RADIOACTIVE LABELLING OF ACIDIC REGIONS IN THE ADENOVIRUS HEXON PROTEIN THROUGH METABOLIC CONVERSION OF [^{14}C]ACETATE

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1. Introduction

The hexon protein is the main capsomer of adenovirus and is produced in excess in infected cells during viral synthesis. It is composed of three identical polypeptide chains with subunit mol. wt 100 000–120 000 [1–9], and has an acetylated N-terminus [10]. The latter has been labelled by preparation of the protein from infected cells grown in the presence of [^{14}C]-acetate. After proteolytic digestion, it could be isolated in a small peptide by chromatography on Dowex-50 [10]. In attempts to extend this method to larger fragments containing the acetylated N-terminus of hexon, unexpected labelling results were noticed, as presently reported.

Internal regions of the protein were found to be labelled by metabolic conversion of the acetate into common non-essential amino acids. In this way, regions rich in acidic and amidated residues were detectable. These results demonstrate complications in attempts at specific in vivo labelling of known acetylated structures, but also show a possibility of detecting particular regions in some proteins.

2. Materials and methods

2.1. *Labelled protein*

Adenovirus type 2 was grown in spinner cultures of KB cells in Eagle's spinner medium containing both essential and non-essential amino acids [11]. The cells were labelled in vivo by addition of [^{14}C]-acetate (500 μCi, 8.3 μmol) to 1 litre of culture medium as in [10]. The isotope was added 16–20 h after infection, and cells were harvested after 30–40 h. Soluble hexon produced in excess was purified on DEAE-cellulose, followed by repeated crystallizations [8]. It was then reduced with dithioerythritol, and carboxymethylated with nonradioactive iodoacetate in buffered 6 M guanidine–HCl, pH 8.1 [8].

2.2. *Peptide analysis*

The in vivo labelled protein (100 mg) was digested at 37°C for 4 h with TPCK-trypsin (1%, by wt) in 0.1 M ammonium bicarbonate. The peptide mixture was fractionated on Sephadex G-50 (2.5 × 100 cm) in the same buffer. Pooled fractions were purified further by high-voltage electrophoresis and by chromatography [8]. Peptides were detected by staining with Cd-ninhydrin [12] or with fluorescamine ([13], Fluram-Roche) in acetone (4 μg/ml), and by autoradiography. Total compositions were determined with a Beckman 121 M amino acid analyzer. End-groups and amino acid sequences were analyzed with the dansyl-Edman method as in [8]. TLCK-chymotrypsin, a staphylococcal extracellular protease, pepsin, and thermolysin were used for redigestion of peptides [14]

3. Results

Fractionation on Sephadex G-50 of a tryptic digest of hexon (100 mg, 4 × 10^6 cpm) labelled in vivo with [^{14}C]acetate is shown in fig.1. Six radioactive peptides were detected when material from different areas of the chromatogram was purified by

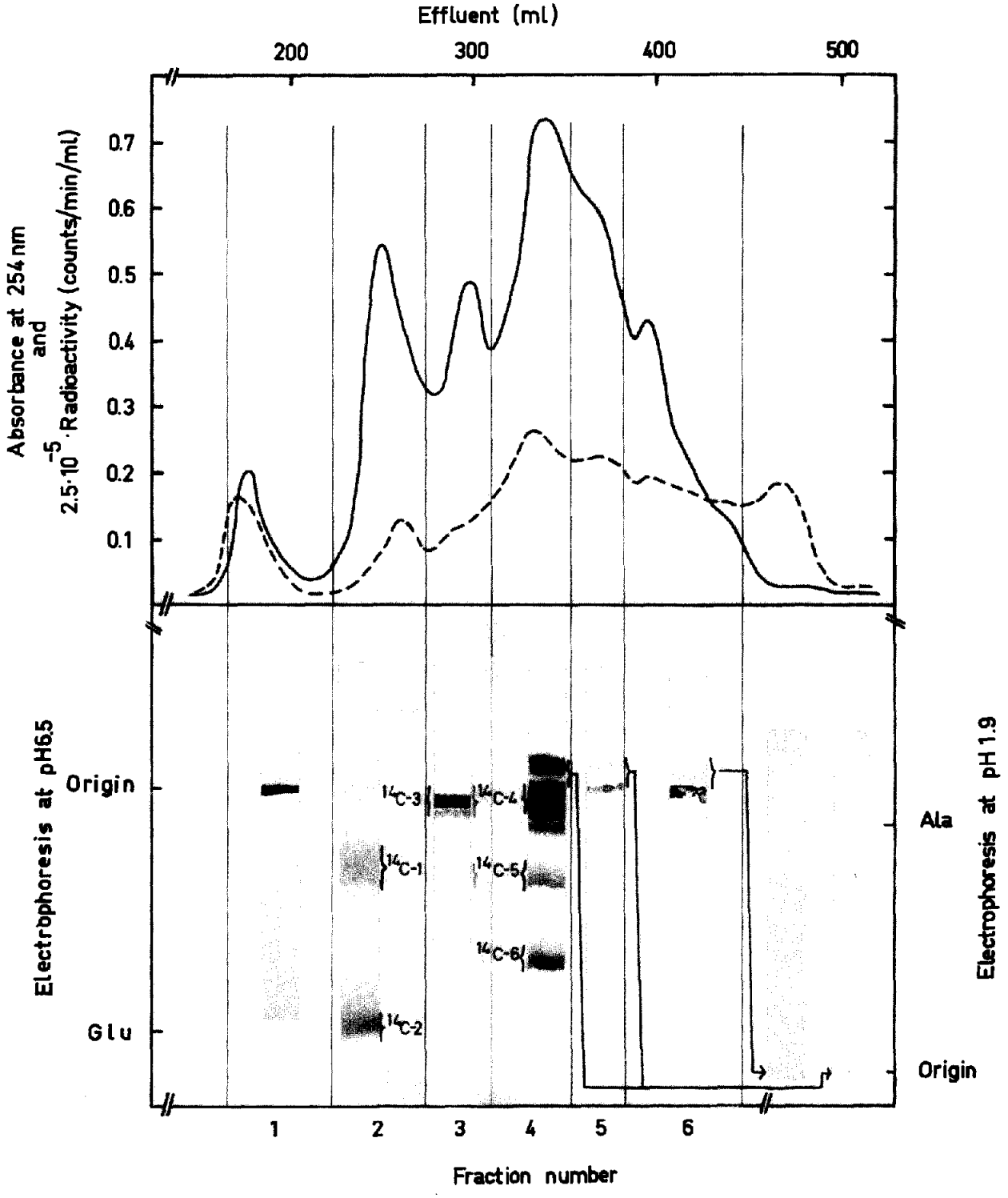
Effluent (ml)
200
300
400
500
Absorbance at 254 nm and 2.5·10⁻⁵ · Radioactivity (counts/min/ml)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
Electrophoresis at pH6.5
Origin
Glu
14C-3
14C-4
14C-1
14C-5
14C-6
14C-2
Ala
Origin
Electrophoresis at pH 1.9
1
2
3
4
5
6
Fraction number

Fig.1

Table 1
Radioactive tryptic peptides from hexon labelled by growth in [^{14}C]acetate

Peptide	Composition		
	N-terminal acetyl groups	Asx + Glx	Total residues
^{14}C-1	–	10	33
^{14}C-2	–	18–19	23–24
^{14}C-3	–	8	29
^{14}C-4	1	5	32
^{14}C-5	–	8	31
^{14}C-6	–	6	16

paper electrophoresis. One of them (^{14}C-4, fig.1) is the N-terminal tryptic peptide with the expected α-amino acetyl label. The other peptides are derived from internal regions of the protein, but have in common a high content of dicarboxylic residues, as shown in table 1.

Radioactivity measurements of residues removed during sequence degradations of these peptides showed that the ^{14}C-label was recovered mainly in the dicarboxylic residues and their amides. They had specific radioactivities of up to a few percent of that observed for the N-terminal acetyl group, and more in glutamic acid than in aspartic acid. The corresponding amides were less radioactive, and some label was also shown in proline, together with trace amounts in serine. Insignificant levels or no labels at all were found for other residues analyzed, including alanine and glycine. Quantitatively, the radioactivities recovered were about 25, 15 and 5–10 cpm/nmol for glutamic acid, aspartic acid and amides/proline, respectively, versus a value of the order of 1000 cpm/nmol for the blocking N-terminal acetyl group. The labels of dicarboxylic residues explain the distinct autoradiographic bands in fig.1, and directly reveal acidic regions in the protein.

The material recovered from radioactively labelled regions was further analyzed by sequence degradations of the intact peptides and of fragments obtained by secondary digestions with different enzymes. The results are shown in table 2. Total compositions of all peptides show exact agreement with values expected from the sequence results. Peptides ^{14}C-5 and ^{14}C-6 (fig.1) contain carboxymethylcysteine and have been recovered before in differently labelled forms after alkylation (peptides A and E, respectively [8]). In the latter peptide, however, a tryptophan residue was previously not detected, which may also have affected two amide assignments. The tryptophan could now be positioned, and the correct structure is given in table 2. The extremely acidic peptide ^{14}C-2 (charge −8) has an electrophoretic mobility like free glutamic acid (fig.1) in spite of a size of about 25 residues. The peptide has only 5 types of residues and all the non-dicarboxylic ones occur at either end (table 2). The high glutamic acid content is not due to contamination, since both total compositions and charges of secondarily obtained fragments are consistent.

4. Discussion

In vivo labelling of hexon with [^{14}C]acetate was investigated in order to allow detection of the known acetylated N-terminus in a large fragment from proteolytically derived peptide mixtures. The expected fragment was preferentially labelled and directly detectable (^{14}C-4, fig.1). This permitted an extension of the known part of the structure in the N-terminal region of hexon. Most of the total ^{14}C-label in the hexon was confined to the N-terminal peptide as in [10] where the N-terminal thermolysin fragment was shown to contain 31% of the radioactivity. Further protein labelling was now found, due to metabolic conversion of the acetate into amino acid residues. Distinct radioactive peptides are still produced since labelled residues are not evenly

Fig.1. Top: Exclusion chromatography in 0.1 M ammonium bicarbonate on Sephadex G-50 (2.5 × 100 cm) of tryptic peptides from hexon labelled by growth in [^{14}C]acetate. Radioactivity measured in a Packard scintillation counter (continuous line) and optical density in an LKB Uvicord flow cell at 254 nm (broken line). Bottom: Autoradiographic pictures of fractions after electrophoretic separations at pH 6.5. In the case of neutral peptides (fractions 5 and 6) the radioactivity is not confined to single peptides, but represented by several weakly labelled fragments that are hardly detectable after further electrophoretic separations, at pH 1.9.

Table 2
Structures of ^{14}C-labelled tryptic peptides

Peptide ^{14}C-1:
Ser-Gly-Leu-Gln-Ile-Gly-Ser-Asp-Asn-Ala-Glu-(Asx_2,Thr,Ser,Glx_4,Pro_3,Gly_2,Ala_2,Val,Ile,Tyr_2,Trp,Lys,Arg).

Peptide ^{14}C-2:
Ala-Val-Ala-Glu-(Asx_3,Glx_{8-9})-Glu-Glu-Glu-Glu-Gln-Asn-Ala-Arg.

Peptide ^{14}C-3:
Val-Val-Leu-Tyr-Ser-Glu-Asp-Val-Asn-Met-Glu-Thr-Pro-Asp-Thr-His-Leu-Ser-Tyr-Lys-Pro-Gly-Lys-Gly-Asp-Glu-Asn-Ser-Lys.

Peptide ^{14}C-4:
Ac-Ala-Thr-Pro-Ser-Met-(Met)-Pro-Gln-Trp-Ser-Tyr/Met-His-Ile-Ser-Gly-Gln-Asp-Ala-Ser-Glu-Tyr-Leu-Ser-Pro-Gly-Leu-Val-Gln-Phe/Ala-Arg.

Peptide ^{14}C-6:
Gly-Ala-Pro-Asx-Ser-Cys-Glx-Trp-Glu-Gln-Thr-Glx-Asx-Ser-Gly-Arg.

Sequences were determined by analyses of the intact peptides and of fragments obtained by redigestions with different enzymes or CNBr. Peptide ^{14}C-5 is given in [8]

distributed in hexon. The radioactive peptides thus obtained contain an excess of glutamic acid and aspartic acid or the corresponding amides (table 1). This is consistent with the close metabolic connection via transaminative steps between these acids (or the amides with one more biosynthetic step) and, respectively, α-ketoglutarate and oxaloacetate in the Krebs' cycle. Similarly, the fifth labelled residue, proline, is biosynthetically near glutamic acid (two reductive steps). Further significant incorporation of labelled acetate into other common, non-essential amino acids was not detected. This is not surprising since their biosynthesis from acetate is less direct (although transamination is one of the steps in the pathway to alanine). In general, labelling patterns will of course also be influenced by turnover rates and sizes of intracellular pools for the various products. The direct labelling of dicarboxylic residues, however, explains the distinct autoradiographic bands in fig.1.

In vivo labelling of hexon with [^{14}C]acetate thus gives preferential incorporation into the acetylated N-terminus and into regions containing many carboxylic residues. Previously unknown acidic structures are directly identified. The structure of peptide ^{14}C-2 (table 2) is particularly noticeable. The positions in the entire protein of this and the other labelled peptides are not yet established, but further work (not shown) indicates that at least some of the peptides may be close in the primary structure, still more emphasizing the acidic nature of certain regions. In general, the results demonstrate one complication in labellings of protein acetyl groups, but also suggest a method to detect structures rich in clustered acidic amino acids in suitable proteins.

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