AN ACETYLATED N-TERMINUS OF ADENOVIRUS TYPE 2 HEXON PROTEIN

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Summary. An acetylated N-terminus of adenovirus type 2 hexon protein was characterized using radioactively labeled protein and mass spectrometry. The labeled protein, obtained by synthesis in a medium containing ^{14}C-acetate, was digested with proteolytic enzymes. A radioactive peptide, Acetyl-Ala-Thr-Pro-Ser, recovered in good yield, was the only N-terminal structure detected in the protein.

The hexon protein of adenovirus is a capsid protein produced in excess during viral infection. Its size and quarternary structure are not firmly established but it contains subunits. Three, or a multiple of three, similar polypeptide chains per protein molecule have been proposed (1-5). The molecular weight of these subunits is in the order of 100,000-120,000 daltons, although lower values have also been reported (1-8). An N-terminal amino acid was first suggested (9,10) but the pure protein is now recognized to lack free N-terminal residues (6,7), possibly because of blocking acetyl groups (7). The present work was performed in order to identify the blocked N-terminus of the subunits and to establish the primary structure of this region. The hexon protein was therefore labeled with ^{14}C-acetate, and a blocked radioactive peptide separated after digestion with proteolytic enzymes.

MATERIALS AND METHODS

Adenovirus type 2 was grown (11) and the hexon protein

isolated and purified to homogeneity as previously described (1). The ^{14}C-labeled derivative was obtained by the addition of ^{14}C-acetate (500 µCi (8.3 µmole) to 3 x 10^8 cells in 1 l of culture medium) 16 h after infection, which corresponds to the time of maximal synthesis of structural proteins. Cells were harvested at 40 h. 19 mg of the salt-free hexon protein, containing 470,000 c.p.m., was dissolved in 2 ml of 6 M guanidine-HCl, 0.1 M Tris, 2 mM EDTA, pH 8.1, bubbled with nitrogen, reduced with 7.5 µmole dithiothreitol for 2 h at 37°C and carboxymethylated with 25 µmole iodoacetate for 3 h at room temperature. Reagents were removed by dialysis against distilled water. The carboxymethylated ^{14}C-acetate labeled protein was digested at 37°C with 0.5 mg of TLCK-treated (12) chymotrypsin in 0.1 M ammonium bicarbonate for 8 h and then with 0.5 mg of thermolysin in 0.2 M ammonium acetate, 5 mM $CaCl_2$, pH 8.5, for 4 h. The lyophilized peptide mixture was dissolved in water and applied to a column of Dowex 50W-X2, 200-400 mesh, H^+ form (13) which was eluted with water. Non-adsorbed material was purified by high-voltage paper electrophoresis (14) at pH 6.5 and 1.9 (15) and analyzed for total composition and primary structure. Amino acids were determined with a Beckman 120 B amino acid analyzer after hydrolysis for 24 h in 6 N HCl under reduced pressure. The dansyl method (16) and hydrazinolysis (17) was used for estimation of terminal residues. For amino acid sequence determination, the permethylated (18) derivative was analyzed on an LKB mass spectrometer 9000.

RESULTS

Separation of an acetyl-blocked N-terminal peptide. The peptide mixture obtained after chymotryptic and thermolytic clea-

vage of 19 mg of the ^{14}C-labeled protein, prepared as described above, was applied in water to a column (2 x 22 cm) of Dowex 50W-X2, H^+ form. With water as eluent, radioactive material appeared with the front at about 35 ml. The first 70 ml of the eluate, sufficient to contain all non-adsorbed material, was pooled and freeze-dried. No additional radioactivity was recovered after elution with 1 N NH_3 containing 1 N NaCl. The pooled fraction revealed only one radioactive peptide after high-voltage paper electrophoresis at pH 6.5 and 1.9, as shown in Fig. 1. The characteristics of this peptide are given in

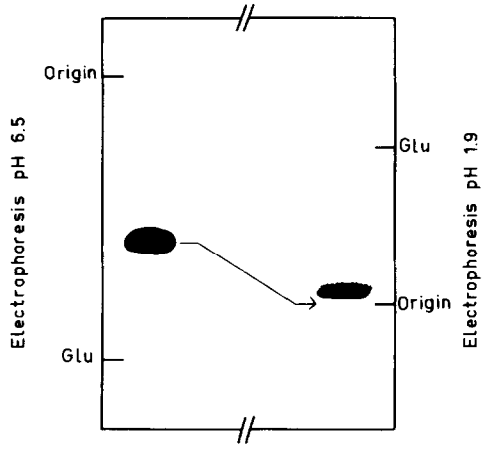

Fig. 1. Autoradiographs obtained after high-voltage paper electrophoresis of the ^{14}C-labeled N-terminal peptide.
After separation at pH 6.5, followed by autoradiography, the radioactive material was cut out, stitched onto a new paper, separated at pH 1.9, and again revealed by autoradiography.

Table 1. The peptide had all the properties expected for an acetyl-blocked N-terminal peptide. It was thus radioactive, ninhydrin-negative, had low mobility on electrophoresis at pH 1.9 and no detectable N-terminus with the dansyl method (16). No ^{14}C-label was found in the constituent amino acids by auto-

Table 1. Characteristics of the N-terminal peptide.

The molar recovery is based on a protein subunit molecular weight of 100,000 daltons. Recovery of radioactivity indicates the amount of the original protein radioactivity that is recovered in the purified peptide.

Electrophoretic mobility (19)	0.53 at pH 6.5 0.06 at pH 1.9
Molar recovery (%)	48
Recovery of radioactivity (%)	15
Composition	Thr 1.0(1) Ser 1.1(1) Pro 0.9(1) Ala 0.9(1) Sum 4
N-terminus	None
C-terminus	Ser

radiographic analysis of electropherograms of the acid hydrolysate, suggesting that the label was confined to ^{14}C-acetate.

Determination of the primary structure of the peptide. Serine was found to be the C-terminal residue (Table 1). After hydrazinolysis (17) at 110°C for 6 h, this was the only free amino acid detected, both by electrophoresis at pH 1.9 and by analysis in the amino acid analyzer. No residues were released by carboxypeptidase A. Subtilisin or partial acid hydrolysis also failed to produce smaller fragments of value for sequence determination. The peptide was therefore permethylated (18)

and submitted to analysis by mass spectrometry. With a direct inlet probe, an electron beam energy of 25 eV and a trap current of 60 μA the peptide appeared at 105°C as estimated from the temperature indicator of the spectrometer. All major fragments identified fit expected fragmentation patterns as shown in Table 2. The same pattern was also observed when the peptide

Table 2. Sequence analysis by mass spectrometry of the permethylated N-terminal peptide.

Identified mass peaks	Corresponding fragments (20)	
100	Acetyl-Ala(Me)-	less CO
128	"	intact
199	Acetyl-Ala(Me)-Thr(Me)$_2$-	less CH_3-C-O-CH_3
225	"	less CH_3OH
257	"	intact
354	Acetyl-Ala(Me)-Thr(Me)$_2$-Pro-	
468	Acetyl-Ala(Me)-Thr(Me)$_2$-Pro--Ser(Me)$_3$	less CH_3OH
500	"	intact
Amino acid sequence	Acetyl-Ala-Thr-Pro-Ser	

was treated with acetic anhydride before permethylation. These results conclusively show that the structure of the peptide is Acetyl-Ala-Thr-Pro-Ser.

DISCUSSION

The structure of the peptide as determined by mass spectrometry, is in agreement with the total composition (Table 1), with the C-terminal determination and with the properties of

the peptide during purification. The position of the proline residue may probably also explain why no residues were released during digestion of the peptide with carboxypeptidase A. The molar recovery of 48% (Table 1) is remarkably high in view of losses during enzymatic digestions and peptide purification, and suggests that the peptide may be derived from all subunits. The recovery of radioactivity of 15% (Table 1) is also high and is deduced, as described above, to be confined to the acetyl group. This shows that the ^{14}C acetate labeling *in vivo* gives a relatively good specificity for labeling of the blocking acetyl group.

By ^{14}C-labeling *in vivo* and mass spectrometry an N-terminus of adenovirus hexon protein, Acetyl-Ala-Thr-Pro-Ser, was thus identified on a small scale. This is the only N-terminal structure found, and it is recovered in good yield. It is therefore suggested that the polypeptide chains of the protein are identical in this part, explaining the absence of free N-terminal residues in the native protein.

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