COMPARATIVE OPTICAL PROPERTIES OF FREE AND ASSEMBLED HEXON CAPSOMERES OF HUMAN ADENOVIRUS TYPE 2

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1. Introduction

The proteinic capsid of adenovirus has been shown to be in the form of an icosahedron 65–80 nm in diameter, and composed of 252 morphological subunits, the so-called capsomeres. Of these capsomeres, 240 are located at the faces and edges of the icosahedron, and, since they have 6 neighbours, they are called hexons. Each of the 12 vertex capsomeres is surrounded by 5 neighbouring hexons and is called penton [1,2].

The hexons are produced in large excess by the infected cell and can be isolated as soluble free subviral components [3–7]. The human adenovirus type 2 hexon is mol. wt 300 000–360 000 for the intact native capsomere, which is composed of three identical polypeptide units of 100 000–120 000 [8–10].

Under mild conditions of disruption with detergents or chemicals [11–13], the adenovirus particle releases a nucleoprotein core and groups of nine hexons arranged with a 3-fold symmetry [14]. In this hexon ninemer, 6 hexons originate from an icosahedral triangular face, and 3 from its adjacent edges [15].

The aim of the present study was to compare the optical properties of free hexon capsomeres and of interlocking hexons in ninemers, in order to determine possible changes of conformational structure of the protein chain upon capsomere assembly, and also to elucidate the nature of the amino acid residues involved in their linkage within this ninemeric edifice.

2. Experimental

2.1. *Cell and virus*

Human adenovirus type 2 was propagated on KB cells maintained in spinner culture in Eagle's basal medium supplemented with 5% horse serum.

2.2. *Isolation of free adenovirus hexon capsomeres*

The isolation and purification of adenovirus type 2 soluble hexons from infected cell extracts have been described in detail [7]. It comprises a fluorocarbon extraction of cell lysate, ammonium sulfate precipitation, DEAE-Sephadex and hydroxyapatite chromatography. The final product was homogeneous as regard to the following criteria: two-dimensional immunoelectrophoresis, analytical ultracentrifugation, analytical acrylamide gel electrophoresis, and electrophoresis on sodium dodecyl sulfate-containing acrylamide gel after denaturation of the protein with 1% sodium dodecyl sulfate (SDS)–2% 2-mercaptoethanol.

2.3. *Isolation of groups of nine hexons*

Hexon ninemers were isolated from purified adenovirus particles. Adenovirus were purified from fluorocarbon extract of cell lysate by two successive cycles of CsCl bandings, as in conventional techniques [16]. The virus band from CsCl gradient was dialysed against 5 mM Tris–HCl buffer, pH 7.8, and dissociated by heating at 56°C with 0.5% (w/v) sodium deoxycholate for 90 s. The dissociation was evidenced by the sudden disappearance of the opalescence of the virus suspension [12]. The cleared virus suspension

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(0.5–1 ml) was then layered on top of a 10–40% (w/v) glycerol gradient in 11 ml 5 mM Tris–HCl buffer pH 7.8 and centrifuged at 35 000 and 4°C for 80 min in a MSE-6 × 14 ml swinging-bucket rotor. A 1 ml cushion of 80% (w/v) glycerol was placed at the bottom of each gradient. Each gradient fraction (0.6 ml) was monitored by A_{260} and A_{280} and the different peaks thus obtained examined in electron microscopy. Each fraction was also analyzed by SDS–polyacrylamide gel electrophoresis after reduction by 2-mercaptoethanol and SDS-denaturation.

The fractions containing groups of nine hexons were pooled, dialysed against 50 mM sodium phosphate buffer, pH 6.8, and groups of nine further separated from contaminating free capsomeres by chromatography on DEAE-Sephadex A-50 equilibrated in the same buffer. Proteins were eluted with a linear gradient of NaCl made in the same buffer and ranging from 0.0–0.6 M NaCl.

2.4. *Circular dichroism (CD) measurements*

The CD spectra in far and near ultraviolet were recorded with a Jobin-Yvon R. J. Mack III Dichrograph, at 24°C, under a stream of dry N_2, with a cell path length of 0.01 cm, 0.05 cm and 0.1 cm. Monomeric and ninemeric hexons were dissolved in 10 mM Na phosphate buffer, pH 6.8. Protein concentrations were adjusted to give absorbances between 0.5 and 1.0. The protein estimations were carried out by the technique described [17], using bovine serum albumin as standard. The molar ellipticity $[\theta]$ expressed as degrees × cm^2 × $dmol^{-1}$ was calculated on the basis of a mean residue weight of 111 for hexon protein, value obtained from the amino acid composition [5,6]. A nonlin program, NLG version [18] was used to resolve the intrinsic CD curve into gaussian bands.

3. Results

3.1. *Isolation of adenovirus 2 hexon ninemers*

As shown on fig.1, groups of nine hexons sedimented with an apparent sedimentation coefficient of 50–60 S, intermediate between adenovirus cores (185–200 S) and free capsomeres (10–12 S). The fractions 11–16 of the gradients, containing the hexon ninemers were pooled, dialysed against 50 mM sodium phosphate buffer, pH 6.8, and chromatographed on DEAE–Sephadex A-50. The hexon ninemers eluted in the excluded peak, whereas the other contaminating viral soluble components were retained on the ion-exchanger (not shown). Pentons were eluted at 0.1 M NaCl and free hexons at 0.35 M NaCl [7].

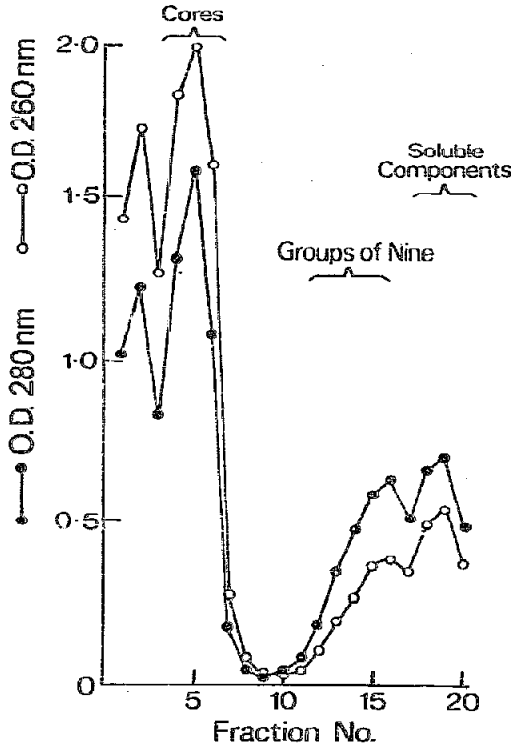

Fig.1. Isolation of groups of nine hexons from deoxycholate-disrupted adenovirus on a 10–40% linear glycerol gradient. The presence of DNA in the adenovirus cores is evidenced by the higher A_{260}.

3.2. *CD spectra of free and assembled hexons*

The shapes and intensities of the intrinsic dichroic spectra from 180–250 nm were identical for both kinds of hexons (not shown; see the CD spectra of free hexons [19,20]). Resolution of the CD curves into gaussian bands yielded positive maxima at 191 nm and negative maxima at 198, 207, 214 and 222 nm. The α-helix content was estimated from the n–π^* transition, i.e., the band centered at 220 nm, according to [21], taking the value of −35 000 for the helix as standard. The amount of β structure was

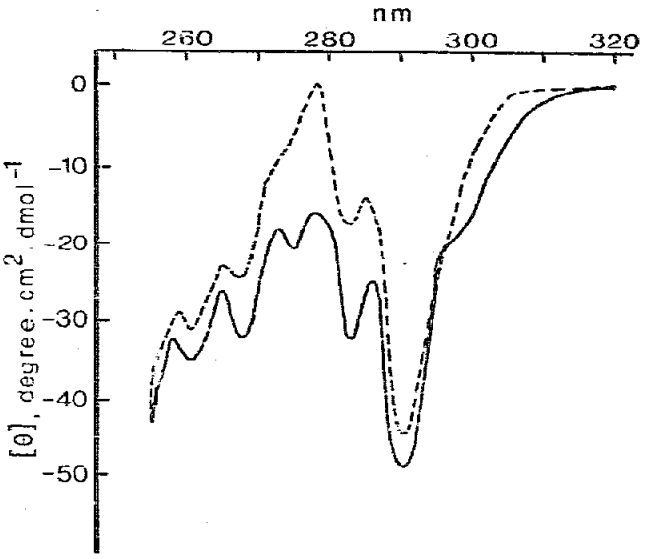

Fig.2. Circular dichroism spectra in near ultraviolet of free hexon capsomeres (------) and of hexon capsomeres assembled in groups of nine generating the faces and part of the edges of the adenovirus icosahedron (———). The nature of the linkage between the hexons affected the magnitude of the dichroic bands between 283 nm and 302 nm, specific for the chromophore group of tryptophan.

estimated in a similar way, using the band at 214 nm, and taking — 9200 for the β standard [21]. This resulted in about 16% α-helix and about 25% β-structure. The rest of the polypeptide backbone was apparently in aperiodic loop and bend conformation.

The CD spectra for hexon monomers and ninemers from 250–320 nm are given in fig.2. All bands were negative and showed slight but significant differences between both samples. More specifically, a shoulder was observed at 302 nm for ninemers, which was not found in the hexon monomer spectra. At 290 nm, and 283 nm the dichroic bands were more intense for the hexon ninemers than for the monomers. In addition, a dichroic band was visible at 275 nm, only in the ninemer spectra. Since the amino acid composition of both samples was identical, these changes of amplitude in the ninemer CD spectra were likely due to a stronger organization of the protein chains within the oligomers.

The CD bands concerned could be correlated to specific residues involved in the assembly of hexons. The marked shoulder at about 300 nm suggested that tryptophan residues were in a disturbed electronic environment within the ninemers [22]. The band at 290 nm was also induced by tryptophan indole rings and corresponded to the so-called 0–0L_b band [22]. In some cases a companion CD band with the same sign can occur from 6–8 nm on the short wavelength side [22]. This band at 283 nm was also probably due to tryptophan residues, but this was difficult to assess, since the tyrosyl CD maximum is located between 275 nm and 282 nm: tyrosyl bands could therefore superimpose the tryptophan band in this spectrum domain.

4. Discussion

Adenovirus type 2 hexon is a trimeric protein [9] which can assemble in more complex edifices such as groups of nine hexons and virus shells [23]. Hexons have a natural tendency to self-aggregate spontaneously or after limited proteolysis [24]. Cross-linking in vitro with glutaraldehyde, as well as limited proteolysis, seems to generate a preferred hexon multimeric species, hexon trimers [9,19].

Groups of nine hexon have been shown to be constituted of the regular arrangement of three groups of three hexons with a 3-fold symmetry [14]. The mechanism of adenovirus capsid construction and the resulting bonds between the different capsomeres are still unclear. Low molecular weight viral proteins have been assigned as cementing material between the major capsid subunits. The polypeptide IX, according to the accepted nomenclature for adenovirus proteins, of mol. wt 12 500 is associated with groups of nine hexons [25]. Scanning of the analytical SDS–acrylamide gel of hexon ninemers stained with Coomassie blue or autoradiographed after labeling with [^{3}H]-valine revealed that IX accounted for less than 5% of the total protein material (not shown).

Comparative analysis of the CD spectra of free and assembled hexons capsomeres has been made to elucidate the nature of the bonds between the hexons and the possible consequences of hexon interlocking on the conformational structure of the protein. Both experimental and theoretical considerations indicate that some particular amino acid side-chain interactions cause more intense CD than others. For example, from a qualitative point of view, it has been estab-

lished that when a tyrosyl or tryptophanyl side chain is at less than 10 Å from another aromatic side chain, the near ultraviolet CD may be particularly intense, due to μ–μ coupling. Coupling between the near ultraviolet transition of a tyrosyl or tryptophanyl side chain and the π–π^* transitions of peptide bonds within about 8 Å may also give important CD intensity.

The CD spectra in near ultraviolet of free and assembled hexons showed discrete but significant differences. Marked negative bands at 300, 290 and 283 nm suggested that some critical tryptophanyl side chains were involved in the hexon bonding within the ninemers without important pertubations of the tertiary structure of the hexon molecule upon assembly. The same kind of bonding has been found implicated in the aggregation of tobacco mosaic virus protein [26]. The contribution of the minor hexon-associated polypeptides IX to the change of ellipticity amplitude in the 300–283 nm domain of hexon ninemers, compared to free hexons, could not be established since the tryptophan content IX is still unknown. The tryptophan content of hexon protein is 1.2–1.5% [5,6] and the ultraviolet spectrum of the native protein shows a shoulder at 293 nm, characteristic of tryptophan ([20], personal observations). The absence of appreciable variation in the intensity of far ultraviolet CD spectra between free hexons and hexon ninemers, suggested that the assembly of hexons into groups of nine was not accompanied by any folding or alteration of the secondary structure of the hexon molecule. Hexon protein has been shown to have a low helical content [19,20] - no modification of this α-helix structure was found.

Mild disruption of a virus capsid and analysis of resulting subviral components offers therefore an interesting route to study the interactions between the capsomers at different stages of assembly. Groups of nine hexons maintained by tryptophan-mediated hydrogen bonds seem a rather stable and preferred edifice of the adenovirus complex capsid. Hexon ninemers can also re-aggregate in vitro at low pH values into icosahedral shells [23]. As inferred from biochemical [11–15] and structural data [27] the bonding between two neighbouring hexon ninemers in the capsid is weaker than bonding within a group of nine hexons. The nature of this interninemeric bonds remains to be eludicated.

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