products at the higher protease concentrations indicates that they are partial cleavage products. Since CNBr mapping implies that the lipid-protein cross-links in Pr65 are located within either p15 or p12, and since the smallest p12-containing fragment from Pr65 is over 20-kDa (26), we infer that the 10-kDa fragment is derived from within p15. Such a fragment should be generated from the NH2 terminus of p15 based on the DNA sequence.

In support of this identification, V8 cleavage of mature p15 after in situ cross-linking of [14C]phosphatidylethanolamine-labeled Rauscher MuLV also produces a lipid-containing 10-kDa fragment. This result is shown in lane g of Fig. 5. The 10-kDa fragment released from p15 co-electrophoreses with the 10-kDa fragment released from Pr65 (lane e), suggesting that the two lipid-containing fragments are identical. Parallel cleavage of [14C]glycine-labeled p15, demonstrating the extent of cleavage, is shown in lane b. Under these conditions, V8 protease only cleaves p15 once and the smaller of the two cleavage fragments is not observed since it does not contain...
glycine. The slight differences in mobility of the lipid-containing fragments and the corresponding fragments without cross-linking can be attributed to the perturbation resulting from covalent attachment of the phospholipid.

**Protein-Protein Interactions within Pr65**—When Gazdar MSV is treated with 0.3 mg/ml DMS, dimers, trimers, and higher molecular weight complexes of Pr65 are generated. This result is shown in lane j of Fig. 4. The electrophoretic complexes are marked with subscripts. These complexes are not detected without added cross-linker (lane g) and are observed over a narrow concentration range of DMS. Above 2 mg/ml DMS, all of the Pr65 is converted into complexes too large to enter the gel (not shown). Prior treatment of the virus with the nonionic detergents 1% Triton X-100 or 1% Nonidet P-40 does not affect the pattern of cross-linked products, while prior treatment with the ionic detergents 1.4% sodium cholate, 1.4% sodium deoxycholate, or 1% SDS blocks the appearance of cross-linked products (not shown).

The protein-protein cross-links that generate the three DMS-induced dimers can be localized within Pr65 by the peptide-mapping technique. For each of the three dimers, we have localized the cross-linking to a single cleavage fragment based on differences in the cleavage profiles for cross-linked and uncleaved Pr65. These results are shown by the cleavage profiles for the three DMS-induced dimers in tracks b–d of Fig. 6. The most prominent differences that distinguish the cleavage pattern of dimer 1 (track b) from the cleavage profile of Pr65 (track a) are the reductions in intensity of three spots: fragments 4 (denoted by arrow) and the two fragment 4-containing partials (4-3, 4-3-2). The six other Pr65 cleavage products are unchanged in this dimer. The small amounts of fragment 4 and its partials that remain visible in track b (less than 10% of the theoretical yields based on densitometry) derive from background smearing of Pr65-containing species throughout the gel track, since similar amounts of these bands are generated by CNBr cleavage of proteins of the original gel strip both above and below the dimer bands. New spots are also observed that presumably contain additional fragment combinations generated by the protein-protein cross-linking. However, these products have not been characterized. We interpret the absence of fragment 4-containing cleavage products to mean that the DMS linkages(s) in dimer 1 are homotypic, coupling fragment 4 on one molecule of Pr65 to the same region on another. By similar arguments, we infer from the cleavage profiles of the other dimers (tracks c and d), which themselves are nearly identical, that they were generated by homotypic cross-links in fragment 1. We have not attempted to determine the origin of the mobility difference of the two fragment 1 cross-linked dimers.

Fragment 4 consists of p10 plus four amino acid residues from the COOH terminus of p30. These four amino acids include a lysine, which in mature MuLV is a site at which reversible cross-linker dithiobispropionimidate and the cross-linked products analyzed by diagonal SDS-polyacrylamide gel electrophoresis (31), we observed that the 45- and 15-kDa fragments are the major cleavage products (lane f, Fig. 4). When gel slices containing the 45-kDa fragment are treated with CNBr and the cleavage fragments analyzed by SDS-urea polyacrylamide gel electrophoresis (lane j, Fig. 6), a pattern is obtained that is consistent with the reported structure of this fragment. Like Pr65, the 45-kDa protein is expected to be cleaved into nine products, but we detected only eight of these. The ninth product, the remaining 11-kDa portion of fragment 1, could not be identified. Of the eight cleavage products detected, six are identical with cleavage products from Pr65. These correspond to products that contain fragments 2, 3, and 4. The two fragment 1-containing partials are absent; two new bands can account for the missing products. A 33-kDa product just below fragment 2-3 corresponds to fragment 1-2 (48 kDa) minus murine p15. And a 43-kDa fragment that migrates faster than fragment 2-3-4 corresponds to fragment 1-2-3 (58 kDa).

When DMS-cross-linked Gazdar MSV is treated with avian p15 in the presence of Triton X-100, the Pr65 dimer is cleaved and a new band (45-kDa), is generated that upon electrophoresis migrates more slowly than Pr65. This result is shown in lanes k–l of Fig. 4. The electrophoretic analysis of this product, after excision from the gel and cleavage with CNBr, is shown in lane g of Fig. 6. Five of the eight cleavage products from the 45-kDa protein (lane f) are contained in the dimer. These products contain fragment 3, fragment 2, and the modified fragment 1. Only fragment 4-containing products are missing. This implies that the 45-kDa dimer is formed as a consequence of a homotypic cross-link in fragment 4. Since the substrate in these experiments was cross-linked Gazdar MSV that contained the three DMS-induced dimers, and after cleavage by avian p15 only fragment 4-linked 45-kDa dimers remained, we interpret this result to mean that both fragment 1-linked homotypic dimers are cross-linked in p15.

**Protein-Protein Interactions in Rauscher MuLV** To assess the role of the gag precursor interactions in maturation, we have investigated the structure of processed proteins in Rauscher MuLV, a mature C-type virus, under the same conditions of cross-linking used for Gazdar MSV. Previous studies with mature murine viruses demonstrated that p15 (7) and p30 (9) both form homotypic oligomers after cross-linking. The observed reduction in the intensity of the p10 band after cross-linking (9) suggested that p10 also forms dimers, although no discrete oligomers were observed by those authors. When Rauscher MuLV is treated with the reversible cross-linker dithiobispropionimidate and the cross-linked products analyzed by diagonal SDS-polyacrylamide gel electrophoresis (31), we observed that in fact p30, p15, and p10 all form homotypic complexes. This result is shown in Fig. 7. The monomeric species released from the major cross-linked complexes are denoted a, b, and c. In each instance, only one protein is released from a single complex, suggesting that the associations in the complex are homotypic. The three complexes are not detected without cross-linker or after pretreatment with ionic detergents such as 1% SDS (not shown). An additional oligomeric complex discernible in Fig. 7, the env gene product gp70-p15E, has been characterized previously (9).
Fig. 7. Identification of Rauscher MuLV protein complexes by two-dimensional SDS-polyacrylamide gel electrophoresis. [14C]Glycine-labeled Rauscher MuLV (25,000 cpm) was treated with 0.3 mg/ml dithiobisproprionimidate for 2 h at 24°C and cross-linked complexes were analyzed by diagonal gel electrophoresis as previously described (31). First dimension, no reducing agent; second dimension, 3% 2-mercaptoethanol. The radioactive proteins were visualized by fluorography. a–c indicate species released from the most prominent cross-linked products. At the left is a gel strip from a first dimension gel that was fluorographed directly.

This most prominent cross-linked complex detected in Rauscher MuLV is the p30 dimer. This complex is formed over a wide range of cross-linker concentrations (0.1–3 mg/ml), spanning the concentration used in the experiment in Fig. 7. Dimers of p30 also are generated with the uncleavable analogue DMS. Other bifunctional reagents tested, DFDNB and PDM, do not induce formation of p30 dimers (not shown). These latter reagents were tested over concentration ranges (0.03–0.3 and 0.003–0.03 mg/ml, respectively) that resulted in significant cross-linking of Pr65 in Gazdar MSV.

DISCUSSION

We have investigated lipid-protein and protein-protein interactions in the immature or A-type virions of Gazdar MSV by a combination of cross-linking and peptide mapping. The results are summarized schematically in Fig. 8. Lipid-protein cross-links in Pr65 are located in a 10-kDa fragment derived from its NH2 terminus, which is the same fragment that is cross-linked to lipid in Rauscher MuLV p15. Homotypic protein-protein cross-links in Pr65 are located in p15 and in p10, which also are sites of cross-links observed for mature proteins in Rauscher MuLV. Although the analysis of Pr65 presented is based on work with the replication-defective mutant Gazdar MSV, similar cross-linking patterns were observed for Pr65 in wild type virus (not shown). However, the low level of Pr65 in wild type MuLV makes peptide-mapping analysis of these cross-linked products impossible. Together the results from the cross-linking analysis of Gazdar MSV and of Rauscher MuLV suggest that many of the interactions of the gag proteins in the mature virion occur prior to cleavage of the gag precursor. These data provide strong evidence that the precursor itself is central to the organization of mature proteins in C-type particles.

Some of the gag proteins in mature virus particles have very different reactivities to cross-linker than in immature virus particles. One major difference appears to be the susceptibility of p30 to cross-linking. In the mature Rauscher MuLV, the DMS-induced p30 dimer is the major cross-linked product over a wide range of cross-linker concentrations. The predominant linkage in this dimer is between fragment 2 and 3 on adjoining proteins (not shown). By contrast, we could not detect any cross-links of this nature in Pr65 from immature Gazdar MSV. A second difference is the reactivity to DFDNB. In Gazdar MSV, Pr65 is extremely reactive to cross-linking by DFDNB. We could not detect any cross-linked products in Rauscher MuLV, even with a 10-fold increase in reagent concentration (data not shown). The DFDNB-induced cross-links in Pr65 were localized within the p30-p10 region of the precursor by a combination of proteolytic cleavage (MuLV-associated protease) and CNBr mapping, but could not be more precisely defined. Together the DMS and DFDNB cross-linking results suggest that protein-protein interactions involving p30 change during viral maturation. These differences may reflect the structural changes that accompany core condensation.

The ability to localize cross-linked products within Pr65 relies on a two-dimensional electrophoretic mapping technique that we have developed for ordering peptides. This technique is based on nearest neighbor analysis of cleavage products that are released from partial cleavage fragments in gel slices. We have found that the mapping technique has several limitations. Successful interpretations of the cleavages with this type of analysis depend heavily on the resolution of the cleavage products. The analysis rapidly becomes ambiguous when fragments cannot be identified or when fragments co-migrate. A second problem is that the rate of cleavage by
CNBr in some instances appears to be influenced by the amino acid sequence containing the methionine, making it difficult to generate a representative collection of cleavage fragments. For example, the cleavage site in Pr65 that gives rise to fragment 4 required extremely high CNBr concentrations to generate any of the product, which explains why it was not observed in previous studies (28). The concentrations used here are almost 1 order of magnitude greater than those used routinely for other proteins (not shown). An additional problem that has been described for cleavage of Pr65 by CNBr is that in some instances CNBr appears to generate a representative collection of cleavage fragments, mapping peptides by their release from partial fragments. For example, the cleavage site in Pr65 that gives rise to fragment 4 is used here are almost spurious. This difference presumably reflects the short fragment analysis, usually involving sequencing of overlap- ping fragments, mapping peptides by their release from partial cleavage products enables a direct and rapid ordering of cleavage products. This approach should be applicable to cleavage reactions other than with CNBr.

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REFERENCES
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