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Synthesis In Vitro of Type 5 Adenovirus Capsid Proteins[1]

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Reaction mixtures containing cytoplasmic extracts and ribosomal fractions prepared from KB cells infected with type 5 adenovirus were able to carry out incorporation of amino acids into protein. The in vitro product included proteins which reacted specifically with antisera to adenovirus capsid proteins; in control experiments with extracts from uninfected cells, no reactions with the antisera were found. The viral proteins were synthesized in vitro on small polyribosomes, were released from them, and significant numbers of the free polypeptides were assembled in vitro into multimeric adenovirus capsid structures.

Upon infection of mammalian cells, adenovirus deoxyribonucleic acid (DNA) enters the nucleus (11) where it replicates and accumulates (3). The capsid proteins, however, are synthesized on cytoplasmic polyribosomes, but after release they rapidly migrate into the nucleus (20) and about 10% combines with viral DNA to form virions (2, 13, 14). To explore the control of synthesis, morphogenesis, and transport of adenovirus capsid structures, an in vitro system was developed. This communication reports that virus-specific proteins can be made in vitro and that this process shows striking similarities to events observed in cell cultures (19). Viral proteins were synthesized in cytoplasmic extracts (or with partially purified ribosomal fractions) on rather small polyribosomes and were released as chains which sedimented with an average sedimentation coefficient of 3*S*. The material synthesized in vitro was immunologically reactive, and a sizable fraction of the nascent polypeptides was assembled into structures with the sedimentation characteristics of adenovirus hexon, penton base, and fiber.

MATERIALS AND METHODS

Virus and cell cultures. The methods for cell growth, viral propagation, and assay have been described (1, 7). KB cells in suspension cultures were infected with 100 to 200 plaque-forming units of type 5 adenovirus. From 18 to 20 hr later, cells were collected and washed twice with cold 0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, *p*H 7.6, containing 0.15 M sodium chloride. Uninfected cells taken directly from suspension cultures were also employed.

Preparation of cytoplasmic extracts and ribosomal fraction. The washed cells were suspended in RSB [0.01 M Tris-hydrochloride (*p*H 7.6), 0.0015 M $MgCl_2$, and 0.01 M KCl] containing 0.001 M dithiothreitol (1 ml per 4×10^7 packed cells), allowed to swell for 10 min, and disrupted in a glass Dounce homogenizer. Nuclei and unbroken cells were separated by centrifugation at $750 \times g$ for 10 min; the supernatant fluid was centrifuged again and used as cytoplasmic extract. The protein concentration was generally 12 to 14 mg/ml. Ribosomes were sedimented from the cytoplasmic extracts in a Spinco angle rotor no. 65 at 40,000 rev/min for 2 hr. The pellet was resuspended in RSB containing dithiothreitol and termed the ribosomal fraction.

Incorporation of amino acids in vitro. Cytoplasmic extracts or ribosomal fractions were mixed with one volume of an assay mixture containing the following ingredients (in final concentrations): Tris-hydrochloride (*p*H 7.6), 0.05 M; ammonium chloride, 0.050 M; magnesium chloride, 0.006 M; potassium chloride, 0.005 M; dithiothreitol, 0.001 M; adenosine triphosphate (ATP), 0.002 M; guanosine triphosphate (GTP), 0.0002 M; potassium phosphocreatine, 0.010 M; phosphocreatine kinase, 20 μg/ml; ^{14}C-valine, 0.4 μCi/ml, 1.6 nmoles/ml; or ^{14}C-amino acid mixture, 4 μCi/ml total; and unlabeled amino acids, 4 nmoles/ml each.

Standard conditions. Standard conditions consisted of incubating 0.2-ml mixtures at 37 C for 30 min (exceptions are noted in the legends to figures and Tables). Incorporation of ^{14}C-amino acid into protein was assayed by precipitation of protein with excess 5% trichloroacetic acid and heating of the mixture in boiling water for 10 min. Precipitates were collected on filter paper discs, washed with trichloroacetic acid, ethanol, and acetone, and dried. Radioactivity was determined in a toluene-based scintilla-

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tion fluid utilizing a Packard Tricarb scintillation spectrometer.

Antisera and purified viral proteins. Antisera to purified adenovirus and viral components were prepared by methods described previously (19, 20). Antiserum to adeno-associated virus type 4 was a generous gift of D. M. Hoggan, National Institutes of Health. ^{14}C-Labeled adenovirus hexon and fiber were prepared by modification of described methods (10) to be detailed elsewhere (P. Dorsett and H. S. Ginsberg, *in preparation*).

Immunological precipitation of proteins. (i) Direct precipitation. Samples of incubation mixtures were diluted to 0.4 ml with PBS (0.01 M phosphate buffer, *p*H 7.2, containing 0.15 M NaCl) and mixed with 0.2 ml of a dilution of antiserum which was shown to give optimal antigen precipitation. Sodium dodecyl sulfate (SDS) was added to a final concentration of 0.05%. After 2 hr of incubation at 37 C, antigen-antibody complexes were collected by centrifugation (Servall angle rotor SM 24; 4,500 × *g*, 10 min) and washed three times in cold 0.15 M NaCl. Precipitates were dissolved in 0.1 M NaOH, precipitated with cold 5% trichloroacetic acid, heated, and washed as described for determination of radioactivity.

(ii) Coprecipitation. Coprecipitation was used for assay of the small quantities of viral proteins in fractions from polyribosome gradients. Samples were mixed with 0.05% SDS (final concentration) and antiserum in a volume of 0.4 ml. After incubation as above, 0.2 ml of the optimal dilution of goat anti-rabbit gamma globulin was added, and the mixture was incubated for an additional 2 hr. The precipitate formed was collected and assayed as described above.

Sucrose density centrifugation of polyribosomes. After the addition of 0.5% sodium deoxycholate (final concentration), the in vitro reaction mixtures were layered on 28-ml, 15 to 30% linear sucrose gradients in RSB and centrifuged for 90 min at 4 C in a Spinco SW 25.1 rotor at 22,500 rev/min. Fractions (1 ml) were collected from the bottom of the tube and assayed for absorbance at 260 nm, trichloroacetic acid-precipitable radioactivity, immunologically precipitable radioactivity, or both. Sedimentation coefficients were estimated from the position of the monoribosome peak (74*S*).

Sucrose density sedimentation of proteins. Generally, 0.2-ml samples of in vitro incubation mixtures or purified viral proteins as reference markers were layered on 5-ml, 5 to 20% linear sucrose gradients in PBS containing 0.001 M sodium ethylenediaminetetraacetate and centrifuged for 17.5 hr at 20 C in a Spinco SW 65 rotor at 32,000 rev/min. Five-drop fractions (40–45 total) were collected directly on filter paper discs which were washed sequentially with hot and cold 5% trichloroacetic acid and acetone, dried, and assayed for radioactivity. Sedimentation coefficients were estimated from the position of viral hexon at 12*S* and fiber at 6*S* (10, 15, 16).

Sources of materials. Radioactive amino acids were products of New England Nuclear. ATP, GTP, and phosphocreatine kinase were obtained from Sigma Chemical Company; phosphocreatine was purchased from Calbiochem.

RESULTS

Requirements for and kinetics of in vitro amino acid incorporation. Cytoplasmic extracts of KB cells infected with type 5 adenovirus incorporated radioactive amino acids into trichloroacetic acid-insoluble material. Most experiments were performed with ^{14}C-valine, but ^{14}C-phenylalanine, ^{14}C-lysine, or a mixture of fifteen ^{14}C-amino acids were also incorporated into protein. The incorporation (Fig. 1) was linear for 15 min, after which the rate declined and incorporation was virtually complete after 30 min at 37 C. Amino acid incorporation was dependent on the presence of the cell extract (Table 1), an ATP-generating system, and a critical concentration (ca. 6 mM) of magnesium ion. The addition of cycloheximide, puromycin, or exogenous ribonuclease inhibited incorporation, but chloramphenicol did not affect the reaction. Sodium fluoride and pactamycin, inhibitors of initiation of mammalian protein synthesis (5, 9), also blocked the incorporation. These data implied that the amino acid incorporation measured resulted from in vitro protein synthesis and not only from incorporation of amino acids into proteins of contaminating whole mammalian cells or bacteria.

When the cytoplasmic extract was centrifuged at 100,000 × *g* to remove ribosomes, the supernatant fluid did not incorporate amino acids (≦2% of the original extract). When the ribosomal pellet was suspended in buffer and supplied with the required materials (Table 1), however, protein synthesis ensued as previously described (Table 2). Although the ribosomal mass in each final incubation mixture was equiva-

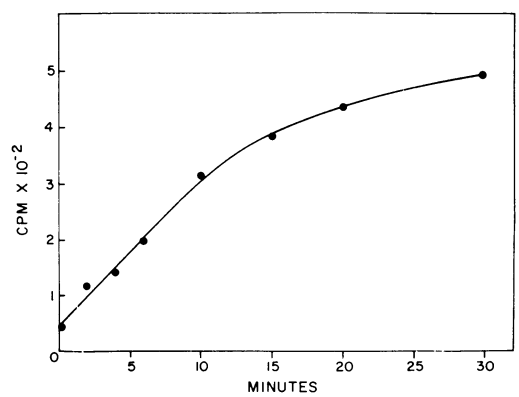

FIG. 1. *Kinetics of incorporation of* ^{14}*C-valine into protein by cytoplasmic extract of infected cells. The volume of the reaction mixture was 2 ml; the contents are described in Materials and Methods. Samples (0.1 ml) were assayed for trichloroacetic acid-precipitable radioactivity.*

TABLE 1. *Requirements for in vitro amino acid incorporation and response to inhibitors*

Conditions[a]	^{14}C-Valine in protein (counts/min)
Expt 1	
Complete	1,030
No extract	41
Trichloroacetic acid at zero time	68
No ATP-generating system[b]	81
Expt 2 and 3	
Complete—1 mM Mg^{2+}	56
5.8 mM Mg^{2+}	1,090
11 mM Mg^{2+}	210
Complete—3.3 mM Mg^{2+}	190
5.8 mM Mg^{2+}	1,110
8.3 mM Mg^{2+}	705
Expt 4	
Complete	906
+ Cycloheximide (1 mM)	123
+ NaF (10 mM)	210
+ Puromycin (0.1 mM)	55
+ Ribonuclease (5 μg/ml)	50
Expt 5	
Complete	920
+ Chloramphenicol (1 mM)	980
Expt 6	
Complete	850
+ Pactamycin (0.001 mM)	740
+ Pactamycin (0.005 mM)	492
+ Pactamycin (0.01 mM)	450

[a] Protein synthesis under standard conditions (see Materials and Methods) with cytoplasmic extracts from infected cells. The total incubation mixtures were assayed for trichloroacetic acid-precipitable radioactivity.

[b] Adenosine triphosphate (ATP), phosphocreatine, and phosphocreatine kinase were deleted from complete system.

lent in the experiments summarized in Tables 1 and 2, the amino acid incorporation was markedly increased when only the ribosomal fraction was employed. These results could be accounted for by the difference in specific activity of the radioactive valine in the two systems. The added radioactivity was diluted with unlabeled valine contained within the cells when they were disrupted to yield the extract, whereas the unlabeled valine pool was removed when the ribosomes were sedimented and resuspended. The following findings support this explanation. An infected culture was equilibrated with ^{14}C-valine, and a cytoplasmic extract and ribosomal fraction were prepared. The concentration of valine in these preparations, relative to the culture fluid, was

TABLE 2. *In vitro protein synthesis with ribosomes isolated from infected cells*

Conditions[a]	^{14}C-Valine in protein (counts/min)
Complete	6,250
+ NaF (10 mM)	1,730
+ Cycloheximide (1 mM)	1,180
+ Ribonuclease (5 μg/ml)	134
− ATP-generating system	108

[a] Protein synthesis under standard conditions with the ribosomal fraction of infected cells. The total incubation mixtures were assayed for trichloroacetic acid-precipitable radioactivity. ATP, Adenosine triphosphate.

determined by measurement of acid-soluble radioactivity in the fluid, the extract, and the ribosomal fraction. From these data, the specific activity of the ^{14}C-valine in the reaction mixture containing the ribosome fraction was calculated to be sixfold higher than in the extract mixture; the addition of undialyzed ribosomal supernatant fluid to ribosomes reduced the incorporation to the level of the extract alone, whereas the dialyzed ribosomal supernatant fluid stimulated ^{14}C-valine incorporation slightly.

In vitro protein synthesis was also examined in reaction mixtures containing extracts from uninfected cells. The requirements and responses to inhibitors were similar to those noted in Tables 1 and 2. However, the product of the uninfected cell reaction mixture differed greatly from that of the infected cells (see below).

Polyribosomes in the in vitro reaction mixture. In cells infected with type 5 adenovirus, the viral polypeptides are synthesized on polyribosomes with a peak sedimentation coefficient of about 200*S* (19). To characterize further the nature and kinetics of the in vitro protein-synthesizing system, samples of the reaction mixture were centrifuged on sucrose density gradients after increasing incubation periods. After 3- or 10-min incubation periods, during the period of linear protein synthesis, nascent polypeptides were labeled on polyribosomes of 180 to 200*S* (Fig. 2) which were similar in size to the polyribosomes in adenovirus-infected cells (19). The most active polyribosomes encompassed those in tetramers to hexamers.

As would be predicted from the kinetics displayed in Fig. 1, the polyribosomes were functional in protein synthesis for 3 and 10 min; during this period the completed proteins were released from polyribosomes and found at the top of the gradients (Fig. 2A and B). However, after 25 min, protein synthesis had essentially

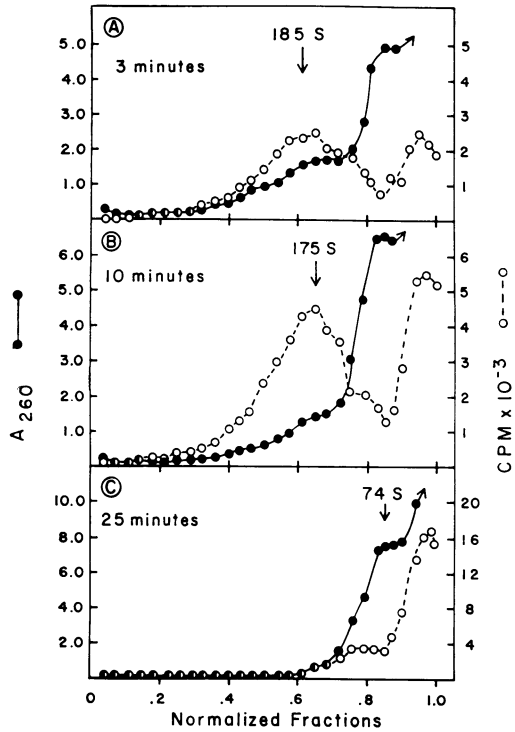

FIG. 2. *Sucrose density sedimentation analysis of polyribosomes after various periods of in vitro protein synthesis with a cytoplasmic extract of infected cells. Mixtures of 2 ml containing the ^{14}C-amino acid mixture were incubated for 3 min (A), 10 min (B), 25 min (C) and centrifuged in a linear 15 to 30% sucrose gradient for 90 min in a Spinco SW 25.1 rotor at 22,500 rev/min. Fractions (1.0 ml) were collected. A_{260} (●); trichloroacetic acid-precipitable radioactivity (○). Sedimentation coefficients of the peak fractions of polyribosome incorporation were estimated from the position of the monoribosomes (74S).*

ceased (Fig. 1); the polyribosomes had been converted to monoribosomes, and over 80% of the nascent proteins had been released (Fig. 2).

Products of in vitro protein synthesis. Immunoprecipitation procedures were employed to characterize the products of the reaction mixtures prepared with the cytoplasmic extract of infected or uninfected cells. After the reaction mixture was incubated until protein synthesis had ceased, a major fraction (20–40% in various experiments) of the radioactive product from an infected system reacted with antibodies to purified type 5 adenovirus. Only 1 to 5% of this product was precipitated by normal rabbit serum or by sera from rabbits immunized with type 4 adeno-associated virus or simian virus 40 (Table 3). In contrast, the in vitro product of the reaction mixture containing a cytoplasmic extract from uninfected cells reacted to the same low level with either antiviral serum or normal serum (Table 3, experiment 5). Moreover, when the labeled in vitro product of an uninfected system was mixed with an unlabeled infected cell extract, either normal serum or adenovirus antiserum precipitated approximately equal amounts of the label (Table 3, experiment 6).

To determine further whether virus-specific proteins were made in vitro, the product was analyzed immunologically with antisera from rabbits immunized with purified viral capsid proteins. The results summarized in Table 4 show that the protein synthesized in vitro was precipitated with antibodies directed against either the adenovirus hexon, the major macromolecular subunits of the virus (6), or a mixture of fibers and pentons, structures which form the corner

TABLE 3. *Immunological precipitation of protein synthesized in vitro*

Conditions of protein synthesis[a]	Antiserum to	Per cent labeled protein precipitated
Expt 1: Cytoplasmic extract, infected	Virion[b]	40
	NRS[c]	5
Expt 2: Cytoplasmic extract, infected	Virion	23
	NRS	1
Expt 3: Ribosomal fraction from same preparation as expt 2	Virion	24
	NRS	1
Expt 4: Cytoplasmic extract, infected	Virion	28
	AAV[d]	1
	SV40[e]	1
	NRS	2
Expt 5: Cytoplasmic extract, uninfected	Virion	3
	NRS	2
Expt 6: Cytoplasmic extract, uninfected	Virion	2
	NRS	1
Plus unlabeled, infected extract after protein synthesis[f]	Virion	4
	NRS	3

[a] Protein synthesis in incubation mixtures of 1 ml. Samples of 0.2 ml were assayed for total trichloroacetic acid-precipitable radioactivity or radioactivity precipitable by antisera. Levels of ^{14}C-valine incorporation were comparable to those of Tables 1 and 2.

[b] Virion = antiserum to purified type 5 adenovirus.

[c] NRS = normal rabbit serum; sera from unimmunized animals.

[d] AAV = type 4 adeno-associated virus.

[e] SV40 = simian virus 40.

[f] Before assay of radioactivity precipitable by antisera, these samples were mixed with equal volumes of a synthesis system prepared with an infected extract and ^{12}C-valine.

TABLE 4. *Immunological precipitation of in vitro synthesized proteins by antisera to specific structural proteins of type 5 adenovirus*

Reaction mixture[a] containing infected cell material	Antiserum to	Per cent labeled protein in complex
Expt 1: cytoplasmic extract	Virion[b]	21
	Hexon[c]	7
	Fiber-penton[d]	1
	NRS[e]	1
Expt 2: ribosomal fraction from same preparation	Virion	26
	Hexon	14
	Fiber-penton	21
	NRS	3

[a] Protein synthesis in incubation mixtures of 1 ml. Samples of 0.2 ml were assayed for total trichloroacetic acid-precipitable radioactivity or radioactivity precipitable by antisera. Levels of ^{14}C-valine incorporation were comparable to those of Tables 1 and 2.

[b] Antiserum to purified type 5 adenovirus.

[c] Antiserum to purified adenovirus hexon.

[d] Antiserum to an isolated adenovirus fiber and penton-base complex.

[e] NRS = normal rabbit serum.

units of the virion (6). These data indicate that specific viral proteins were made in vitro in reaction mixtures containing either whole cytoplasmic extracts or crude polyribosomes from infected cells.

Since the nascent polypeptides from polyribosomes in infected cells are immunologically reactive (20), similar experiments were done to determine whether the polypeptides made in vitro would also react with antibodies to viral proteins. The nascent chains from polyribosomes of 170*S* reacted with antiserum to whole virus, and about 30% of the label could be precipitated with antiserum (Fig. 3). More specifically, the nascent chains from polyribosomes reacted immunologically with antibodies to purified capsid-proteins, i.e., the viral hexon and penton-fiber (Fig. 4). It is striking that these nascent proteins appeared to be made on relatively small polyribosomes.

Since proteins made in vitro showed immunological reactivity, experiments were devised to inquire whether the polypeptides could be assembled in vitro into specific multimeric viral capsid structures. Reaction mixtures, after incubation for 25 min, were centrifuged in sucrose density gradients to display structures of sedimentation coefficients less than 15*S*. About 80% of the in vitro product was routinely recovered in

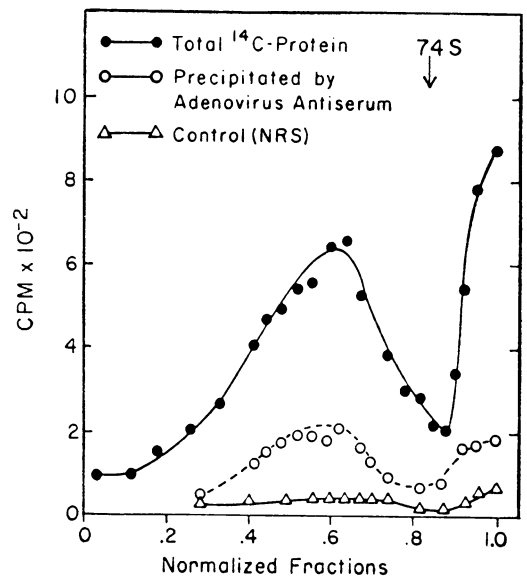

FIG. 3. *Immunological reactivity of polypeptides synthesized on polyribosomes in vitro. A 2-ml mixture containing the ^{14}C-amino acid mixture was incubated for 3 min and analyzed on a sucrose density gradient as in Fig. 2. Samples (0.25 ml) of each fraction were assayed for total trichloroacetic acid-precipitable radioactivity (●); radioactivity precipitable by antiserum to purified adenovirus particles (○) or by serum from non-immunized animals (NRS, normal rabbit serum) (△).*

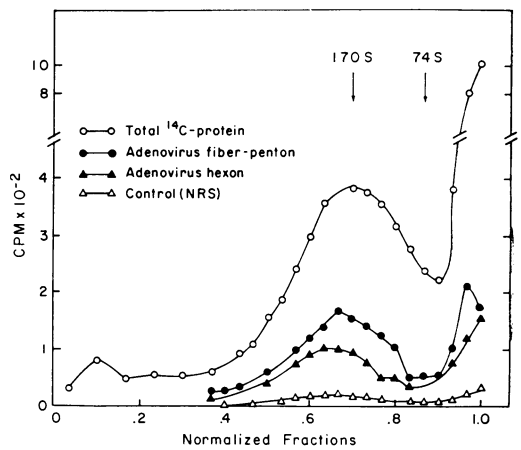

FIG. 4. *Immunological reactivity of specific viral polypeptides on polyribosomes after in vitro synthesis. A reaction mixture similar to that of Fig. 3 was analyzed on a sucrose density gradient after incubation for 3 min. Samples (0.2 ml) of each fraction were assayed for total trichloroacetic acid-precipitable radioactivity (○); and radioactivity precipitable by antiserum to viral penton-fiber (●), by antiserum to viral hexon (▲), and by NRS (△).*

these gradients; the remainder was in the ribosomal pellet at the bottom of the tube. This finding correlated closely with the observation that 80% of the in vitro product was released from ribosomes after cessation of amino acid incorporation (Fig. 2). About one-half of the in vitro product of an infected cytoplasmic extract sedimented as a symmetrical peak with an average sedimentation coefficient of about 3S (Fig. 5). This finding is similar to in vivo observations, in which after a short pulse the radioactive amino acids are first detected in proteins with a sedimentation coefficient of about 3S (20).

It is even more striking that portions of the in vitro product consistently sedimented at the positions of the multimeric capsid proteins (Fig. 5). Proteins were present having the sedimentation characteristics of the adenovirus hexon at 12S, the penton base at 9S, and the fiber (a small shoulder) at 6S (20). The sedimentation coefficients of these peaks of in vitro protein were estimated from the positions of adenovirus hexon and fiber markers ($S_{20,w}$ = 12.1 and 6.1, respectively; reference 10) in parallel gradients.

These proteins were most probably viral because they were precipitated with antiserum to purified adenovirus (Fig. 6). The 3S region of the gradient had the lowest immunological reactivity; this region presumably contains single polypeptide chains which are precursors to the multimeric assembled structures. The in vitro proteins from the 12, 9, and 6S regions of the sucrose gradient also had the immunological properties of virus-specific structures when tested with specific antisera (Table 5). Protein from the 12S region reacted specifically with antiserum to hexon;

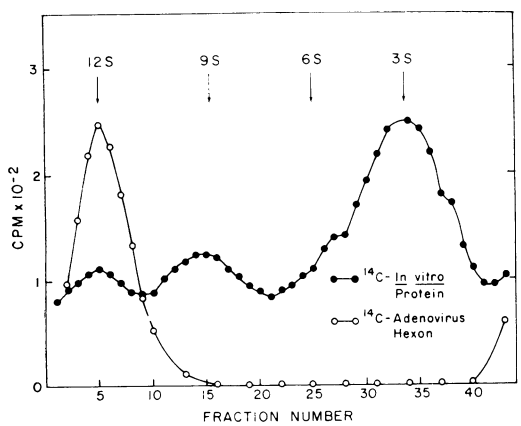

FIG. 5. *Sedimentation characteristics of adenovirus proteins synthesized in vitro. The in vitro proteins of a standard reaction mixture were labeled from a ^{14}C-amino acid mixture and centrifuged in a linear 5 to 20% sucrose gradient for 17.5 hr in a Spinco SW 65 rotor at 32,000 rev/min. Fractions (5-drop) were collected and analyzed for trichloroacetic acid-precipitable radioactivity. Proteins synthesized in vitro with a cytoplasmic extract of infected cells (●). ^{14}C-Labeled, purified adenovirus hexon ($S_{20,w}$ = 12.1, reference 10) centrifuged in parallel (○). Purified adenovirus fiber ($S_{20,w}$ = 6.1, reference 10) centrifuged under the same conditions displayed a peak at the position marked 6S (data not shown).*

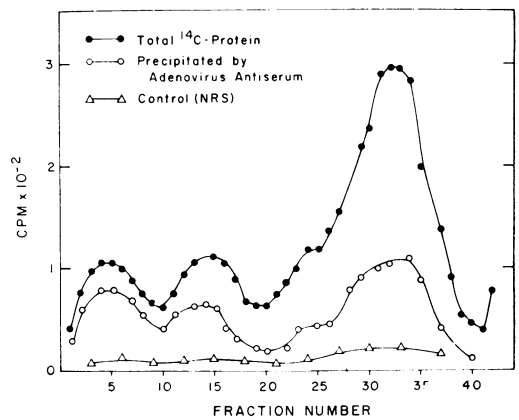

FIG. 6. *Immunological reactivity of the in vitro product after separation by sedimentation. A standard incubation mixture containing a cytoplasmic extract of infected cells was analyzed as in Fig. 5. A sample of each fraction was assayed to determine total trichloroacetic acid-precipitable radioactivity (●), and the remainder was assayed for radioactivity precipitable by antiserum to adenovirus (○) or by NRS (△).*

TABLE 5. *Immunological precipitation of in vitro protein from various regions of a sucrose density gradient*[a]

Protein from	Antiserum to	Per cent of labeled protein precipitated
12S Region	Hexon[b]	76
	Fiber-penton[b]	18
	NRS[b]	16
9S Region	Hexon	15
	Fiber-penton	35
	NRS	11
6S Region	Hexon	17
	Fiber-penton	64
	NRS	12

[a] A standard incubation mixture containing a cytoplasmic extract of infected cells was analyzed as in Fig. 5. A sample of each fraction was assayed for total trichloroacetic acid-precipitable radioactivity, and the remainders were pooled appropriately. These materials were assayed with various antisera by technique ii (Materials and Methods).

[b] See Table 4 for explanation.

antiserum to fiber-penton precipitated the majority of the protein from the 6S region. Material from the 9S region was most reactive with the fiber-penton antiserum, but only 35% of the labeled protein was precipitated. However, if the 9S in vitro peak is a penton base, the low reactivity might be expected since the antiserum employed was prepared by immunization with a mixture containing predominantly fiber and less than 5% penton (L. F. Velicer, Ph.D. thesis, Univ. of Pennsylvania, Philadelphia, 1969).

When the in vitro product of an uninfected cytoplasmic extract was analyzed (Fig. 7), the sedimentation pattern was distinctly different from that seen in Fig. 5 and 6, i.e., there was neither a large 3S peak nor proteins in the positions of identifiable viral structures. Furthermore, when the product of a reaction mixture containing a cytoplasmic extract from uninfected cells was mixed with an unlabeled cytoplasmic extract from infected cells, incubated in the presence of cycloheximide to prevent further protein synthesis, and then sedimented, the distribution of labeled proteins was identical to that shown in Fig. 7 for the protein products of the uninfected cell extract. Thus, proteins made in vitro did not aggregate nonspecifically with preformed viral structures.

The conversion of the in vitro synthesized polypeptides to adenovirus hexons, penton bases, and fibers was also observed when biosynthesis was performed with the ribosomal fraction from infected cells. Moreover, the activity responsible for synthesis and assembly of viral proteins could not be sedimented at 20,000 × *g*; it therefore appeared to consist of free polyribosomes and not to be present in large membrane-bound structures. These findings should be contrasted to the results obtained with the in vitro synthesis of poliovirus proteins in extracts of HeLa cells (17, 18) in which most of the activity was sedimented at 20,000 × *g* and appeared to be membrane bound. The suggestion that membrane-bound polyribosomes may not be significantly involved in the synthesis of adenovirus protein was strengthened by the following observation. In uninfected KB cells, 12 to 15% of total cytoplasmic ribosomes were membrane-bound, but 18 hr after infection when 75 to 80% of the proteins synthesized are virus-specific (1, 19) only 6 to 8% of the ribosomes were associated with membranes (P. Luciw and J. M. Wilhelm, *unpublished observations*).

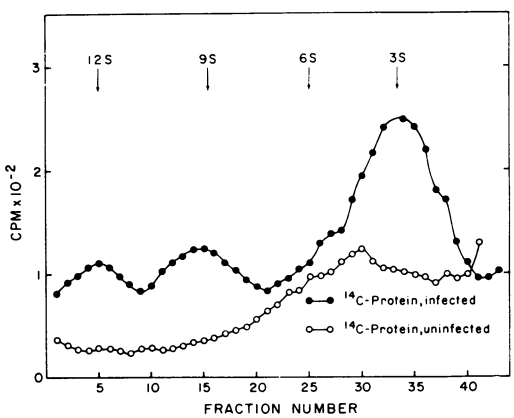

FIG. 7. *Comparison of the sedimentation properties of the in vitro product of adenovirus-infected cells with that of uninfected cells. Standard incubation mixtures containing cytoplasmic extracts were analyzed as in Fig. 5. Product of an infected extract (●). Product of an uninfected extract (○). Sedimentation coefficients were assigned from an accompanying analysis of ^{14}C-labeled viral hexon and fiber.*

DISCUSSION

The experiments in this communication demonstrate not only that the polypeptide chains of adenovirus-specific proteins are synthesized in vitro but also that the polypeptides are assembled into multimeric structures having the physical and immunological characteristics of the hexon, penton base, and fiber. Thus, the viral messenger ribonucleic acid (RNA) species appear to be faithfully translated. It is striking that although these distinctive structures are assembled one of the final morphogenetic events, the attachment of the fiber to the base to form the intact penton, apparently cannot be accomplished.

Caffier et al. (4) likewise showed in vitro synthesis of adenovirus proteins and presented direct evidence that the in vitro protein synthesis was initiated with methionine. The polypeptides made were identified as virus specific by electrophoresis in polyacrylamide gels.

It has been generally considered that a relationship exists between the size of a polypeptide and the size of the polyribosomes engaged in its synthesis. Thus, hemoglobin (16,000 daltons) is made on ribosomal pentamers (21), whereas collagen chains (95,000 daltons) are made on polyribosomes composed of about 30 ribosomes (8). In contrast, however, adenovirus proteins appear to be synthesized, both in vivo (19, 20) and in vitro (Fig. 3 and 4), on polyribosomes that are considerably smaller than would be predicted from the sizes of the peptide chains of the capsid proteins, which range from molecular weights of 61,000 daltons for the fiber (P. H. Dorsett and H. S. Ginsberg, Bacteriol. Proc., p. 223, 1971) and 70,000 daltons for the penton base (11), to the uncertain values of 60,000 (U. Pettersson, Ph.D. thesis, Univ. of Uppsala, Sweden, 1970), 93,000

(Dorsett and Ginsberg, *unpublished data*), or 120,000 (12) for the hexon polypeptides. Indeed, even polyribosomes of 180 to 200*S* seem too small for the synthesis of polypeptides with sedimentation coefficients of 2.5 to 3*S* (20; Fig. 2–4). It is unlikely that the small size of the polyribosomes is an artifact of preparation and analysis, since the polyribosomes from uninfected cells are consistently found to be larger, having their peak of biosynthetic activity at approximately 300*S*. If such small polyribosomes were produced by nuclease action on messengers when infected cells are broken, one would not expect the observed extensive production and release of labeled protein from ribosomes since the initiation and termination points of the messenger would have been separated. The anomolous size distribution of the polyribosomes may reflect an unusual spacing of ribosomes on the adenovirus messenger RNA species as contrasted to other mammalian systems. Alternatively, the viral proteins may be made as small polypeptides which are subsequently joined, through peptide bonds, to yield the single chains of mature virions.

Previous studies suggested that after release from cytoplasmic polyribosomes, the nascent adenovirus polypeptides were rapidly transported into the nucleus where morphogenesis of the multimeric capsid proteins occurred (20). The data presented, however, clearly indicate that assembly of the capsid structures can occur in vitro in cytoplasmic extracts (Fig. 5 and 6) and therefore raise questions about the role of the nucleus in viral morphogenesis. Since the nuclei of infected cells leak viral proteins (19), however, cytoplasmic extracts could contain nuclear factors that participate in the assembly reaction. But if this putative activity exists, it sediments with ribosomes and does not appear to be bound to membrane structures. In this context, it should be noted that in vitro there was only a limited assembly of viral fiber and the attachment of fibers to penton bases to form intact pentons apparently did not occur.

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