

## Hexon Peptides of Type 2, 3, and 5 Adenoviruses and Their Relationship to Hexon Structure

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Peptides of hexons from type 2 and 5 (subgroup III) and type 3 (subgroup I) adenoviruses were produced by treatment with cyanogen bromide and were separated by isoelectric focusing in polyacrylamide gels containing 8 M urea. Peptides with identical isoelectric points, but from different hexon types, were considered to have structural similarities. According to this criterion for chemical relatedness, about two-thirds of the type 2 and 5 hexon peptides may be considered similar. In contrast, the majority of the type 3 hexon peptides differed chemically from peptides of type 2 and 5 hexons. Virions and free hexons were iodinated with <sup>125</sup>I in the presence of lactoperoxidase and H<sub>2</sub>O<sub>2</sub>. When <sup>125</sup>I-labeled virions were disrupted and the hexon was purified, the highly labeled cyanogen bromide peptides had pI values greater than 6.8; some unique as well as some common peptides were labeled. When purified hexons from the excess cellular pool were iodinated, peptides common to types 2, 3, and 5 (peptides 12 and 14) were most extensively labeled. Thus, hexons assembled in virions and those free in solution were iodinated differently. The data suggest that immunologically the hexons in viral capsids react differently from unassembled hexons because the polypeptide chains assume slightly different folding configurations in the two hexon forms and therefore expose different regions of the protein to antibodies.

The icosahedral capsid of adenoviruses consists of 12 pentons at the vertexes and 240 hexons (7, 10, 28). The hexon has a group-specific antigen that is characteristic of all serotypes of mammalian origin and a type-specific antigen. The following observations indicate that the antigen purported to be responsible for the induction of neutralizing antibodies (19, 33) is on the surface of the adenovirus virion and that it is predominantly the type-specific antigen of the hexon. Each virus serotype is neutralized only by antibody prepared against the respective type-antigenic determinant in virions or purified hexons (19, 33). Virions react mainly as type-specific antigens in complement fixation and agglutination tests when antivirion sera are used (27). In addition, only virions incubated with antisera against homotypic hexons are coated with antibodies when viewed by electron microscopy (18). The major group antigen, however, is readily detectable in purified hexons (19, 21, 32).

The physical structure of the hexon has been well defined. X-ray crystallographic analysis indicates that type 5 and 2 adenovirus hexons are composed of three crystallographic asymmetric subunits of 94,000 to 105,000 (3) and 110,000 to 119,000 daltons (4), respectively. The difference in protein mass may be due to difficulties in accurately determining the density of the supernatant solution (3). The polypeptide chains in the hexon of a given type appear to be identical (6, 20) and interact to form a polygonal structure (35) that is approximately 8.0 nm wide by 11.0 nm high, with a central hole of approximately 2.5 nm in diameter (20). There are no detectable immunological differences between hexons isolated from the virion or as soluble antigens from infected cells (6, 33).

The presence of a group antigen, the characteristic morphology of the adenovirus hexon, and the requirement that three polypeptide chains interact to form a capsomer suggested that regions of common primary amino acid sequences may exist in hexons of different types. However, the type-specific determinants should consist of variable regions in the polypeptide chain. To determine whether hexons do

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indeed consist of such variable and common regions, this investigation was designed to analyze chemically hexons of three different adenovirus serotypes that belong to either subgroup I (type 3 adenovirus) or III (type 2 and 5 adenoviruses). The results indicate that regions of structural similarity exist and are critically located. Some structural differences are probably related to the unique type-specific antigenic determinants.

## MATERIALS AND METHODS

**Virus.** The prototype strains of adenovirus types 2, 3, and 5, which had been plaque purified three times, were used. After infecting monolayers of KB cells, virus was quantitated by plaque (12) or fluorescent focus assay (5).

**Tissue culture, viral infection, and labeling.** Virus was propagated by infecting KB cells with 100 PFU/cell, as previously described (2, 12, 13). Viruses and the viral soluble antigens were labeled 12 h after infection by adding L-amino acid mixtures (New England Nuclear Corp) of either 0.5 mCi of  $^{14}\text{C}$  or 1.25 mCi of  $^3\text{H}$  per liter of cells. For labeling of the viral proteins, KB cells (150,000 cells/ml) were maintained in spinner culture in Eagle minimal essential medium containing one-half the normal concentration of amino acids and 5% calf serum.

**Purification of hexon.** Type 2 and 5 hexons were purified as described previously (13, 30), with the following modifications. Cell lysates were treated with 0.025 mg of DNase (Worthington) and 0.010 mg of RNase (Worthington) per ml. No proteolytic enzymes were used. After three extractions with Freon (trichlorotrifluoroethane; DuPont), virus was separated from soluble antigens by rate zonal centrifugation into a preformed discontinuous CsCl gradient consisting of 1.4 and 1.2 g/ml. The virions banded at approximately 1.33 g/ml; the material above the virions was considered soluble antigens. The virions were further purified by isopycnic centrifugation in a preformed 1.2- to 1.4-g/ml CsCl gradient. Soluble antigens, which had been dialyzed against 0.01 M phosphate buffer, pH 7.2, containing 0.001 M EDTA, were treated with streptomycin (0.01 g/ml). After centrifugation, the supernatant was dialyzed against 0.01 M phosphate buffer, pH 8.0, containing 0.001 M EDTA, and the antigens were precipitated by adding saturated ammonium sulfate (Baker) to 45% saturation at pH 8.0. After dialysis against 0.001 M phosphate buffer, pH 7.2, without 0.001 M EDTA, the antigens were fractionated by chromatography on hydroxyapatite (Bio-Rad); the hexon obtained was subsequently chromatographed on DEAE-cellulose (Whatman) as described previously (13).

Type 3 hexon was purified as described above except that hydroxyapatite and DEAE-cellulose chromatography were not used because these procedures did not effectively separate type 3 hexon from fiber. Instead, type 3 soluble antigens were fractionated by exclusion chromatography on 6% agarose (Bio-Rad) according to Pettersson (21). Preparative polyacrylamide gel electrophoresis (PAGE) was the final step

in purification (21); the "fast"-electrophoresing population of hexons (21) was used for the chemical analyses to be described.

To prepare hexons from type 2 adenovirus particles, referred to as "virion hexon," the virions were disintegrated by incubating for 1 h at room temperature in 10% pyridine (vol/vol), pH 9.0, according to Prage et al. (24). Subsequently, the mixture was placed on a 5 to 25% linear sucrose gradient and centrifuged for 2 h at  $95,000 \times g$ . The capsomers, which were near the top of the sucrose gradient, were dialyzed against 0.01 M phosphate buffer, pH 7.2. The virion hexons were further separated from pentons and fibers by hydroxyapatite chromatography. All hexon preparations were submitted to preparative PAGE (20).

**PAGE.** Hexon preparations were concentrated for preparative PAGE by pressure dialysis against 0.075 M Tris-glycine buffer, pH 9.0, containing 0.001 M EDTA. Preparative PAGE was carried out with a Buchler polypreparative apparatus, and the proteins were electrophoresed through a 6% polyacrylamide gel cross-linked with 0.16% *N,N'*-methylene bisacrylamide in 0.1 M Tris-glycine, pH 9.5, 0.1% *N,N,N',N'*-tetramethylethylenediamine, and 0.07% ammonium persulfate (Baker). The anode and cathode reservoirs contained 0.2 and 0.1 M Tris-glycine buffer, pH 9.5, respectively. The elution, which was monitored spectrophotometrically, was accomplished in 0.075 M Tris-glycine buffer, pH 9.5. Acrylamide (Eastman) and *N,N'*-methylene bisacrylamide (Eastman) were recrystallized (14). The gel was electrophoresed at 20 mA for 16 h at 5 C before adding the sample. Samples were electrophoresed at 20 mA and at 5 C for 8 to 12 h.

Analytical PAGE, using nondisrupted hexons, was done in 7% gels according to Wilhelm and Haselkorn (36) except that 6 M urea was present in the sample and in the gel.

**Cleavage of hexon.** Purified hexon was suspended in 70% formic acid, 1 mg of cyanogen bromide (CNBr) per mg of protein was added, and the mixture was incubated at 37 C for 24 h to obtain cleavage at the methionine residues according to the method of Gross and Witkop (9). Water, 10 times the volume of the reaction mixture, was added, and the formic acid and CNBr were removed by lyophilization. Samples were resuspended in deionized 10 M urea to 10 to 15 mg/ml and stored at -20 C.

**Isoelectric focusing in polyacrylamide gels (IFPA).** Samples were isoelectrophoresed in cylindrical and thin-layer 5% polyacrylamide gels as previously described (1, 16, 31, 37) except for the following modifications. All samples were electrolyte free and contained 10 M urea that had been deionized by passing through an Amberlite MB-3 (Mallinckrodt) column. For cylindrical gels, approximately 25,000 counts/min was polymerized in the gel mixture. For thin-layer gels, 1 mg of protein in 0.05 ml was added into holes punched in the gel.

To fractionate the peptides, gels containing a mixture of 1.2% (pH 3 to 10), 0.4% (pH 3 to 5), and 0.4% (pH 8 to 10) carrier ampholytes (LKB) were electrophoresed in glass tubes (0.5 cm [inner diameter] by 20 cm) or in 20-cm thin-layer plates. The gels also contained deionized 8 M urea, 0.05%

*N,N,N',N'*-tetramethylethylenediamine from a 5% stock solution that had been adjusted to pH 7.0 with 5 N HCl, and 0.0264 mg of riboflavin (Nutritional Biochemicals). Gels were polymerized with fluorescent light.

The electrolyte reservoirs for cylindrical gels contained 8 M urea adjusted to pH 2.5 with 18 M H<sub>2</sub>SO<sub>4</sub> for the anode and to pH 11.0 with 5 N NaOH for the cathode. Thin-layer gels on glass plates were placed onto carbon electrodes coated with 5% phosphoric acid for the anode and 5% monoethanolamine for the cathode. Cylindrical gels were electrophoresed at 300 V while not exceeding 1 mA/gel at 5 C for 18 h. Electrophoresis in thin-layer gels was carried out at 300 V while not exceeding 7 mA at 5 C for 18 h. The pH of cylindrical gels was measured with a microelectrode after adding 0.5 ml of water to each 1-mm gel slice and incubating at 5 C for 30 min. For thin-layer gels, a 5-mm cork borer was used to remove plugs of gel, and the pH was determined as with slices of cylindrical gels.

**Determination of radioactivity.** All gels containing <sup>3</sup>H or <sup>14</sup>C were cut into 1-mm slices and solubilized by heating at 37 C for 16 to 20 h in Protosol (New England Nuclear Corp.) solution containing 9 parts Protosol, 1 part water, and 10 parts toluene. Subsequently, 10 parts of an Omnifluor (New England Nuclear Corp.)-toluene mixture was added and the radioactivity was counted in a Packard scintillation spectrometer. Gels containing <sup>125</sup>I were sliced as described above and the radioactivity was counted in a Packard gamma counter.

**Iodination with <sup>125</sup>I.** Virions and hexons, purified from the infected cell homogenate, were iodinated with <sup>125</sup>I in the presence of excess lactoperoxidase according to the method of Phillips and Morrison (22). To 0.02 ml of purified virions or hexons in 0.005 M Tris-hydrochloride, pH 7.8, containing 0.001 M EDTA was added 25  $\mu$ Ci of carrier-free Na<sup>125</sup>I (New England Nuclear Corp.), 0.01 ml (2 mg/ml) of lactoperoxidase (Sigma Chemical Co.) in 0.005 M Tris-hydrochloride, pH 7.8, and 0.025 ml of 10<sup>-4</sup> M hydrogen peroxide (Baker). After 40 min at room temperature, the reaction was terminated by adding 0.025 ml (1 mg/ml) of Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub> (Baker). Unconjugated <sup>125</sup>I was removed by dialysis and by chromatography on G-25 Sephadex columns.

## RESULTS

**Purity of hexon.** To detect contamination with host proteins, the removal of KB cell extract containing <sup>14</sup>C- or <sup>3</sup>H-labeled amino acids and nucleic acids from hexons during the purification procedure was monitored as has been described (13, 29). To determine whether the putative purified type 2, 3, and 5 hexons were contaminated with other viral proteins, immunoelectrophoresis and analytical PAGE were used. Both methods demonstrated that the preparations contained only hexon protein.

One major species of protein was detectable by analytical PAGE for type 2, 3, and 5 labeled

hexon preparations (Fig. 1). Furthermore, sodium dodecyl sulfate-PAGE of disrupted type 2, 3, or 5 hexon detected only one polypeptide for each purified hexon preparation. Results similar to those in Fig. 1 were reported by Pettersson (21), but the purification procedure had several differences from that used in this investigation. The slow-migrating protein, which represented 1 to 4% of the total radioactivity in each preparation, appeared to be hexon dimers since the protein had a sedimentation coefficient of approximately 18S and was quantitatively precipitated by anti-hexon serum but not by sera containing antibodies for fiber and penton proteins. Hexon dimers have also been detected in type 7 adenovirus hexon preparations (17). In

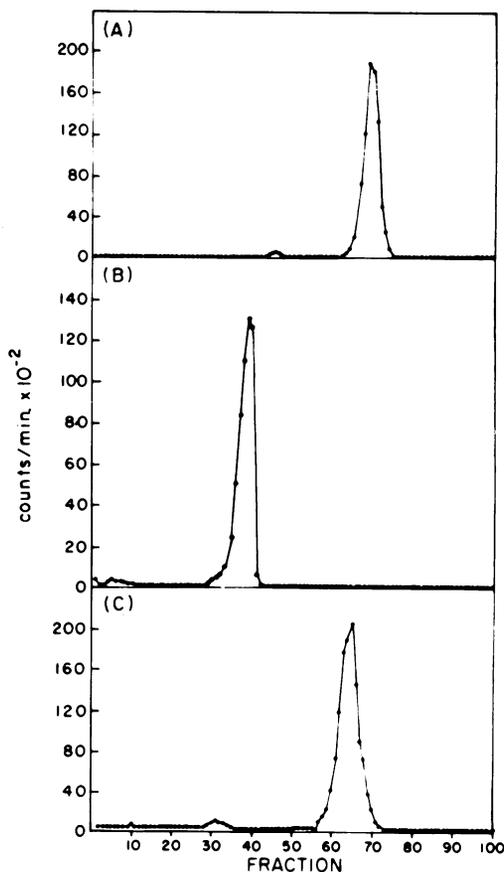


FIG. 1. Analytical PAGE of purified types 2 (A), 3 (B), and 5 (C) adenovirus hexons. The proteins were labeled with a <sup>3</sup>H- or <sup>14</sup>C-labeled amino acid mixture. Electrophoresis was carried out at 3 mA/gel for 4 h and in 7% gels at pH 8.5 containing 6 M urea; origin is at the left and anode is at the right. Gels were sliced into 1-mm fractions from the origin of electrophoresis, solubilized, and counted. Greater than 99.5% of the input radioactivity was recovered from each gel.

some preparations, a "slow excess pool" hexon, which had a sedimentation coefficient of 12S (6, 21), was also noted.

**CNBr peptides of type 2, 3, and 5 hexons characterized by isoelectric focusing.** Type 2, 3, and 5 adenovirus hexons were analyzed to determine similarities and differences in hexon structure. The CNBr peptides were assumed to have little or no secondary structure in IFPA gels because of their low molecular weight and the presence of 8 M urea in the gel. Hence, the pI should be indicative of the peptide's net charge, which is influenced primarily by the amino acid composition. Figure 2 illustrates that 27 to 30 distinct peptides for type 2 and 5 hexons could be detected by Coomassie brilliant blue staining of thin-layer IFPA gels. The number of type 5 hexon peptides detected, 27 to 30, is consistent with the predicted number according to the average methionine content of approximately 2.6 residues per 10,000 daltons (21; Dorsett and Ginsberg, unpublished data) of a protein having a mol wt of 94,000 to 105,000 (3) or 120,000 (15). Amino acid composition analysis of hexon cleaved by CNBr detected less than 0.1 methionine residue per 10,000 daltons (Dorsett and Stinski, unpublished data). These data suggested that complete cleavage of the polypeptide chains was accomplished.

Structural similarities and differences were determined by co-IFPA. The majority of the  $^{14}\text{C}$ -labeled type 5 hexon peptides, but only a minority of the  $^3\text{H}$ -labeled type 3 hexon peptides, isoelectrophoresed at the same pI values as those of  $^3\text{H}$ -labeled type 2 hexon peptides (Fig. 3 and 4). Two peptides were considered chemically similar when the peak of radioactivity was detected at exactly the same pI. Assuming that peptides with identical pI values are chemically similar, these data indicate that there are regions of chemical similarities as well as variable or unique sequences in the hexon types analyzed. The hexons of adenoviruses that are clearly related (e.g., types 2 and 5) have a large proportion of their polypeptide chains chemically similar, whereas hexons from less related types (e.g., types 2 and 3) have many fewer amino acid sequences in common.

**Topological location of common and unique regions of the hexon polypeptide chain.** Hexons assembled in the virion capsid react differently in immunological assays than when present in the reaction mixture as free purified hexons. The assembled virion hexon has only a type-specific antigen detectable with antisera from rabbits immunized with purified homotypic hexons as the immunogen (18, 19, 33). In contrast, purified free hexons have a readily

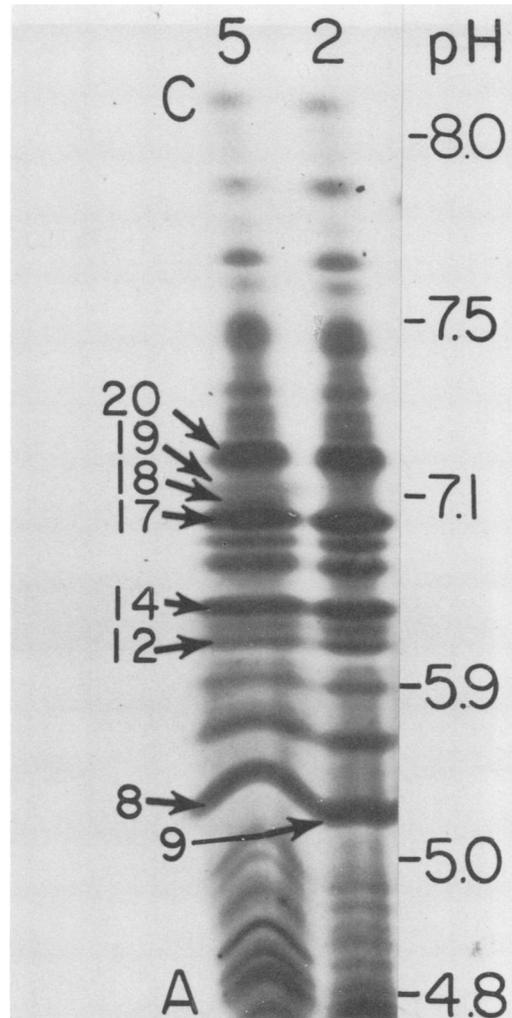


Fig. 2. Profiles of type 2 and 5 adenovirus hexon CNBr peptides fractionated by IFPA. Peptides were obtained by resuspending the protein in 70% formic acid and incubating in an  $\text{N}_2$  atmosphere for 24 h at 37 C with 1 mg of CNBr/mg of protein. Isoelectric focusing was done at 300 V for 18 h at 5 C in 5% polyacrylamide gels containing 8 M urea and carrier ampholytes (pH 3 to 10). Peptides were detected by staining the gel with Coomassie brilliant blue and were numbered consecutively from anode (A) to cathode (C). Therefore, peptides with the same pI derived from different hexon types do not necessarily have the same number.

detectable group-specific antigen and type-specific antigen (19, 21, 32). These findings imply either that the polypeptide chains assume different folding configurations or that different regions of the polypeptide chains are exposed to macromolecular interactions when the capsomer is held within the rigid restraints

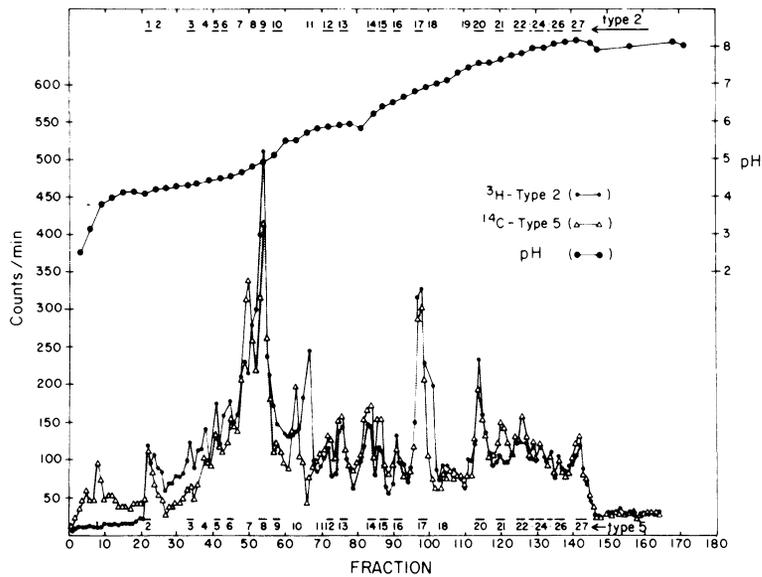


FIG. 3. Co-isoelectrophoresis of  $^3\text{H}$ -labeled type 2 and  $^{14}\text{C}$ -labeled type 5 hexon CNBr peptides. Conditions for cleavage and isoelectric focusing are described in Fig. 2. Cylindrical gels were used and were sliced into 1-mm fractions. The method of peptide numbering is based on bands detected by Coomassie brilliant blue staining of parallel cylindrical gels and correlated with peaks of radioactivity. Cross-hatched areas and numbers marked with a bar indicate peptides with identical  $pI$  values, although peptides with the same  $pI$  do not necessarily have the same number as described in Fig. 2. The  $^3\text{H}$  counts were corrected for  $^{14}\text{C}$  counts occurring in the channel set for  $^3\text{H}$  counting, which was approximately 11% of the total counts per minute.

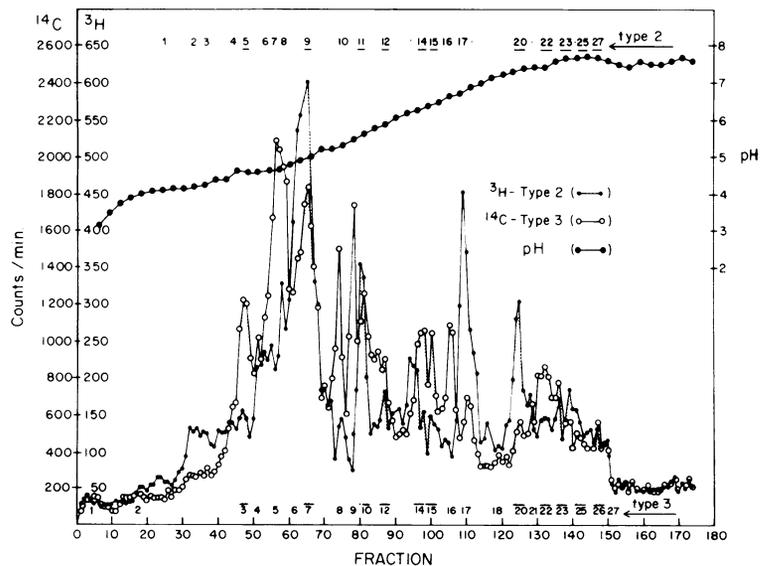


FIG. 4. Co-isoelectrophoresis of  $^3\text{H}$ -labeled type 2 and  $^{14}\text{C}$ -labeled type 3 hexon CNBr peptides. The experimental conditions and definitions are described in Fig. 2 and 3. The  $^3\text{H}/^{14}\text{C}$  correction is described in Fig. 3.

of the capsid or when the hexon is free in solution. To test these possibilities, the type 2 hexon was iodinated with  $^{125}\text{I}$  while the capsomer was assembled in the virion or when the purified hexon was in solution. When the hexon

is part of the virion, only tyrosines and histidines in the exposed regions of the polypeptides should be labeled, including those in the exposed type-specific antigenic reactive site. Similarly, certain regions of the polypeptides should

not be labeled until the capsomers are free of the capsid and free of any proteins attached to the hexons. Therefore, it should be possible to determine whether common or unique peptides are labeled and whether the physical state of the hexon influences the regions of the polypeptide chains exposed.

In the experiments to be described, proteins having 50,000 counts/min of  $^{125}\text{I}$  were in the gel. The amount of radioactivity associated with a particular peptide reflects the topology of the polypeptide chain during iodination (22) and the tyrosine and histidine content of the peptide (11). Therefore, the distribution of the radioactivity in the pH gradient is influenced by three factors: (i) the degree of exposure to the surface of that region of the polypeptide from which the peptide originates; (ii) the tyrosine and histidine content of individual peptides; and (iii) the number of peptides labeled.

**Iodination of virions.** Procedures for  $^{125}\text{I}$  labeling in the presence of lactoperoxidase and  $\text{H}_2\text{O}_2$ , disruption of type 2 virions after labeling, and purification of the virion hexons were described in Materials and Methods. The type 2 virion hexons were cleaved with CNBr and the peptides were fractionated by IFPA. Five of the virion hexon peptides were highly labeled when the virion was iodinated with  $^{125}\text{I}$ ; these peptides isoelectrophoresed between pH 6.8 and 8.0 (Fig. 5). In this region of the pH gradient, each hexon type yielded some common and unique peptides after CNBr cleavage (Fig. 2-4). The unique peptides may have originated from the type-antigenic determinants. Although some of the type 2 and 5 hexon CNBr peptides had distinct pI values in the low region of the pH gradient (Fig. 3), these peptides were poorly iodinated with  $^{125}\text{I}$  when the hexon was part of the virion (Fig. 5A) or as purified capsomer (Fig. 5B).

**Iodination of purified hexon.** When purified type 2 hexons were labeled with  $^{125}\text{I}$  in the presence of lactoperoxidase and then cleaved by CNBr, the distribution of radioactivity in the isoelectric gradient was different (Fig. 5B). Two peptides, 12 and 14, were predominantly labeled. Peptide 17 was also moderately labeled. Peptides 12 and 14 (Fig. 5B) had the same pI values, respectively, for all hexon serotypes analyzed (Fig. 3 and 4) and therefore have chemical similarities. This interpretation was confirmed by co-IFPA in cylindrical gels with  $^{125}\text{I}$ -labeled type 2 hexon peptides prepared as described above and  $^{14}\text{C}$ -labeled type 3 hexon peptides, as well as by the type 2 and 5 hexon peptides separated in thin-layer gels and stained with Coomassie brilliant blue (Fig. 2).

Peptide 17 derived from type 2 and 5 hexons had identical pI values (Fig. 3), but peptide 17 from type 3 hexon had a slightly higher pI (Fig. 4). Peptides 17 to 21 from iodinated type 2 hexons (Fig. 5B) were also labeled in the presence of lactoperoxidase, but the amount of radioactivity was decreased in comparison with hexons iodinated in the intact virion (Fig. 5A). This result was apparently not a reflection of a lower tyrosine and histidine content since peptides 17 to 21 contained relatively the same amount of radioactivity when iodination was done in the presence of chloramine T. It is possible that in free hexons the structural conformation of the segment of the polypeptide chain from which peptides 17 to 21 originate is partially folded to the interior of the capsomer; therefore this region may not be readily accessible to the high 78,000-mol-wt enzyme lactoperoxidase (25), whereas the 282-mol-wt

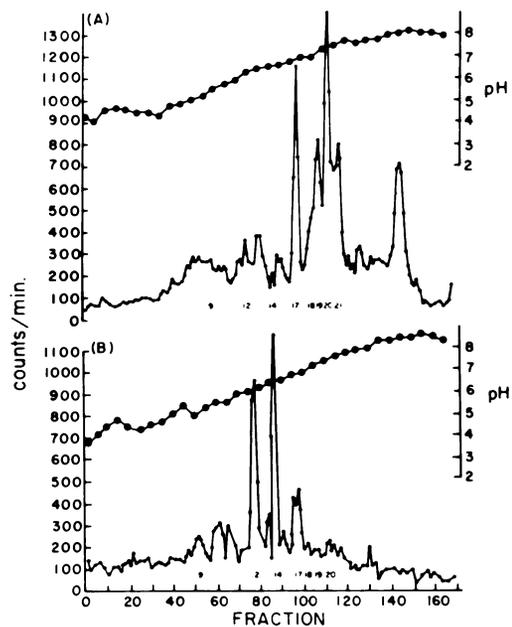


FIG. 5. Profiles of  $^{125}\text{I}$ -labeled CNBr peptides from the type 2 adenovirus hexons derived and purified from virions or from infected cell homogenates. The proteins were iodinated with  $^{125}\text{I}$  in the presence of lactoperoxidase and  $\text{H}_2\text{O}_2$ , cleaved with CNBr, and fractionated by IFPA as described in Fig. 2 and 3. The method of peptide numbering was based on bands detected by Coomassie brilliant blue staining of parallel cylindrical gels as described in Fig. 3. (A) Purified type 2 virions were iodinated with  $^{125}\text{I}$  and then disrupted in 10% pyridine, and the virion hexon was purified before CNBr cleavage. (B) Hexon, purified from the infected cell homogenate, was iodinated with  $^{125}\text{I}$  before CNBr cleavage.

chloramine T (11) can penetrate to this region of the polypeptide.

### DISCUSSION

Each type hexon has immunological type and group specificity. The type-specific antigen is thought to be on the surface of the virion because antibody to this antigen neutralizes the virus (18, 19, 33). The group-specific antigen is apparently buried when the hexon is part of the capsid (18), but it becomes accessible to antibodies in all purified hexon preparations (19, 21, 32). Hexon polypeptide chains must contain specific regions that confer these antigenic characteristics, and therefore different hexon types were analyzed to identify the extent of the common and variable segments in their respective polypeptide chains. Hexons of type 2, 3, and 5 adenoviruses were selected because type 2 and 5 adenoviruses have many similar properties and are classified in the same subgroup, III; type 3 adenovirus was analyzed because it belongs to subgroup I and has many properties that are different from type 2 and 5 viruses (8, 23, 26). The profiles of the CNBr peptides of type 2, 3, and 5 hexons in isoelectric gradients were compared. Since the peptides were maintained in 8 M urea and were of low molecular weight, little or no secondary structure should have been present. Consequently, the pI should be indicative of the net charge of the peptide, which is influenced primarily by its amino acid composition. Thus peptides from different hexon types and with the same pI values were considered to be chemically similar. Preliminary confirmation has been obtained with a few of the peptides that were extracted from gels and trypsinized; the generated tryptic peptides from common type 2 and 5 hexon CNBr fragments co-chromatographed on a Beckman peptide column with pyridine-acetic acid buffers (unpublished data). By these criteria, approximately one-third of the peptides of the three hexon types appeared to be structurally alike. Additional peptides, consisting of about another one-third of the protein mass, had similar pI values for the more closely related type 2 and 5 hexons. These data imply that the similar peptides originated from a common segment(s) of the polypeptide chain and that the peptides with distinct pI values were from the variable segment(s) of the polypeptide. In comparing the CNBr peptides of type 2 and 3 hexons, however, the inconstancy of the  $^3\text{H}/^{14}\text{C}$  ratios of the peptides that co-electrophoresed (Fig. 4) suggests that even some of these may not be identical. According to these criteria, the

amount of variability was far greater between type 2 and 3 hexons than between the hexons of types 2 and 5.

Some of these variable sequences should be responsible for the type-specific antigenic reactivity of the hexon assembled in the virion. Since the immunological reactions of hexons differ when they are aligned within the viral capsid and when they are free in solution and unencumbered by other proteins, it would be expected that different regions of the polypeptides are available to antibodies when the hexons are bound or free. Evidence supporting these postulates was obtained when hexons assembled in the virion and those free in solution were iodinated by the lactoperoxidase method. When virion hexons were iodinated, one set of peptides with pI values greater than 6.8 was iodinated; the set contained unique as well as some common peptides. Therefore, it is reasonable to suppose that the unique peptides originated from the type-specific antigenic determinants. These data suggested that the hexon is oriented in the capsid so that a specific region of the polypeptide chains, which contained these unique peptides as well as some common peptides, may fold to form the type-specific antigenic determinant involved in the neutralization reaction. It was striking that the surface peptides labeled in unassembled purified hexons were predominantly different from those in the virion hexons. The purified hexon peptides predominantly labeled (peptides 12 and 14) were peptides with common sequences in all three hexon types studied (Fig. 3 and 4). The purified hexon peptides that were labeled less efficiently consisted of common and variable regions of the polypeptide chains. The finding that the peptides exposed when hexons are held in capsids are not as readily exposed in unassembled purified hexons obtained from cell homogenates implies that the polypeptide chains may be in slightly different configurations in the two classes of hexons. These data suggest that the immunological differences between purified hexons in the cell excess pool and those assembled into capsids may be not only a reflection of the unique peptides in the virion hexon but also a variation imposed by slightly different secondary and tertiary structures of the two classes of hexons.

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