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Purification of Splicing Factor SF1, a Heat-Stable Protein That Functions in the Assembly of a Presplicing Complex

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Splicing factor SF1 represents one of the proteins that function early in the splicing of nuclear pre-mRNA in the formation of a presplicing complex. SF1 was purified to homogeneity from HeLa cell nuclear extracts by column chromatography. It consists of a single polypeptide of 75 kDa and is distinct from other protein factors that function early in spliceosome assembly. SF1 activity is completely resistant to temperatures of up to 100°C. The purified protein does not appear to be associated with RNA-binding, RNA-annealing, or ATPase activity.

Splicing of introns from nuclear mRNA precursors (pre-mRNAs) occurs in large ribonucleoprotein complexes, termed spliceosomes or splicing complexes (for reviews, see references 16 and 17). The major constituents of these structures are small nuclear ribonucleoprotein particles (snRNPs) which bind to the pre-mRNA in an ordered fashion. First, U1 snRNP interacts with the pre-mRNA in the absence of ATP, and a complex which is committed to the splicing pathway is generated (30, 33, 36). Presplicing complex A is then formed in an ATP-dependent reaction after binding of U2 snRNP to the branch site (10, 21). U4/U6 and U5 snRNPs join the presplicing complex as a preformed triple snRNP which leads to the assembly of splicing complex B (2, 7, 21, 41). This complex is converted to the active spliceosome (complex C) after a conformational change which results in destabilization of the base pairing interaction between U4 and U6 RNAs (3, 7, 21, 32). The biochemical transesterification reactions, i.e., 5′ splice site cleavage-lariat formation and 3′ splice site cleavage-exon ligation, that generate mature mRNA are initiated either concomitantly with the assembly of complex C or immediately after this structure is formed.

In addition to snRNPs, a number of non-snRNP splicing factors have been identified in mammalian cells (for a review, see reference 16). Most of these factors function in the formation of the presplicing complex, i.e., at a stage in the reaction at which the correct splice sites are defined. U2AF (34, 43), SF1, SF3 (25), and ASF/SF2 (14, 23) have been detected in fractions derived from mammalian splicing extracts. With antibodies prepared against isolated splicing complexes, two proteins, Sc35 (11) and an 88-kDa polypeptide (1), have been identified. Furthermore, in addition to general splicing factor ASF/SF2 (14, 22), heterogeneous nuclear RNP (hnRNP) protein A1 (29) and a factor termed DSF (18) function in the selection of alternative 5′ splice sites; however, it is not clear whether these proteins play a role in constitutive splicing.

Other proteins have been detected by the ability to bind the pre-mRNA substrate. These are IBP (15, 40), PTB (13), and hnRNP proteins A1, C, and D (39). Although roles for hnRNP protein C during the splicing reaction have been reported (8, 38), it remains to be shown whether these RNA-binding proteins represent general splicing factors.

Only limited information is available about the exact function of these proteins during presplicing complex formation. IBP, U2AF, and PTB bind to sequences at the 3′ end of an intron (13, 15, 40, 43). ASF/SF2 is associated with general RNA-binding and RNA-annealing activities (23). Both U2AF and Sc35 are required for U2 snRNP to bind to the branch site (12, 34), and Sc35, in addition, plays a role in the initial binding of U1 snRNP to the pre-mRNA (12). Furthermore, Sc35 and an 88-kDa protein are integral components of the spliceosome (1, 12); U2AF and PTB have been shown to be present in the presplicing complex (13, 44).

We have previously separated HeLa cell nuclear extracts into five fractions containing splicing proteins (SF1 to SF4 and U2AF) and two fractions that are enriched in the spliceosomal snRNPs (26, 27). All of these fractions are required to splice an RNA substrate derived from the adenovirus type 2 major late (AdML) transcription unit. SF1, SF3, and U2AF, in combination with snRNPs, are sufficient for the generation of presplicing complex A (27). Since both SF1 and SF3 activities are represented by rather crude chromatographic fractions, it has not been possible to compare these activities with other splicing factors that function early during the splicing reaction. By employing presplicing complex formation as an assay to monitor SF1 activity, this splicing factor has been purified to homogeneity. SF1 consists of a polypeptide of 75 kDa which exhibits unusual heat stability and appears to be distinct from other protein factors that have been identified.

MATERIALS AND METHODS

Purification of SF1. HeLa cell nuclear extract was fractionated by DEAE-Sepharose Fast Flow chromatography (Pharmacia-LKB) as previously described (27). Proteins in the flowthrough fraction (DS100) were precipitated after addition of solid ammonium sulfate to 50% saturation, and after collection by centrifugation, they were dissolved in 1/30 of the original volume of buffer A (20% [vol/vol] glycerol, 20 mM *N*-hydroxyethylpiperazine-*N*′-2′-ethanesulfonic acid [HEPES]-KOH [pH 7.9], 50 mM KCl, 3 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM dithiothreitol). After dialysis against an excess of the same buffer, the material was loaded onto a Blue-Sepharose column (Pharmacia-LKB; 2.5 by 26 cm; 1.2 mg of protein per ml; flow rate, 150 ml/h) equilibrated with buffer B (10% [vol/vol] glycerol, 20 mM HEPES-KOH [pH 7.9], 3 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 1 μg of leupeptin per ml) plus 0.05 M KCl. The column was washed with 250 ml of the same buffer, and bound proteins were eluted with a 750-ml

gradient of 0.05 to 1 M KCl and then step eluted with 2 M KCl in buffer B. Aliquots of individual fractions were dialyzed against buffer A and tested for activity. SF1 activity eluted in a broad peak from 0.4 to 1 M KCl. Fractions containing SF1 activity were pooled and concentrated by ammonium sulfate precipitation as described above. Proteins were dissolved in buffer A and dialyzed against the same buffer. The material was applied to an 8-ml Mono S fast protein liquid chromatography column (Pharmacia-LKB; 4.6 mg of protein per ml) in buffer B plus 0.05 M KCl at a flow rate of 60 ml/h. Unbound material was removed with 40 ml of the same buffer. Elution was performed with a 160-ml gradient of 0.05 to 0.5 M KCl followed by step elution with 1 M KCl in buffer B. Fractions of 4 ml were collected, and activity was tested without prior dialysis. An aliquot of a Mono S fraction enriched in SF1 activity (3.5 ml) was concentrated to 0.25 ml by Centricon-10 (Amersham) centrifugation for 2 h at 4,300 × *g*. The concentrate was heated to 100°C for 10 min, and insoluble material was removed by centrifugation in an Eppendorf microcentrifuge. The cleared supernatant was applied to a Superose 12 fast protein liquid chromatography gel filtration column (HR10/30; Pharmacia-LKB) equilibrated with buffer B plus 0.1 M KCl. The column was run at 30 ml/h, and 0.5-ml fractions were collected. The following protein standards were fractionated under identical conditions: thyroglobulin, 669 kDa, 8.5 nm; catalase, 232 kDa, 5.25 nm; aldolase, 158 kDa, 4.81 nm; bovine serum albumin, 67 kDa, 3.55 nm; ovalbumin, 43 kDa, 3.05 nm; chymotrypsinogen, 25 kDa, 2.09 nm.

This purification procedure resulted in a final yield of about 50 μg of purified SF1 from 100 ml of nuclear extract (prepared from 80 liters of HeLa cells at 500,000 cells per ml).

Alternatively, SF1 was fractionated on poly(U)-Sepharose (Pharmacia-LKB) following Mono S chromatography and heat denaturation (as described above). Proteins were applied to the column in buffer B plus 0.05 M KCl and eluted with a gradient of 0.05 to 1 M KCl in the same buffer. SF1 activity eluted between 0.1 and 0.3 M KCl.

Protein concentrations were determined as described by Bradford (5), with bovine serum albumin as the standard (Bio-Rad).

Preparation of RNA substrates. Pre-mRNA was prepared by in vitro transcription with SP6 RNA polymerase (25). The resulting RNA is derived from the AdML transcription unit and contains the 5′ and 3′ splice sites of the first intron (RNA1; reference 10).

Splicing assays. Formation of presplicing complexes was analyzed in a total volume of 10 μl for 30 min at 30°C as previously described (25), in the presence of 1 μl of SF3 (Mono Q), 1 μl of U2AF (HS1000), 0.5 μl each of U1 and U2 snRNPs (Mono Q), and the amounts of SF1 indicated in the figure legends. Analysis of splicing complexes was done in 4% polyacrylamide gels in 25 mM Tris–25 mM boric acid–1 mM EDTA (25).

Splicing reactions (25 μl) were performed for 3 h at 30°C as previously described (25, 26). They contained either 5 μl of nuclear extract or 1 μl of SF2 (ammonium sulfate-concentrated HS100), 3 μl of SF3 (Mono Q), 3 μl of SF4 (Mono Q), 2 μl of U2AF (HS1000), 2 μl of U1 snRNP, 1 μl of U2 snRNP (both Mono Q), and 0.5 μl each of the SF1-containing fractions (for a description of the fractions used, see references 26 and 27). Analysis of isolated reaction products was done in denaturing 12% polyacrylamide gels. (Note that the splicing factor SF2 used here is different from the SF2 defined by Krainer et al. [23, 24].)

Renaturation of SF1 after SDS-polyacrylamide gel electrophoresis. Fifty microliters of a poly(U)-Sepharose fraction containing partially purified SF1 (~5 μg) was precipitated by addition of 200 μl of acetone at −80°C. Proteins were pelleted by centrifugation, washed once with cold acetone, dissolved in sodium dodecyl sulfate (SDS) loading buffer (28), and incubated for 30 min at 37°C. Proteins were separated in an SDS–9% polyacrylamide minigel. The gel lane was cut into 15 slices (0.3 cm each), and proteins were eluted into 250 μl of 50 mM Tris-HCl (pH 7.9)–0.1 mM EDTA–5 mM dithiothreitol–0.15 M NaCl–0.1% SDS for 2 h at room temperature. Eluted proteins were precipitated with acetone as described above and suspended in 20 μl of 6 M guanidinium hydrochloride–0.1% (vol/vol) Nonidet P-40–0.1 M KCl. After incubation at room temperature for 1 h, the samples were spun through 400 μl of Sephadex G-25 equilibrated with buffer A plus 50 mM KCl and 0.05% (vol/vol) Nonidet P-40. After 1 h at room temperature to allow refolding of the polypeptide chains, the samples were tested for SF1 activity. This procedure was adapted from that of Wang et al. (42).

SDS-gel electrophoresis. Unless indicated otherwise, proteins were precipitated by addition of 2 volumes of 20% trichloroacetic acid and the precipitates were washed twice with cold acetone and dissolved in SDS loading buffer (28). Electrophoresis was performed in SDS–9% polyacrylamide gels (28). Proteins were visualized by staining with Coomassie brilliant blue, and this was followed by staining with silver where indicated.

RESULTS

Previous characterization of the properties of SF1 indicated that SF1 is resistant to 15 min of incubation at 70°C, whereas SF3 and U2AF are inhibited at temperatures of 50 and 45°C, respectively (27). This unexpected heat stability was investigated further by preincubation of the factor at different temperatures and subsequent analysis of presplicing complex formation in the presence of SF3, U2AF, and snRNPs. Upon titration of an SF1 fraction that was either kept on ice or incubated at 65 or 100°C, no loss of SF1 activity in this assay was apparent (Fig. 1), demonstrating that SF1 is completely resistant to heat. Although the experiment shown was performed with a partially purified fraction, similar results were obtained with the initial SF1 fraction (DS100; data not shown). In addition to its heat resistance, SF1 activity is also unaffected by treatment with *N*-ethylmaleimide (27). These properties could suggest that SF1 is not a protein. However, preincubation of an SF1-containing fraction with papain, followed by inactivation of the enzyme, resulted in complete inhibition of presplicing complex formation (data not shown), which supports the conclusion that SF1 activity resides in a protein.

About 10% of the total protein present in the DS100 fraction remained soluble after incubation at 100°C. SDS-gel electrophoresis of this material revealed a uniform distribution of polypeptides over the entire range of molecular masses (data not shown). Nevertheless, the heat stability of SF1 allowed identification of the corresponding polypeptide at a later purification stage (see below).

SF1 was fractionated from the DS100 fraction by Blue-Sepharose and Mono S chromatography. Figure 2A shows that the activity eluted from Mono S in fractions 18 to 32 at KCl concentrations of 0.1 to 0.3 M. Analysis of the protein contents of individual Mono S fractions (Fig. 2B) revealed the presence of a multitude of proteins and that no correla-

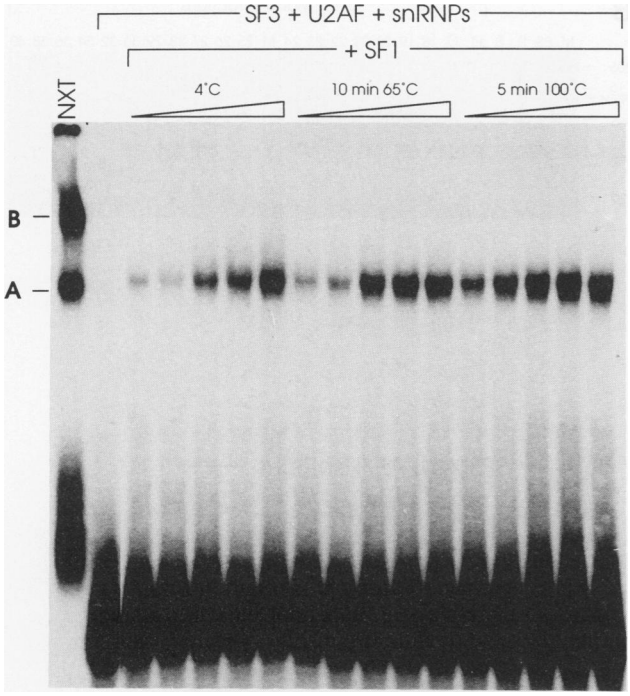

FIG. 1. Heat stability of SF1 activity. A fraction containing SF1 activity was either kept on ice or incubated for 10 min at 65°C or 5 min at 100°C and then centrifuged for 5 min in an Eppendorf microcentrifuge. Dilutions of the supernatants (0.1, 0.2, 0.5, 1.0, and 2.0 μl) were then tested in a standard assay in the presence of SF3, U2AF, and snRNPs for presplicing complex assembly and processed as described in Materials and Methods. The positions of presplicing complex A and splicing complex B are indicated on the left. The radioactivity at the bottom of the gel represents the RNA substrate that has been assembled into complex H, which is believed to represent unspecific interactions of general RNA-binding proteins with the pre-mRNA. NXT, nuclear extract.

tion between SF1 activity and any of the polypeptides is possible. However, when a Mono S fraction was analyzed for heat-stable polypeptides after incubation at 100°C and removal of denatured material, it became apparent that only a small number of polypeptides remained soluble and most of the proteins present in the fraction analyzed were found in the precipitate (Fig. 2C). A protein of 40 kDa was distributed in both fractions, whereas a major polypeptide of 75 kDa was quantitatively recovered in the supernatant together with at least two minor polypeptides of 47 and 28 kDa. Since SF1 activity is completely resistant to exposure to 100°C (Fig. 1), one would expect the corresponding polypeptide to be present predominantly in the supernatant fraction. Hence, the three polypeptides found exclusively in this fraction are good candidates for SF1.

To correlate the association of SF1 activity with one of these polypeptides, partially purified, heat-treated SF1 was subjected to SDS-gel electrophoresis, the gel lane was cut into individual slices, and proteins were recovered by elution. After renaturation, the eluted proteins were tested for SF1 activity. Presplicing complex A was assembled in the presence of proteins eluted from gel slice 6, which contained the 75-kDa polypeptide (Fig. 3). Although only a small proportion (~5%) of the protein used for the experiment was recovered in the eluted fraction, SF1 activity was still detected in the presplicing complex assay, consistent with the finding that the protein is active at rather low concentrations (Fig. 1). The 47- and 28-kDa proteins which were eluted from gel slices 10 and 15, respectively, are not active in presplicing complex formation.

Inspection of the SDS-gel of the Mono S fractions (Fig. 2B) reveals the presence of a prominent 75-kDa protein in fractions 19 and 20. In the remaining fractions that also display SF1 activity, this polypeptide cannot easily be observed. Titration of SF1 activity in individual fractions suggests that fraction 19 contains about 50 times more SF1 activity than fraction 21 (Fig. 2D), which correlates well with the amount of the 75-kDa protein present.

For further purification of SF1, Mono S fractions were concentrated, the concentrate was boiled for 5 min, denatured material was removed by centrifugation, and the cleared supernatant was applied to a Superose 12 gel filtration column. SF1 activity is present in fractions 21 to 34 (Fig. 4C). Quantitation of the amount of RNA substrate that became incorporated into complex A in this assay revealed a peak of activity in fractions 22 to 26, with a gradual decrease of presplicing complex formation in the following fractions (data not shown). Figure 4B shows that heat treatment of the input fraction by itself resulted in considerable purification and the 75-kDa protein was again quantitatively recovered in the supernatant fraction. The protein eluted from the Superose 12 column in fractions 22 to 30 (Fig. 4B), and lower concentrations of the polypeptide were seen up to fraction 36 in the original gel. The few minor polypeptides present in these fractions did not appear to cofractionate with SF1 activity. The faint band visible just below the 75-kDa polypeptide probably represents a proteolytic product of the 75-kDa protein and was not observed in several other preparations. Taken together, these results demonstrate that SF1 activity resides in a heat-stable polypeptide of 75 kDa.

Figure 4D shows a splicing assay in which SF1-containing fractions were tested in the presence of other splicing factors partially purified from HeLa cell nuclear extracts (26, 27). In the absence of SF1, a low background of splicing activity was apparent (lanes 3 and 7); this was probably caused by the presence of a small amount of SF1 activity in one of the complementing fractions. Upon addition of samples from the Superose 12 column, splicing activity was detected in fractions 22 to 31 (lanes 12 to 16). Thus, fractions that contain the 75-kDa polypeptide exhibit activity in both presplicing complex formation and the complete splicing reaction.

Comparison of the elution volume of SF1 with those of known proteins (Fig. 4A) allowed determination of a Stokes radius of approximately 4.5 nm. The sedimentation constant of SF1 was determined to be 2.75S after centrifugation in a glycerol gradient (data not shown). From these values and an assumed partial specific volume of 0.73 ml/g, a native molecular weight of 52,000 was calculated (37). Although this estimate is lower than the molecular weight of the denatured protein, this result suggests that SF1 is present in solution as a monomer.

DISCUSSION

Splicing factor SF1 has been purified to homogeneity from HeLa cell nuclear extracts. It is represented by a polypeptide of 75 kDa and exhibits unusual resistance to temperatures of up to 100°C. As demonstrated previously, SF1 functions in the assembly of presplicing complex A and is required for the cleavage-ligation reactions that generate spliced RNA (25, 27).

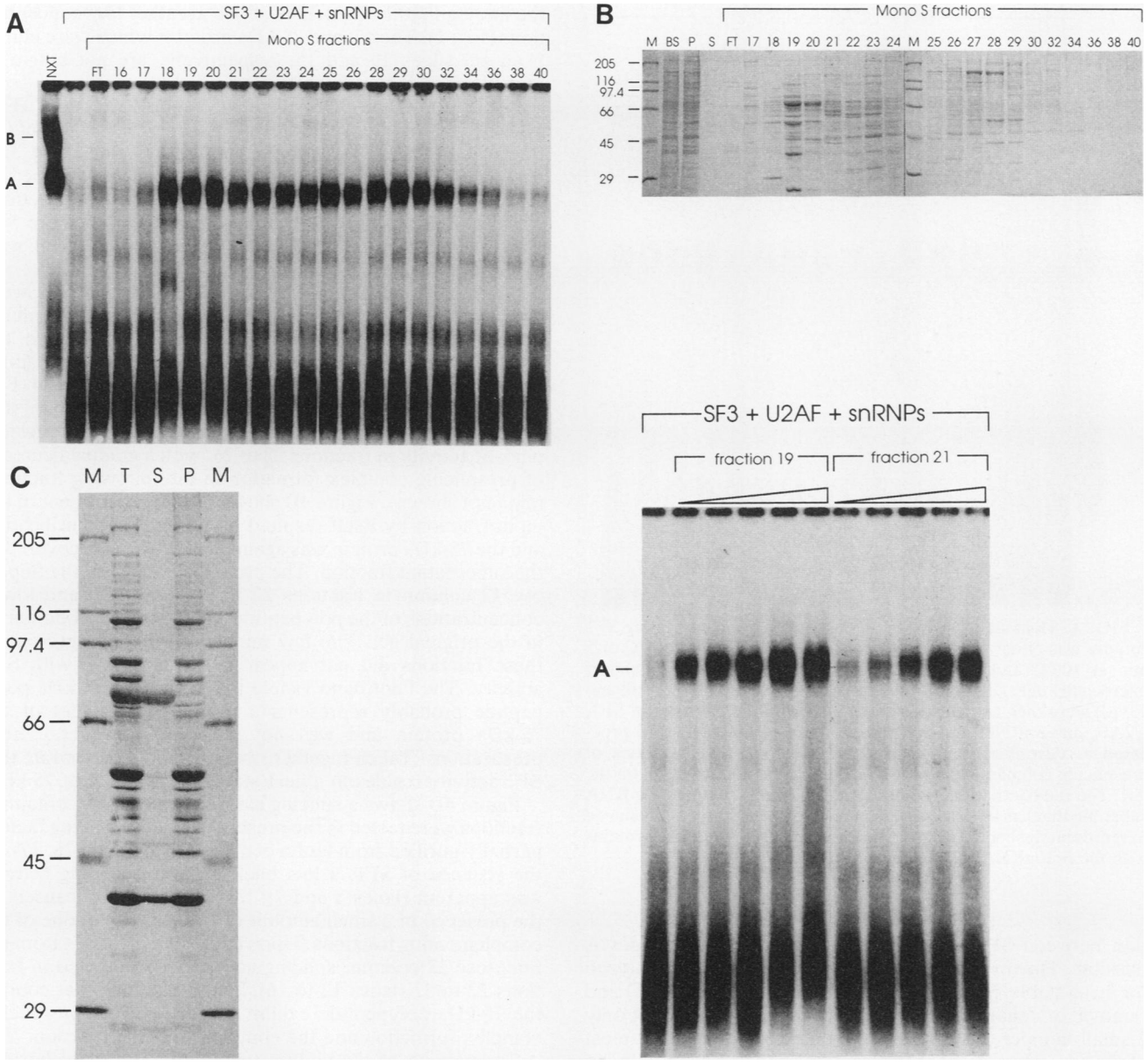

FIG. 2. Mono S chromatography of SF1 and analysis of heat-resistant polypeptides. (A) Individual Mono S fractions (0.5 μl; as indicated above each lane) were added to a standard reaction containing SF3, U2AF, and snRNPs and tested for presplicing complex formation. The reaction shown in the first lane was performed in the presence of 3 μl of nuclear extract (NXT). The positions of presplicing complex A and splicing complex B are indicated. FT, Mono S flowthrough action. (B) Proteins from the Blue-Sepharose pool of SF1 (BS; 100 μl), the dissolved ammonium sulfate precipitate (P; 5 μl), the ammonium sulfate supernatant (S; 200 μl), and individual Mono S fractions (20 μl; as indicated above each lane) were separated in SDS–9% polyacrylamide gels and stained with Coomassie brilliant blue. The positions of marker proteins (lanes M; sizes are in kilodaltons) are indicated on the right. (C) A Mono S fraction (25 μl) containing SF1 activity was incubated for 5 min at 100°C, and denatured proteins were removed by centrifugation. Proteins in the input fraction (lane T) and the supernatant (lane S) and the dissolved denatured proteins (lane P) were analyzed by electrophoresis in an SDS–9% polyacrylamide gel. Proteins were stained with silver. The positions of marker proteins (lanes M) are indicated on the left. (D) Dilutions of Mono S fractions 19 and 21 (0.01, 0.05, 0.1, 0.5, and 1 μl) were tested for presplicing complex A formation in a standard assay in the presence of SF3, U2AF, and snRNPs.

Although SF1-containing fractions can be diluted considerably without a concomitant decrease in activity in presplicing complex assembly, splicing in the presence of the purified protein is rather low. The most drastic reduction of splicing activity is evident after chromatography on Blue-Sepharose. When complete splicing reactions were analyzed for spliceosome assembly, presplicing complex formation was equally efficient with all of the SF1-containing fractions tested. However, formation of splicing complexes B and C was severely reduced in the presence of SF1 derived from Blue-Sepharose fractions or later stages of purification (data not shown). This result argues that the decrease in splicing efficiency is not caused by partial inactivation of SF1 but rather by separation of a component(s) required for conversion of presplicing complex A into complex B. Mixing experiments in which different Blue-Sepharose fractions

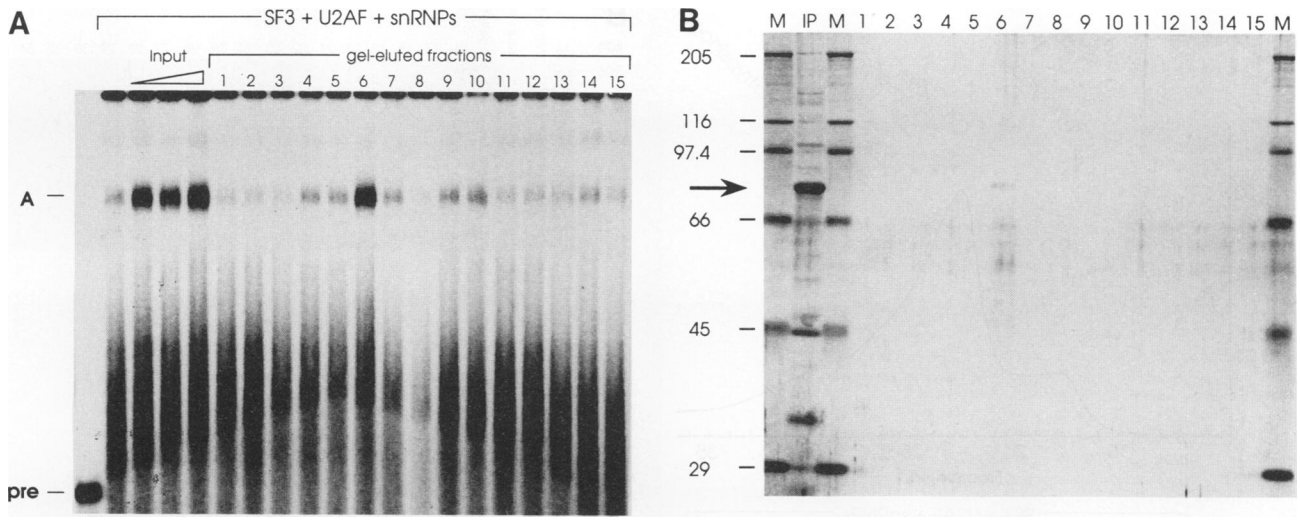

FIG. 3. Analysis of SF1 activity after SDS-gel electrophoresis and renaturation of the eluted proteins. Proteins from 50 μl of a partially purified SF1 fraction were subjected to SDS-gel electrophoresis and processed as described in Materials and Methods. (A) A standard presplicing complex formation assay was performed in the presence of 0.1, 0.2, and 0.5 μl of the input fraction or 2 μl of the eluted and renatured proteins, as indicated above each lane. The positions of the pre-mRNA (pre) and presplicing complex A are indicated. (B) Proteins from 20 μl of the input fraction (lane IP) or 15 μl of the eluted proteins (lanes 1 to 15) were separated in an SDS-polyacrylamide gel and silver stained. The positions of marker proteins (lanes M; sizes are in kilodaltons) are indicated on the left. The arrow points to the 75-kDa polypeptide.

were combined in one assay have failed to identify such a component.

Garcia-Blanco et al. (13) have purified a protein, PTB, that binds to the polypyrimidine stretch at the 3′ end of introns and have suggested that PTB acts early during spliceosome assembly. Like SF1, PTB does not bind well to DEAE-substituted resins but is retained by Blue-Sepharose (4, 6, 13, 19, 31). During the fractionation procedure employed here, PTB was detected in Blue-Sepharose fractions that eluted at 0.8 M KCl, and therefore, the protein partially overlaps with SF1 (unpublished data). It further coeluted with SF1 from Mono S, but the two proteins were separated by fractionation on poly(U)-Sepharose. This step yielded a PTB preparation that did not contain any additional polypeptides as judged by Coomassie blue or silver staining of SDS-gels. PTB was identified as a characteristic doublet or triplet of polypeptides of 56 to 57 kDa, by its ability to bind to the AdML pre-mRNA substrate in UV cross-linking experiments and by cross-reaction with an antibody prepared against the rat homolog of PTB (6; unpublished data). Additional experiments (data not shown) indicated that purified PTB cannot substitute for SF1, SF3, or U2AF in presplicing complex formation, demonstrating that it is different from these factors. Furthermore, addition of purified PTB to a complete splicing reaction did not result in increased efficiency of the cleavage-ligation reactions. Likewise, stimulation of splicing complex formation in the presence of PTB was not observed; on the contrary, addition of PTB to a splicing complex reaction resulted in inhibition of presplicing complex formation in a concentration-dependent manner. UV cross-linking experiments indicate that this inhibition could be caused by competition between PTB and U2AF (and possibly also hnRNP protein C) for the same or overlapping binding sites in the RNA substrate. In conclusion, these results make it highly unlikely that PTB represents the limiting component in the splicing assay.

Another explanation for the reduced efficiency of the cleavage-ligation reactions in the presence of purified SF1 is that the DS100 fraction contains one of the other partially purified splicing factors (for example, SF2 or SF4) which may contribute to efficient splicing and which could be separated from SF1 during Blue-Sepharose chromatography. Owing to the complexity of the assay and to volume constraints, it has not been possible to increase the concentrations of the other splicing factors in the complete splicing reaction.

With the fractionated components used in this study, at least two additional factors, SF3 and U2AF, are required for the formation of presplicing complex A (27). A number of other protein factors that function at the same stage of the reaction have been identified and, in some cases, purified. On the basis of its size, SF1 is clearly different from ASF/SF2 (14, 23), Sc35 (11), PTB (13), and presumably from an 88-kDa protein (1). SF1 also appears to be unrelated to HRF, which is heat stable in its purified form and restores presplicing complex formation and splicing in heat-inactivated nuclear extracts (9). HRF consists of two polypeptides of 70 kDa and a minor polypeptide of 100 kDa and exhibits chromatographic properties on DEAE-Sepharose different from those of SF1. Probably owing to laboratory variations in the preparation of extracts, I have been unable to reproduce the inactivation protocol used by Delannoy and Caruthers (9); therefore, comparison of the activities of these factors has not been possible. Moreover, the amino acid sequences of several peptides derived from SF1 have not revealed any similarities to protein sequences in current data bases (unpublished data).

The exact function of SF1 during presplicing complex assembly is not clear. The purified protein is not associated with any RNA-binding activity, as determined by electrophoresis in native polyacrylamide gels, UV cross-linking, and nitrocellulose filter binding with the AdML pre-mRNA substrate or an RNA that does not contain intron sequences (data not shown). This property is also reflected in a low

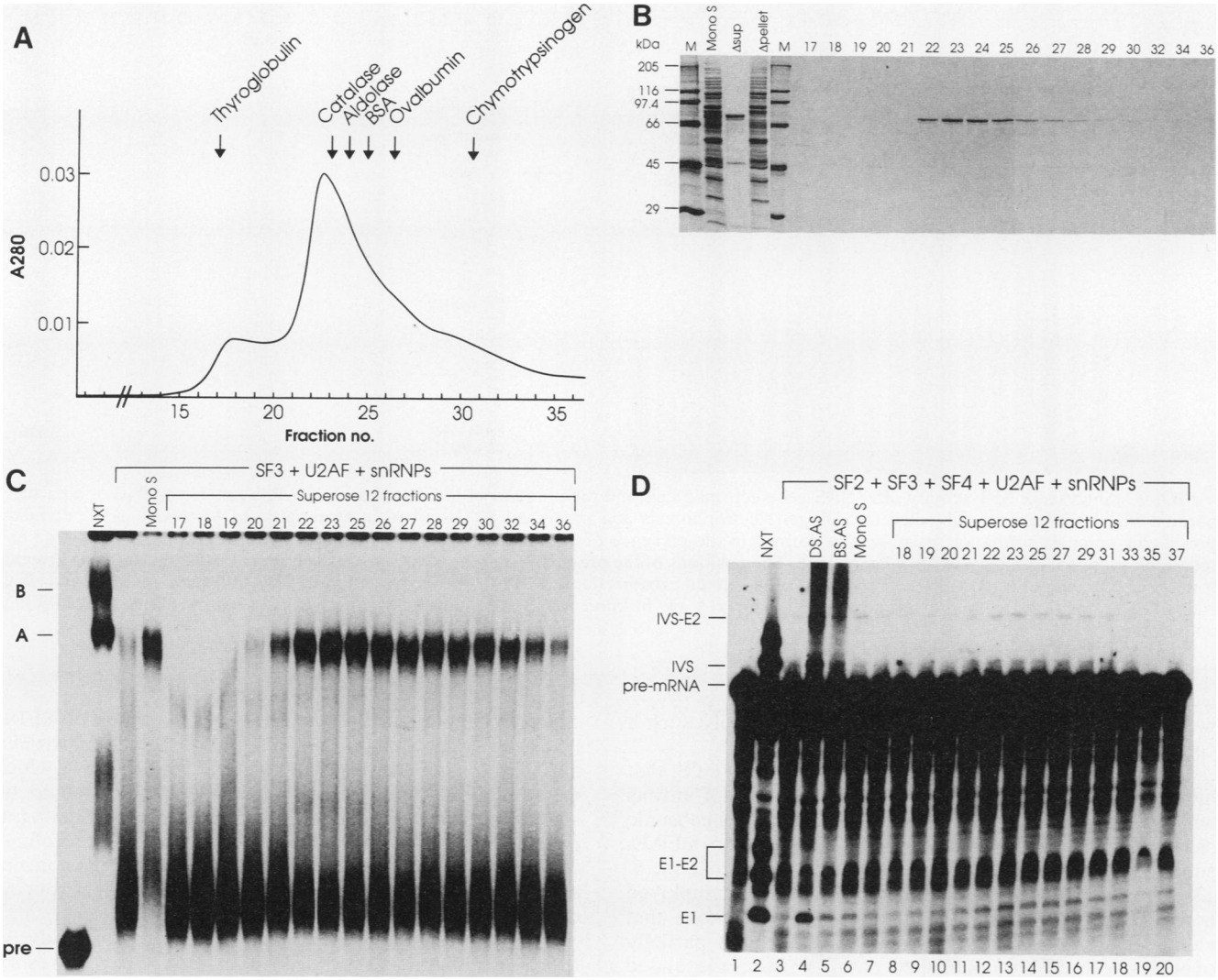

FIG. 4. Superose 12 gel filtration of SF1. Proteins from a Mono S fraction containing SF1 activity were concentrated and separated on a Superose 12 column. (A) The A_{280} of the protein that eluted from Superose 12 was measured. The elution volumes of marker proteins processed under conditions identical to those used for the SF1 sample are indicated. BSA, bovine serum albumin. (B) Proteins present in 0.5 μl each of the concentrated Mono S fraction (S-SF1), the supernatant after heat treatment (Δsup), and the dissolved heat-denatured proteins (Δpellet), as well as 7.5 μl of individual Superose 12 fractions (as indicated above the lanes), were electrophoresed in SDS–9% polyacrylamide gels (without prior precipitation) and stained with silver. The positions of marker proteins (lanes M) are indicated on the left. (C) The concentrated Mono S fraction (0.5 μl) and 0.5 μl of individual Superose 12 fractions were tested for presplicing complex A formation under standard assay conditions. The reaction shown in the first lane was performed in the presence of 3 μl of nuclear extract. The positions of presplicing complex A, splicing complex B, and pre-mRNA are indicated. NXT, nuclear extract. (D) Splicing activity in SF1-containing fractions was analyzed as described in Materials and Methods. The contents of the individual reactions are indicated at the top. The reaction shown in lane 1 was performed in the absence of splicing factors; the reactions shown in lanes 3 and 7 were done in the absence of SF1. DS.AS, ammonium sulfate-precipitated DS100 fraction; BS.AS, ammonium sulfate-precipitated Blue-Sepharose fractions; Mono S, concentrated Mono S fraction that was further purified by Superose 12 gel filtration.

affinity of SF1 for poly(U)-Sepharose. Likewise, RNA-annealing and ATPase activities that are intrinsic to other splicing proteins (20, 23, 35) were not detected (data not shown). RNA-annealing activity was assayed as described by Krainer et al. (23), with complementary RNA strands of the AdML substrate. In a control reaction, purified hnRNP protein A1 efficiently converted the complementary RNAs into an RNase T1-resistant double-stranded RNA. ATPase activity was tested as previously described (20), either in the presence or in the absence of poly(U), with nuclear extract serving as a positive control. Thus, SF1 could associate with other components of the presplicing complex by protein-protein interaction. Whether SF1 transiently interacts with the presplicing complex or is required for possible modification of other components associated with the spliceosome is a subject for further investigation.

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