NOTES

Structural Proteins of Intracisternal A-Particles: Possible Repetitive Sequences

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Peptide maps of tryptic digests of the structural proteins from inner shells of intracisternal A-particles have shown common peptides for all the proteins. The terminal amino group of the three different structural proteins was identified as arginine. The major protein revealed approximately half the number of peptides expected from the amino acid composition. Since evidence for a cross-link bond has not been found, the main structural protein may be a single polypeptide chain containing a total or partial duplication of sequence.

The inner shells of murine intracisternal A-type particles contain a major structural protein with a molecular weight of 73,000 and lesser amounts of proteins in the molecular weight ranges of 46,000 and 30,000 (9, 14). In a previous study these proteins were isolated from particles obtained by the method of Kuff et al. (8) from mouse myeloma and neuroblastoma cells and were found to share common antigens. In addition, the 73,000- and 46,000-dalton components of myeloma-derived A-particles share a significant fraction of peptides released by cyanogen bromide cleavage (9). The relationship between these structural proteins has now been further explored by fingerprinting of their tryptic digests and amino terminal group identification. The results have implications in terms of both the genetic specification and assembly of these virus-like particles.

Figure 1A shows the peptide map of the main structural protein. About 50 peptides were detected in tryptic digests of the main structural protein. This is much below the expected number of 96 calculated from lysine and arginine residues per mole of 73,000-dalton protein (9). Detection of tryptophan-containing peptides using Ehrlich reagent (12) revealed only 5 such peptides in the main protein (Fig. 1A), a number half of that expected from the amino acid composition (9). The amino terminal group was identified as arginine by dansylation of the isolated proteins (6) followed by hydrolysis in 6 N HCl at 105 C for 16 h and bidimensional silica gel thin-layer chromatography (15) of the dansylated amino acid. The chromatography

was run in the first dimension using as solvent toluene-pyridine-acetic acid (150:50:3.5). Plates were dried at 100 C for 7 min, exposed to ammonia vapors for 1 min, and run in the second dimension using as solvent toluene-2-chloroethanol-25% ammonium hydroxide (100:80:6.7). The dry plates were read under UV light and position of standards and amino acid marked with pencil. The 46,000-dalton protein showed about 47 peptides with a distribution very similar to that observed for the main protein and also presented five tryptophan-containing peptides (Fig. 1B). In the lowest molecular weight component several peptides present in the other two proteins were not detected and from the 38 peptides observed only three were tryptophan reacting (Fig. 1C). Both lower-molecular-weight proteins contained amino terminal arginine.

These data extend previous evidence for a close structural relationship between various proteins of the intracisternal A-particle inner shell (9). The metabolic relationships are a subject of current study. The 46,000- and 30,000-dalton proteins could represent independently synthesized products or arise from cleavage of the main protein. Intracellular turnover of the 73,000-dalton A-particle protein has been observed in cultured neuroblastoma cells (K. K. Lueders and E. L. Kuff, Proc. Amer. Ass. Cancer Res. 15:119, 1974), but the products have not yet been identified.

The marked discrepancy between observed and expected number of tryptic peptides in the case of the 73,000-dalton proteins suggests the

FIG. 1. Peptide maps of tryptic digests of purified structural proteins from myeloma intracisternal A-particles. Proteins were alkylated with iodoacetamide, precipitated with 12.5% trichloroacetic acid, washed with acetone, and digested with TPCK-trypsin. In the photograph, the origin is lower left corner of each panel. Thin-layer electrophoresis in pH 2.0 buffer (acetic acid-formic acid-water, 120:5:280) was to the right, ascending chromatography in *n*-butanol-acetic acid-pyridine-water (50:10:40:40). The cellulose plates were dried and dipped into a 0.3% ninhydrin solution in acetone containing 1% pyridine, 1% acetic acid; color was developed at 80 C for 30 min. Tryptophan peptides (→) were detected in the same plates by spraying with fresh Ehrlich reagent (1 volume of *p*-dimethylaminobenzaldehyde 10% in concentrated HCl: 4 volumes of acetone). Ninhydrin positive spots disappear and tryptophan peptides develop a blue color. Tryptophan spots are circled with pencil. Panel A, MOPC 104-E main structural protein; panel B, MOPC 104-E, 46,000 mol. wt. protein; panel C, RPC-20, 30,000 mol. wt. component; panel D, composite diagram of tryptic peptides from structural proteins. Missing peptides in the 30,000 mol. wt. protein are indicated by shaded areas.

presence of repetitive sequence elements. The similarities between the peptide maps of the main protein and the lower-molecular-weight components are also consistent with this possibility. Such repetitive elements could either be distributed tandemly within a single polypeptide chain or contained within two chains that are cross-linked by a covalent bond (other than S-S). We have not been able to find chemical evidence for isopeptide (11) or dityrosine (1) bonds, nor for the aldehyde precursors of aldol-like cross-linkage (10). Furthermore, the above-mentioned bonds are known to be involved in the formation of extensive polymers rather than in dimerization processes. Although there remains the possibility of some undetected but highly specific cross-link between the repeating portions, our evidence thus far suggests that the sequence elements are connected linearly through conventional peptide bonds.

Repetitive sequences in the major structural protein would have important consequences in terms of A-particle assembly, e.g., in determining intermolecular affinities, packing arrangements, and (through these) overall particle morphology. A tandemly repeated sequence could be produced from serially reduplicated genetic material or arise through some posttranscriptional process. There is precedent in bacterial systems for "fusion" or "splicing" of peptides produced by two separate genes (2, 7) but this function has not been established in mammalian cells and cannot yet be considered a likely mechanism to explain the present results. On the other hand, information for A-particle formation appears to be vertically transmitted in mice of many strains and expressed in a programmed manner during normal development (3-5). It is interesting to consider the possibility that a protein with associative properties conducive to assembly in virus form could have been generated by a process of serial gene duplication during mouse evolution or development (cf. 13).

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