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The C Proteins of HeLa 40S Nuclear Ribonucleoprotein Particles Exist as Anisotropic Tetramers of $(C1)_3$ C2

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The C proteins (C1 and C2) of HeLa 40S heterogeneous nuclear ribonucleoprotein particles copurify under native conditions as a stable complex with a fixed molar protein ratio (S. F. Barnett, W. M. LeStourgeon, and D. L. Friedman, J. Biochem. Biophys. Methods 16:87–97, 1988). Gel filtration chromatography and velocity sedimentation analyses of these complexes revealed a large Stokes radius (6.2 nm) and a sedimentation coefficient of 5.8S. On the basis of these values and a partial specific volume of 0.70 cm^3/g based on the amino acid composition, the molecular weight of the complex was calculated to be 135,500. This corresponds well to 129,056, the sequence-determined molecular weight of a $(C1)_3C2$ tetramer. Reversible chemical cross-linking with dithiobis(succinimidyl propionate) and analysis of cross-linked and cleaved complexes in sodium dodecyl sulfate-polyacrylamide gel electrophoresis confirmed that the C proteins exist as tetramers, most or all of which are composed of $(C1)_3C2$. The tetramer is stable in a wide range of NaCl concentrations (0.09 to 2.0 M) and is not dissociated by 0.5% sodium deoxycholate. This stability is not the result of disulfide bonds or interactions with divalent cations. The hydrodynamic properties of highly purified C-protein tetramers are the same for C-protein complexes released from intact particles with RNase or high salt. These findings support previous studies indicating that the core particle protein stoichiometry of 40S heterogeneous nuclear ribonucleoproteins is N(3A1-3A2-1B1-1B2-3C1-1C2), where N = 3 to 4, and demonstrate that the C-protein tetramer is a fundamental structural element in these RNA-packaging complexes. The presence of at least three tetramers per 40S monoparticle, together with the highly anisotropic nature of the tetramer, suggests that one-third of the 700-nucleotide pre-mRNA moiety packaged in monoparticles is associated through a sequence-independent mechanism with the C proteins.

In mammalian cells, pre-mRNA is packaged during transcription by multiple copies of a few abundant nuclear proteins to form a repeating array of regular 40S heterogeneous nuclear ribonucleoprotein particles (hnRNP) (11, 13, 15, 22; W. M. LeStourgeon, S. F. Barnett, and S. J. Northington, *in* S. H. Wilson, ed., *The eukaryotic nucleus*, in press). When isolated from sucrose density gradients, 40S monoparticles possess an ultrastructural morphology indistinguishable from that seen in gently spread transcriptive units (38) and are composed primarily of multiple copies of six major core particle proteins (5, 7, 14, 24, 25, 27, 40) and a 700-nucleotide fragment of RNA (13). The proteins migrate in single-dimension sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as three groups of doublet bands (A1-A2, B1-B2, and C1-C2 doublets) with apparent molecular masses of 32 to 44 kilodaltons (5). Studies with protein-cross-linking reagents have indicated that the basic molar ratio of the core proteins in particles from rapidly dividing HeLa cells is 3A1-3A2-1B1-1B2-3C1-1C2 (27). When antibodies to the core particle proteins are used to isolate hnRNP complexes, several additional proteins are observed, and among these, polypeptides of 68 and 120 kilodaltons have been identified as in vivo components of hnRNP complexes (32).

Interest in the composition and structure of hnRNP complexes and in the role of individual core particle proteins is derived from the large body of evidence indicating that RNA splicing occurs while transcripts exist in a packaged state (3, 4, 15, 22, 30). More specifically, monoclonal antibodies to the C proteins inhibit in vitro splicing of an adenovirus transcript and immunoprecipitate the 60S splicing complex (i.e., the spliceosome) (9). Similarly, polyclonal antibodies to the core particle proteins completely inhibit splicing of human β-globin transcripts in vitro (35). Moreover, immunoadsorption of the C proteins from splicing extracts prevents spliceosome formation and results in loss of splicing activity (9). These observations have suggested an essential role for C proteins in the splicing of pre-mRNA transcripts.

Further interest in C proteins is derived from the recent but different findings of two research groups. Goswami and Goldenberg (20) report that the C proteins present in HeLa nuclear extracts bind mouse RNA in a length- but not sequence-dependent fashion and that binding is dependent on the presence of introns, Mg^{2+}, and ATP. Swanson and Dreyfuss (36), who used similar nuclear extracts, reported that the C proteins specifically bind to the polypyrimidine sequence at the 3′ end of introns in transcripts of the human β-globin and adenovirus genes. It was also reported that C protein failed to bind a bacterial and intronless spliced product and that ATP is not required for sequence-specific binding. Previous studies on the in vitro assembly of 40S monoparticles and polyparticle complexes have shown that correct particle assembly is dependent on RNA length but not on the presence of eucaryotic RNA-processing signals (13, 33, 39). RNA or single-stranded DNA substrates of nearly 700 nucleotides support the assembly of 40S monoparticles, and multiples of this length support the assembly of dimers and polyparticle complexes (13).

We have previously reported a rapid three-step procedure for the purification of native C protein from isolated HeLa 40S hnRNP particles (2). Proteins C1 and C2 copurified in a fixed molar ratio and eluted from analytical gel filtration columns with an apparent mass greater than that of apoferritin (M_r, 443,000). In this report, we show that C-protein

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complexes exist in monoparticles and in solution as stable, highly anisotropic heterotetramers composed of C1 and C2 in a 3:1 ratio. These findings partially confirm previous studies indicating that core particle protein stoichiometry is N(3A1-3A2-1B1-1B2-3C1-1C2), where N = 3 or 4 (27). The presence of at least three C-protein tetramers per monoparticle, the absence of reiterated sequences in the 700-nucleotide length of packaged pre-mRNA, and the potential for C-protein tetramers to bind relatively long lengths of RNA suggest a fundamental sequence-independent RNA-binding mechanism for most if not all of 40S monoparticle-associated C-protein.

MATERIALS AND METHODS

Cell culture, hnRNP particle isolation and C-protein purification. The methods used for cell culture, hnRNP particle isolation, and C-protein purification have been described in detail previously (2; S. F. Barnett, S. J. Northington, and W. M. LeStourgeon, Methods Enzymol., in press). Briefly, nuclei were isolated from exponentially growing HeLa cells. Monomer 40S hnRNP particles were isolated from nuclear sonicates by sedimentation on 15 to 30% sucrose gradients. To isolate C-protein–RNA complexes, monoparticles were adjusted to 350 mM NaCl and chromatographed on Bio-Gel A0.5M in 350 mM NaCl. The C-protein–RNA complexes in the void volume were adjusted to 10% glycerol and 1 mM dithiothreitol, and the RNA was removed by extensive digestion with either micrococcal nuclease (Boehringer Mannheim Biochemicals) or RNase A (Worthington Diagnostics). Final C-protein purification was by anion-exchange chromatography on a Mono Q column (Pharmacia Fine Chemicals) with an NaCl gradient. Proteins C1 and C2 coeluted in 480 mM NaCl. C-protein purity was greater than 95%, as judged by SDS-PAGE (2).

Gel filtration chromatography. Gel filtration chromatography was performed on Bio-Gel A1.5M (1.0 cm [inside diameter] by 75 cm), Bio-Gel P-300 (1.0 cm [inside diameter] by 75 cm), and Superose 6 (1.0 cm [inside diameter] by 30 cm) columns. Buffers contained 20 mM Tris hydrochloride (pH 8.0), 1.0 mM $MgCl_2$, and other components, as indicated. The Bio-Gel A1.5M, Bio-Gel P-300, and Superose columns were eluted at 5, 2, and 12 ml/h, respectively. Void volumes and total column volumes were determined with Dextran Blue 2000 and Phenol Red. The protein standards (and their Stokes radii) used to calibrate the gel filtration columns were bovine thyroglobulin (8.1 nm), bovine immunoglobulin G (5.22 nm), and bovine serum albumin (BSA) (3.5 nm). Protein standards were chromatographed before and after the C-protein complexes to ensure accurate comparisons of elution volumes. In some experiments, hnRNP particles were digested (0°C for 1.5 h) with 10 μg of RNase A per ml or 50 U of micrococcal nuclease per ml before chromatography.

Sucrose density gradient centrifugation. The conditions of centrifugation and determination of sedimentation coefficients were as described by Martin and Ames (29). Sucrose gradients (5 to 20%) were prepared in 20 mM Tris hydrochloride (pH 8.0)–1 mM dithiothreitol–1 mM $MgCl_2$. Other components were varied as described in Results. $MgCl_2$ was omitted when deoxycholate (DOC) was present. The gradients were centrifuged at 188,753 × *g* for 28 h at 4°C. Samples (0.5 ml) were loaded onto 11.5-ml gradients. Bovine immunoglobulin G (7.0S) and BSA (4.3S) were used as standards. Fractions were collected, trichloroacetic acid or ethanol precipitated, and subjected to SDS-PAGE.

Cross-linking with DSP. Purified C protein (1 to 2 ml) was dialyzed against 1.0 liter of cross-linking buffer (10 mM sodium phosphate buffer [pH 7.3], 100 mM NaCl) for 14 h at 4°C. Dithiobis(succinimidyl propionate) (DSP) was freshly prepared by adding 8.0 mg to 1.0 ml of *N'*,*N'*-dimethylformamide at 0°C. This stock solution was further diluted in cross-linking buffer and added to an equal volume of C protein (20 μg/ml) at 0°C. After incubation (0°C, 10 to 30 min), the solution was adjusted to 40 mM lysine by addition of a 1.0 M stock solution (pH 7.5). After 10 min, the solution was adjusted to 20 mM *N*-ethylmaleimide by addition of a fresh 0.5 M stock solution in 80% ethanol. The protein was ethanol precipitated, and the pellet was washed twice with 70% ethanol before addition of electrophoresis sample buffer. Before electrophoresis, the sample was incubated at 37°C for 30 min. DSP cross-links were cleaved by being heated to 100°C for 2 min in sample buffer containing 0.1% β-mercaptoethanol (BME).

Protein determination and SDS-PAGE. Protein was determined by a microassay technique (31) adapted from the method of Lowry et al. (28), with BSA as the standard. Peak fractions of specific proteins in gel filtration or density gradient sedimentation experiments were determined by the method of Fenner et al. (18). This method is based on the spectrophotometric quantitation of Coomassie blue eluted from excised protein bands after separation by SDS-PAGE. SDS-PAGE was by the method of Laemmli (23).

RESULTS

We have recently described a simple protocol for the purification of native C protein from isolated HeLa cell 40S hnRNP particles (2). In those studies, it was observed that C protein elutes from Superose 6 gel filtration columns as a complex composed of C1 and C2 with an apparent mass greater than that of apoferritin (M_r, 443,000). This phenomenon has now been observed with Superose 6 at salt concentrations as high as 2.0 M and with Bio-Gel P columns at salt concentrations as low as 0.1 M. In these studies, it was observed that if C protein devoid of its RNA substrate was applied to agarose-based matrices (Superose 6 or Bio-Gel A1.5M) at salt concentrations below 0.5 M it could not be eluted at salt concentrations as high as 2 M. This problem was not encountered with acrylamide-based gel filtration media such as Bio-Gel P. Analysis of the gel filtration data by the method and equations of Ackers (1) yielded a Stokes radius of 6.2 nm for the C-protein complex. The large Stokes radius suggests either a spherical structure containing more than 15 C-protein monomers or an anisotropic structure containing fewer polypeptides. We distinguished between these two possibilities and defined the protein stoichiometry of the C-protein complex as described below.

The sedimentation coefficient ($s_{20,w}$) of the purified C-protein complex was determined by cosedimentation with BSA (4.3S) and immunoglobulin G (7.0S) in 5 to 20% sucrose density gradients by the procedures of Martin and Ames (29) (Fig. 1). Identical results were obtained at salt concentrations of 0.09, 0.5, 1.0, and 2.0 M. These experiments were conducted in buffers identical to those used for the gel filtration studies. To exclude the possibility that sucrose has an effect on the composition of the C-protein complex, the gel filtration experiments were repeated with buffers containing 10% sucrose. No change in the elution profile was observed (data not shown). On the basis of an analysis of the data in Fig. 1, we define the sedimentation coefficient of the C-protein complex as 5.8S.

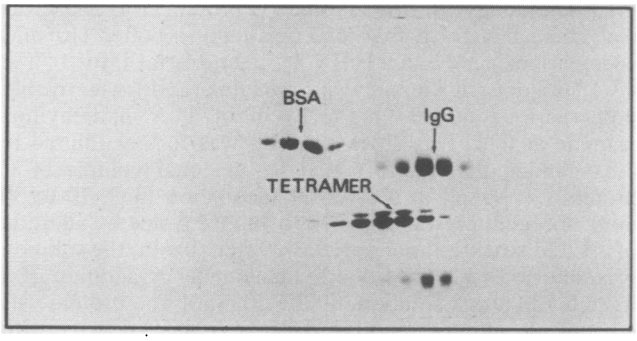

FIG. 1. Sedimentation of purified C protein. Purified C protein was combined with BSA (67 kDa) and gamma globulin (150 kDa) and cosedimented in a 5 to 20% sucrose density gradient as described in Materials and Methods. The sample and gradient contained 0.5% M NaCl. (A) Coomassie blue-stained SDS-PAGE gel showing the distribution of the proteins in the gradient. The peak fractions for each protein were determined by measuring the A_{600} of dye eluted from excised bands. (B) The relative absorbances of the proteins present in each fraction are plotted. The sedimentation coefficient of the C-protein complex was determined to be 5.8, as described in Materials and Methods.

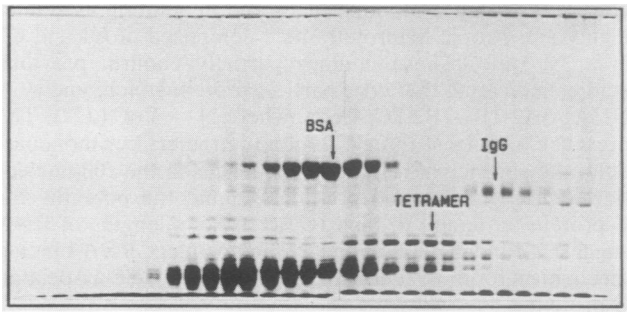

FIG. 2. Sedimentation of the C-protein complex released from monoparticles by digestion with RNase. Digested monoparticles and protein standards (BSA and immunoglobulin G) were combined and sedimented in a 5 to 20% sucrose gradient (90 mM NaCl). The Coomassie blue-stained SDS-PAGE gel reveals the distribution of protein in the gradient. Peak fractions and the sedimentation coefficient of the C proteins were determined as described in the legend to Fig. 1.

The partial specific volume of the C proteins was calculated by the method of Cohn and Edsall (12) on the basis of the sequence-deduced amino acid composition (37). The same value was assumed for both C1 and C2, since they appeared to be extremely similar (8). On the basis of the values obtained for the Stokes radius (6.2 nm), the sedimentation coefficient (5.8S), and the partial specific volume (0.70 cm^3/g), the molecular weight and frictional ratio were calculated to be 135,500 and 2.0, respectively (Table 1). Given the monomer molecular weight of 31,931 (37), these values are most consistent with a tetrameric C-protein complex.

If isolated 40S hnRNP particles are digested with RNase or adjusted to 1.0 M NaCl, the particle proteins dissociate into low-molecular-weight species (5, 13, 25, 27, 34). The C-protein complexes released from particles by these methods have the same hydrodynamic properties as purified C protein. An SDS-PAGE gel showing the sedimentation profile of nuclease-dissociated 40S particle proteins is shown in Fig. 2. A protein with the same electrophoretic mobility as C2 dissociated as did the other particle proteins and sedimented in the upper gradient regions as a monomer (Fig. 2). Unlike proteins C1 and C2, this protein does not bind RNA in a salt-resistant manner and does not copurify with the C-protein complex (2). We (5) and other investigators (32, 40), using two-dimensional gels, have noted the presence of polypeptides which comigrate with C2 in one-dimensional gels.

TABLE 1. Physical properties of the C-protein tetramer

Property[a]	Value
Stokes radius (nm)[b]	6.2
Sedimentation coefficient ($s_{20,w}$)[c]	5.8
Partial specific volume (cm^3/g)[d]	0.70
Calculated M_r[e]	135,500
M_r based on amino acid sequence	127,725
Frictional ratio (f/f_0)[f]	2.0

[a] Stokes radius, sedimentation coefficient, and partial specific volume were determined as described in Materials and Methods.
[b] Based on gel filtration on Superose 6.
[c] Based on sedimentation on 5 to 20% sucrose gradients.
[d] Estimated on the basis of amino acid composition.
[e] Calculated by the equation $M = 6\pi\eta Nas/(1-vp)$.
[f] Calculated by the equation $f/f_0 = a/(3vM/4\pi N)^{1/3}$.

Direct evidence that C protein is a tetramer was obtained through the strategy of Davies and Stark (14) for identifying the subunit structure of oligomeric proteins. The bifunctional, cleavable chemical protein cross-linking reagent DSP (26) was used in these studies. This reagent, in low concentrations, reacts with primary and secondary aliphatic amino groups at neutral pH. Maximum cross-linking was observed at a DSP concentration of 0.2 mM and at a protein concentration of 20 μg/ml. A 5 to 15% gradient gel showing all of the cross-linked products is presented in Fig. 3A. The relative mobilities of the cross-linked products and their molecular weights are shown in Fig. 4. C proteins are known to migrate with an anomalously high molecular weight in SDS-PAGE (37), and in these gels the apparent molecular weight of C1 was 39,000. Therefore, the molecular weights of the cross-linked products are consistent with the presence of dimers, trimers, and tetramers. As is often observed, the electrophoretic mobilities of oligomeric complexes are usually higher than expected on the basis of the molecular weights of the monomeric components because of space filling upon monomer association (10). The maintenance of retarded mobility seen here for dimers, trimers, and tetramers further supports the anisotropic nature of the C-protein tetramer (10). Tetramers were the largest cross-linked products observed (Fig. 4). These findings confirm hydrodynamic studies that indicate that C protein exists in solution as a tetramer.

The presence of multiple cross-linked products and their excellent resolution in gels (Fig. 3A) allowed us to determine that most, if not all, of the tetramers are composed of $(C1)_3C2$. The cross-linked products shown in Fig. 3A were electroeluted from excised gel slices, treated with BME to cleave the cross-links, and resolved by SDS-PAGE (Fig. 3B). Dimers composed of $(C1)_2$ and C1C2 but not $(C2)_2$ were detected. Trimers composed of $(C1)_3$ and $(C1)_2C2$ but not $(C2)_3$ were detected. $(C1)_3C2$ tetramers were the largest cross-linked product obtained, and in other experiments in which the DSP concentration was raised, all of the C protein was converted to the tetramer (data not shown). If tetramers composed entirely of C1 or more than one copy of C2 exist, they were present at levels too low to detect. A summary of the data obtained from the hydrodynamic and cross-linking studies is shown in Table 1. The distribution profile of C protein in the gel filtration and sedimentation experiments

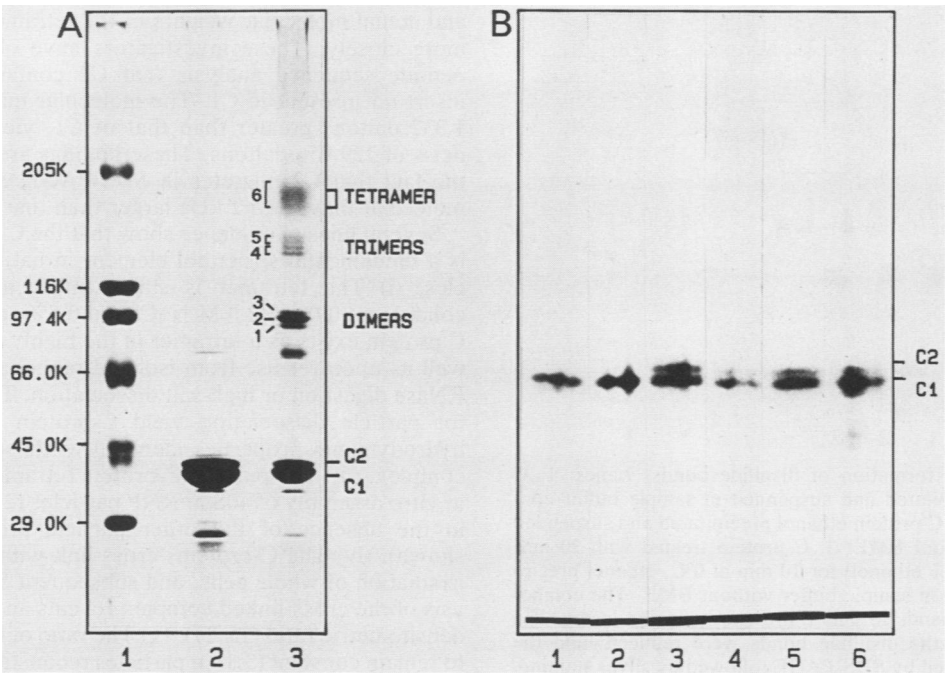

FIG. 3. DSP cross-linking of purified C protein. (A) SDS-PAGE (5 to 15% acrylamide gradient) of molecular weight standards (lane 1), non-cross-linked C protein (lane 2), and DSP-cross-linked C protein (lane 3). The dimer, trimer, and tetramer bands were identified by their molecular weights (see Fig. 4). (B) Composition of specific cross-linked products. Bands were excised, the cross-links were cleaved by reduction, and the products were separated in an 8.75% gel and silver stained. The bands marked 1 to 6 in lane 3 of panel A correspond to the numbered lanes in panel B.

did not reveal the presence of oligomers smaller than tetramers. This argues that the tetramer is not in equilibrium with smaller complexes and is consistent with the stability of the tetramer in extremes of ionic strength.

Protein C1 has a single cysteine at residue 46 (37). We tested the possibility that disulfide bonds could contribute to the stability of the tetramer (Fig. 5). When C protein was precipitated with ethanol and resolved in SDS-PAGE under nonreducing conditions, dimers were detected (Fig. 5, lane 2). Excision of the dimer bands, reduction, and SDS-PAGE under reducing conditions, indicated that the major band was a $(C1)_2$ dimer and the minor band was a C1C2 dimer (Fig. 5, insert). No $(C2)_2$ dimers were detected. If the C protein was treated with a sulfhydryl-modifying reagent (*N*-ethylmaleimide) before ethanol precipitation, no dimers were detected (Fig. 5, lane 3). We therefore conclude that the dimers generated under nonreducing conditions are experimental artifacts and that no disulfide bridges exist between monomers in native C-protein tetramers. This conclusion is supported by an experiment in which C protein was dialyzed extensively against 0.1% BME. Subsequent sedimentation in gradients containing 0.1% BME and 1 M NaCl revealed no effect on the sedimentation rate (data not shown).

Because the C proteins have a carboxy-terminal domain (residues 175 to 290) containing 20 aspartate and 21 glutamate residues, it is possible that these regions could form coordination complexes with divalent cations to stabilize the tetramer. To test this, C protein was dialyzed extensively against buffered 10 mM EDTA and then sedimented in a 5 to 10% sucrose gradient containing 1 mM EDTA and 1M NaCl. The sedimentation coefficient was the same as that demon-

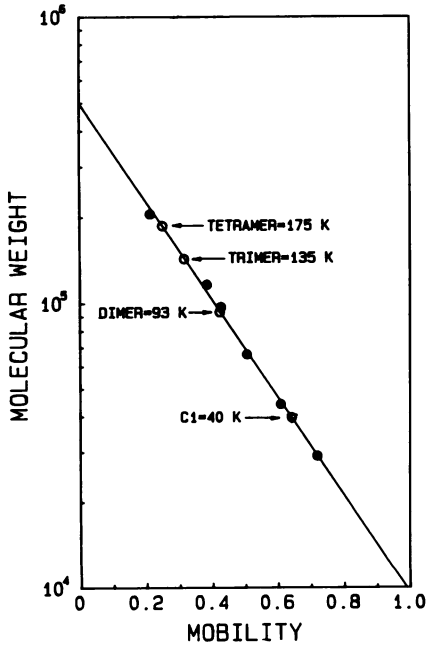

FIG. 4. Molecular weight determinations of the DSP-cross-linked C-protein complexes. The SDS-PAGE gel is shown in Fig. 3A. Protein mobility was measured relative to the bromophenol blue marker. The protein standards were myosin (molecular weight, 205,000), β-galactosidase (116,000), phosphorylase (94,000), BSA (67,000), ovalbumin (45,000), and carbonic anhydrase (29,000).

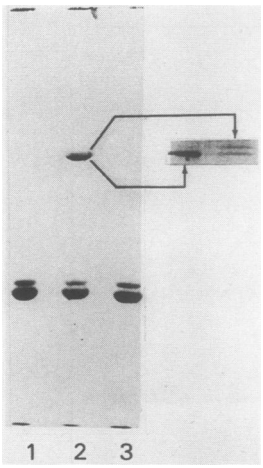

FIG. 5. Artifactual formation of disulfide bonds. Lanes: 1, C protein ethanol precipitated and suspended in sample buffer containing 0.1% BME; 2, C protein ethanol precipitated and suspended in sample buffer without BME; 3, C protein treated with 20 mM *N*-ethylmaleimide (80% ethanol) for 10 min at 0°C, ethanol precipitated, and suspended in sample buffer without BME. The compositions of the dimer bands in lane 2 are shown in the insert. The bands were excised, the disulfide bonds were reduced, and the products were separated by SDS-PAGE followed by silver staining.

strated for the tetramer in the absence of EDTA (data not shown).

Studies on the individual properties of C1 and C2 are complicated by the inability to separate these polypeptides by standard chromatographic techniques. In a previous study, isolated hnRNP particles were reversibly dissociated with 0.5% DOC (33). To determine whether this treatment disrupts hydrophobic interactions and dissociates the C-protein tetramer, purified C protein was dialyzed to remove Mg^{2+}, and solid sodium DOC was added to a final concentration of 0.5%. This material was analyzed in 5 to 20% sucrose gradients containing 0.2% DOC, but dissociation of the tetramer was not observed (data not shown).

DISCUSSION

We have demonstrated that most, if not all, of the C proteins of HeLa 40S hnRNP particles exist in solution and in intact particles as a stable tetramer of $(C1)_3C2$. This confirms the protein cross-linking studies of Lothstein et al. (27), which indicated that the basic molar ratio of these polypeptides in hnRNP is 3:1, and it provides a structural basis for this observation. The approximate mass of monoparticles (1.5×10^6 to 1.8×10^6) (11, 41) and the minimum core particle protein stoichiometry (3A1-3A2-1B1-1B2-3C1-1C2) indicate that the monoparticles must contain three or four C-protein tetramers. While several lines of evidence suggest that 40S monoparticles are regular structures which package 700 ± 20 nucleotides of pre-mRNA (13, 33, 39; LeStourgeon et al., in press), the location of the C-protein tetramers along the packaged length of RNA is unknown.

The values determined here for the Stokes radius (6.2 nm), sedimentation coefficient (5.8S), and partical specific volume (0.70 cm^3/g) of the C-protein tetramer yield a calculated molecular weight of 135,500. The close agreement of this value with the sequence-determined mass of a tetramer composed entirely of C1 (125,725) lends credibility to the above values. Based on recent findings (B. M. Merrill and K. R. Williams, personal communication), the calculated and actual molecular weights of the tetramer, in fact, agree more closely. These investigators have observed through peptide sequence analysis that C2 contains a 13-residue insert not present in C1. The molecular mass of C2 is thus 1,332 daltons greater than that of C1, yielding a tetramer mass of 129,056 daltons. These findings are consistent with the fact that C2 migrates in SDS-PAGE with an apparent molecular mass 1 to 2 kDa larger than that of C1 (5, 7).

Several lines of evidence show that the C-protein tetramer is a fundamental structural element in native hnRNP particles. (i) The tetramer is stable under a wide range of conditions (0.09 to 2.0 M NaCl and 0.5% sodium DOC). (ii) C protein exists as a tetramer in the highly purified state, as well as upon release from isolated monoparticles by either RNase digestion or high-salt dissociation. These procedures for particle dissociation yield C-protein complexes with hydrodynamic properties identical to those of the purified complex. (iii) The purified C-protein tetramer participates in in vitro assembly of 40S hnRNP particles (2) and binds RNA in the absence of the other particle proteins (data not shown). (iv) The C-proteins cross-link with RNA upon UV irradiation of whole cells, and subsequent SDS-PAGE analysis of the cross-linked complex reveals an approximate 3:1 densitometric ratio (15–17). (v) The ratio of C1 to C2 appears to remain constant (3:1) in particles reconstituted in RNAs of various lengths (14). This suggests that C-protein tetramers and not single polypeptides participate in particle assembly. (vi) Previous protein-cross-linking studies performed on intact isolated 40S monoparticles with dimethyl-3,3′-dithiobispropionimidate revealed fundamental trimeric associations of C1 and small amounts of the C-protein tetramer (27).

As originally defined (5), the C proteins of HeLa cell hnRNP were the two major polypeptides of 40S core particles which (i) bind RNA in a salt-resistant manner, (ii) have acidic isoelectric points, (iii) migrate in SDS-PAGE with approximate molecular masses of 42 and 44 kilodaltons, and (iv) are the major phosphorylated species among the six core particle proteins (21). More recent studies have also shown that among the six core proteins, the C proteins most readily cross-link with RNA upon UV irradiation (7, 13) and that both C1 and C2 are specifically bound by the monoclonal antibodies termed 2B12 and 4F4 (8, 17). This report adds the additional characteristic of their tetrameric association.

A final point deserving considration is the anisotropic nature of the C-protein tetramer. The tetramer eluted from gel filtration columns with an apparent molecular weight of nearly 0.5×10^6 and it had a frictional ratio of 2.0. This suggests that the tetramer is a prolate ellipsoid with an axial ratio of 20 or an oblate ellipsoid with an axial ratio of 30 (19). While this analysis does not allow one to predict the actual shape of the tetramer, it is clearly not an efficiently folded spherical structure. The underlying significance here bears on the spatial orientation of the RNA-binding domains. In the sequence analysis of Swanson et al. (37), a putative RNA-binding sequence common to several RNA-binding proteins was identified in the amino-terminal region of C1. Thus, the spatial arrangement of the four RNA-binding domains may determine the length of RNA contacted by a single tetramer. If the binding domains are maximally separated (as on the surface of a tetrahedral structure or spaced in a tandem association of polypeptides), a complex with a Stokes radius of 6.2 nm might contact an RNA fragment with as many as 115 nucleotides. In this context, it can be noted that the single-stranded binding protein of *Escherichia coli* (EcoSSB) binds 65 nucleotides at 0.2 M salt and is a highly

stable tetramer with one-third less mass and one-half the Stokes radius of the C-protein tetramer (6). Because the C proteins make up one-third of the core proteins of 40S monoparticles, representing at least three C-protein tetramers, it is likely that much of the packaged 700-nucleotide pre-mRNA moiety (13) is associated with C protein in a sequence-independent manner.

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