CONFORMATIONAL STUDY OF ADENOVIRUS TYPE 2 HEXON AND FIBER ANTIGENS

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Summary

The conformational state of adenovirus hexon and fiber proteins was investigated by both circular dichroism spectra and optical rotatory dispersion. It was found that native hexon protein has rather low α helix content and high level of random chain and β structure, and that fiber protein is in a highly unordered and β conformation. Sodium dodecyl sulfate treatment reordered most of the random and β forms in helical structures.

Introduction

Hexon and fiber antigens, two of the morphological subunits of the icosahedrally-shaped adenovirus capsid have been extensively purified and studied (1-3). Both proteins can be obtained in a crystallized form (4-7), which permits analysis of molecular structure by X-ray diffraction. Hexon has a molecular weight of 330, 000, calculated from both sedimentation-diffusion constants and crystallographic parameters (5) and is composed of three subunits of molecular weight 110, 000 (5, 8). Fiber is presumably constituted by one single polypeptide chain of molecular weight 70, 000 (2).

However, nothing is known about the conformational structure of the constitutive polypeptide chains of these proteins. Information on their conformational state could contribute to elucidate the mechanism

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of adenovirus capsomere assembly (9). In this study, we report the results of ultra-violet circular dichroism spectrum analysis of adenovirus type 2 hexon and fiber, in the native and denaturated state.

Materials and methods

- Materials

Hexon and fiber purification was carried out as described previously (3) with the following modifications : the high voltage preparative liquid film electrophoresis was replaced by a chromatographic step on DEAE-Sephadex A-50 and purification of adenovirus structural antigens was achieved by preparative polyacrylamide gel electrophoresis ("Prep-PAGE Quickfitt Apparatus", Quickfitt and Quartz Ltd, England) in 5 % acrylamide gel in the discontinuous buffer system of Davis (10). Hexon and fiber prepared by this method were homogeneous with regard to the following criteria : analytical acrylamide electrophoresis, immunoelectrophoresis, and analytical ultracentrifugation.

- Circular dichroism measurements

The circular dichroism (CD) was measured with a Roussel-Jouan Model CD 185 dichrograph in 0.1 cm path length cells at an adsorbance of less than 1.5 optical density unit. The proteins were dissolved in O.15 M NaF. The ellipticity is expressed as mean residual molar corrected ellipticity $[\theta']$ in degrees x cm^2 x $decimole^{-1}$ The mean residue weight was 111 for hexon and 105 for fiber. The ellipticity curves were constructed from at least five recordings. The errors in ellipticity were estimated to be 5 % at 205-225 nm and 10 % at 190-205 nm.

- Optical rotatory dispersion analysis

The optical rotatory dispersion (ORD) analysis was carried out on a Zeiss-Model 370 746 polarimeter at the following wavelengths : 578, 546, 436, 405 and 365 nm.

Results and Discussion

Generally, α helix, β pleated sheet and unordered segments can coexist in the same protein molecule in solution. The utility of CD in studying these three basic conformations depends on the possibility of correlating specific structures with CD spectra. i) CD spectrum of α helix polypeptides is quite well elucidated in terms of wavelengths and ellipticities (11). ii) Polypeptides in the β form exhibit CD patterns depending upon side-chains and solvents (12). iii) For random coil polypeptides, the CD spectra do not correspond, as has been often assumed, to that of a charged chain such as poly-L-lysine (12) : this latter have been shown to have locally ordered structures with characteristic CD spectra (11). Thus, the CD spectra of highly salt concentrated aqueous solutions of poly-L-serine must be used as models for unordered structures (13). The evaluation of protein conformation from theoretical combinations of experimental curves for α helix, β form and random coil has been made by several authors (12, 14), for proteins whose conformations had been previously determined from X-ray diffraction.

The interpretation of the experimental spectra obtained in this study in terms of α helical, β and unordered structures was made according to the recombination method of Greenfield and Fasman (12), except that poly-L-serine was used as reference model for random coil. Fig. 1 and 2 present the far ultraviolet CD spectra of hexon and fiber proteins in the native state, and in the presence of sodium dodecyl sulfate (SDS).

In the native form, hexon spectrum suggests a mixture of the three conformations α helix, β form and unordered structure. That is confirmed by the optical rotatory dispersion data : the calculated A_{225} and A_{193} parameters for hexon do not correspond to Shechter and Blout plot, which is characteristic of a mixture of only α helix and random conformation (15). The Moffit-Yang b_o parameter is particularly suitable for the estimation of the helical content, since this parameter is known to be essentially insensitive to non-conformational effects, and ORD analyses of β or unordered structured polypeptides result in zero or very small values of b_o (16). By this method, one obtains a α helix percentage of

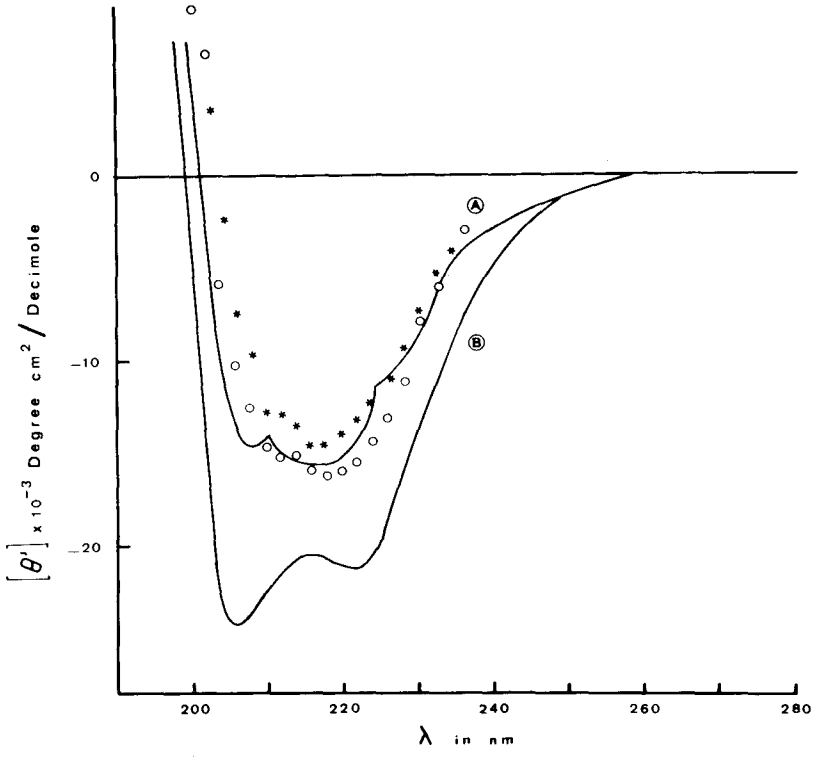

Fig. 1. - The circular dichroism spectra of hexon protein

In the native state (curve A) in 0.15 M NaF aqueous solution, its CD spectrum is characterized by three maxima at 225, 217.5 and 207.5 nm, and a crossover at 201 nm. 20 % α helix 40 % β structure, 40 % random conformation (o), and 30 % α helix, 30 % β structure, 40 % random conformation (*) were calculated from poly-L-Lysine and poly-L-serine reference spectra in water. In 0.15 M NaF aqueous solution containing 0.04 M SDS (curve B), the CD spectrum shows only two negative bands at 222 and 205 nm and a crossover at 198 nm.

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about 20 % for hexon molecule. This result was confirmed by computed data from CD spectra, which indicate the percentages of 20 to 30, 30 to 40 and 40 % for α helix, β form and unordered structure, respectively (Fig. 1 A).

For the native fiber (Fig. 2 A), there is no evidence, in the CD spectrum, for the presence of a negative band or a shoulder at about

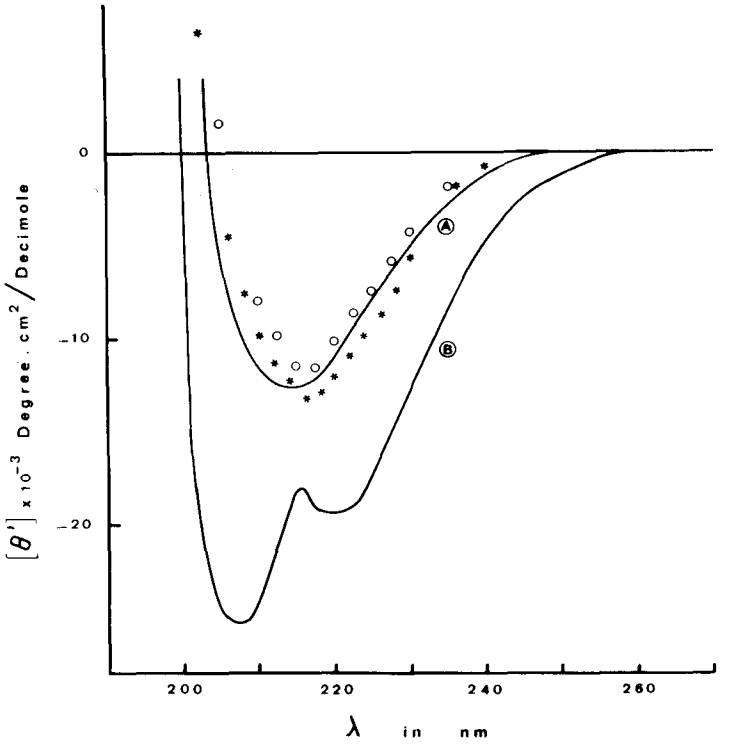

Fig. 2. - The circular dichroism spectra of fiber protein

In the native state (curve A) in 0.15 M NaF aqueous solution, its CD spectrum is characterized by one negative band with a maximum at 214 nm and a crossover at 204 nm. 60 % β structure, 40 % random conformation (o), and 10 % α helix, 50 % β structure, 40 % random conformation (*) were calculated from poly-L-lysine and poly-L-serine reference spectra in water. In 0.15 M NaF aqueous solution containing 0.04 M SDS (curve B), two negative bands are visible at 222 and 206 nm, with a crossover at 200 nm.

222 nm or 207 nm, normally displayed by even small amounts of helical form such as observed in ribonuclease (14, 17). The magnitude of the ellipticities indicates that most of the fiber is constituted of β and unordered structures. Because of the somewhat conflicting data available from different reference compounds, it is difficult to assess which fraction of the protein has adopted the pleated-sheet conformation. However, one can estimate, from CD pattern, that fiber has about 50 to 60 % β conformation

and 40 % unordered structure, although the presence of some α helix (less than 10 %) could not be totally excluded in fiber.

A radical change in CD spectra is induced by SDS as shown in fig. 1 B and 2 B : CD spectra exhibit two extrema, one located at 222 nm (α helix), and a major one at 205 nm. As observed for other proteins (18, 19), SDS-treatment reorders most of the native β form and unordered structures and leads to the formation of helical structure, but CD pattern cannot be perfectly interpreted unless it is supposed that SDS induces the formation of another type of helix : the 205 nm maximum obtained with SDS is consistent with the "extended helix" model of Tiffany and Krimm (11) and the CD pattern is reminiscent of a mixture of α helix and this other form of helix. Presence of such a structure in SDS-treated hexon and fiber proteins is not improbable and is supported by the fact that hydrophobic bonds play a decisive role in the protein conformation and a hydrophobic environment is needed for the formation and stability of the hydrogen-bonded secondary structures (20). Unfortunately, further information on native protein structure cannot be inferred from SDS-CD spectra, for it is impossible to determine which regions and/or which structures of the native molecule are responsible for the formation of each helical form.

The far-ultraviolet circular dichroism spectra show adenovirus hexon and fiber to be nonhelical proteins. The low level of α helical structure and the high percentage of β and unordered conformations in hexon protein is scarcely surprising since hexon presents a compact polygonal structure (1, 21) and since a number of globular proteins (except globin) have proved relatively poor in α helix (16, 19, 22). In the other hand, the presence of major β conformation in fiber polypeptide makes it structurally similar to other rod-like proteins, e. g. β - keratin (23) and silk fibroin (24).

The CD examinations herein reported were carried out on "soluble" hexon and fiber antigens, i. e. adenovirus structural proteins produced in considerable quantities by KB cells infected with adenovirus and released by lysed cells. It is obvious that the molecular structure of hexon and fiber can be different when associated in adenovirus capsid and when dissociated in "soluble" form. It would be of interest to study the molecular conformation of fiber in relationship with penton base as

in penton capsomere, of penton capsomere surrounded by five hexons (21) of dodecon (assembly of twelve pentons (25)) and of associations of three, six and nine hexon capsomeres, as released from desintegrated adenovirus capsids (21, 26).

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