Biochemistry

 Volume 23, Number 2 January 17, 1984

Accelerated Publications

Purification of the Messenger RNA Cap-Binding Protein Using a New Affinity Medium†

Nancy R. Webb, Ravi V. J. Chari, Gia DePillis, John W. Kozarich, and Robert E. Rhoads*

ABSTRACT: The *p*-aminophenyl γ-ester of 7-methylguanosine 5′-triphosphate (m^7GTP) was synthesized and coupled to Sepharose 4B. A 0.5 M salt extract of rabbit reticulocyte ribosomes was passed over a column containing the affinity medium. After extensive washing, a solution of m^7GTP was passed through the column, and a single polypeptide species of 24 kilodaltons (kDa) was eluted. This had an electrophoretic mobility identical with that of the mRNA cap-binding protein. This assignment was confirmed by the fact that the eluted material was enriched nearly 200-fold in the ability to specifically bind ^{32}P-labeled capped oligonucleotides. A control affinity medium consisting of GTP similarly coupled to Sepharose failed to retain the 24-kDa species. The postribosomal supernatant fraction yielded slightly more of the 24-kDa species than the ribosomal wash fraction when passed over this affinity medium.

The recognition of the 7-methylguanosine-containing 5′-terminus of eukaryotic mRNA (cap) marks the entry of mRNA into the cycle of protein synthesis. This step is mediated by binding of the cap to a 24-kilodalton polypeptide termed cap-binding protein (CBP)[1] [reviewed by Banerjee (1980), Ehrenfeld (1982), and Rhoads (1984)]. Other polypeptides of 46 kDa (eIF-4A) and 200 kDa appear to be involved as well (Tahara et al., 1981; Grifo et al., 1983).

CBP has been purified both by conventional methods (Trachsel et al., 1980; Hellmann et al., 1982) and by affinity chromatography (Sonenberg et al., 1979; Rupprecht et al., 1981). The latter approach is decidedly superior since the CBP is present in small quantities, and long procedures cause unacceptable losses. The affinity media used to date (Figure 1, resins **1** and **2**) have been effective. However, they may suffer from a low binding constant to CBP or a lack of specificity, since it has been necessary to purify crude extracts beyond a certain stage before CBP can be successfully bound to the media. Synthesis of these resins is also complex and difficult.

We present here the synthesis of a different affinity medium, the *p*-aminophenyl γ-ester of m^7GTP coupled to Sepharose (Figure 1, resin **3**). Synthesis of this material is simpler than that of resins **1** and **2**. Furthermore, the CBP can be obtained by applying crude ribosomal wash from rabbit reticulocytes without prior purification, suggesting that the affinity of the protein may be higher.

Materials and Methods

Materials. GTP (lithium salt) and DEAE-Sephadex A-25 were purchased from Sigma. Intestinal alkaline phosphatase and phosphodiesterase I (venom) were products of P-L Biochemicals. One unit is defined for both enzymes as the amount which catalyzes the hydrolysis of 1 μmol of substrate per min at 25 °C. Sepharose 4B was obtained from Pharmacia. HPLC analysis of reaction products was performed on a Partisil-10-SAX anion-exchange column. In all cases, the eluting solvent was 0.4 M NaH_2PO_4, pH 3.3, at a flow rate of 1.5 mL/min. DMF was dried and distilled over CaH_2, and *p*-nitrophenol was recrystallized from ethanol–water. All Sephadex chromatography was performed at 4 °C. Buffer A is 20 mM Tris-HCl, pH 7.5 at 4 °C, 0.2 mM EDTA, 1 mM dithiothreitol, and 10% glycerol.

Synthesis of m^7GTP Triethylammonium Salt. GTP (300 mg, 0.5 mmol) was methylated by using dimethyl sulfate according to the procedure of Hendler et al. (1970) with

† From the Department of Biochemistry, University of Kentucky, Lexington, Kentucky 40536 (N.R.W. and R.E.R.), and the Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510 (R.V.J.C., G.D., and J.W.K.). *Received October 18, 1983.* Supported by Grants GM20818 to R.E.R. and GM26985 to J.W.K. from the National Institute of General Medical Sciences. R.V.J.C. is a Yale-Celanese Fellow, and J.W.K. is an American Cancer Society Faculty Research Awardee (1983–1985).

[1] Abbreviations: CBP, 24-kilodalton cap-binding protein; kDa, kilodalton(s); HPLC, high-performance liquid chromatography; DMF, dimethylformamide; TEAB, triethylammonium bicarbonate; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

0006-2960/84/0423-0177$01.50/0

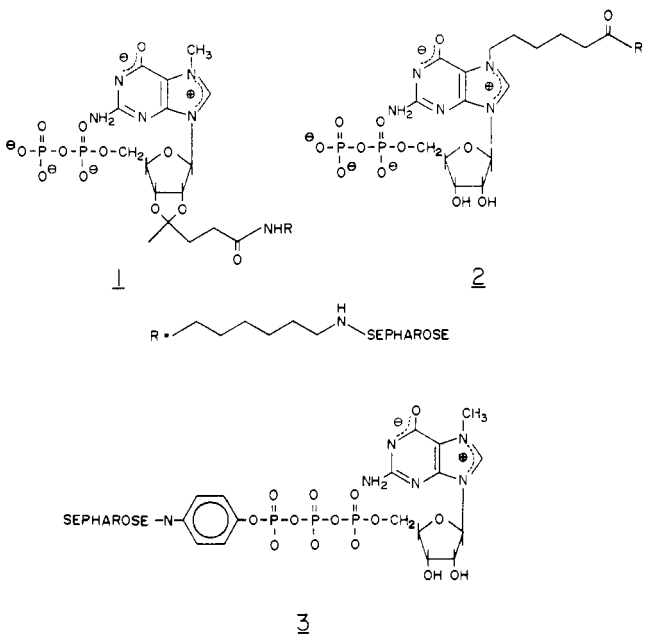

FIGURE 1: Affinity resins for purification of CBP. **1**, 2′,3′-*O*-[1-(2-carboxyethyl)ethylidene]-7-methylguanosine 5′-diphosphate coupled to Sepharose (Sonenberg et al., 1979); **2**, 7-(5-carboxypentyl)guanosine 5′-diphosphate coupled to Sepharose (Rupprecht et al., 1981); **3**, *p*-aminophenyl γ-ester of 7-methylguanosine 5′-triphosphate coupled to Sepharose (see Materials and Methods).

modification. The reaction mixture was neutralized and the product purified on a DEAE-Sephadex column (3 × 50 cm). The column was washed with water and then eluted with a linear gradient of 0–0.8 M TEAB, pH 7.8 (2-L total volume). Twenty-milliliter fractions were collected and assayed spectrophotometrically at 260 nm. m^7GTP eluted at approximately 0.4 M, and the peak was pooled and repeatedly evaporated to dryness from water in vacuo. m^7GTP triethylammonium salt was obtained as a white solid (0.25 mmol, 50% yield).

Activation of m^7GTP. This step was performed by a modification of the procedure of Knorre et al. (1976). m^7GTP triethylammonium salt was converted into the tri-*n*-octylammonium salt by using tri-*n*-octylamine. A solution of m^7GTP (0.24 mmol) in methanol (5 mL) was treated with tri-*n*-octylamine (0.32 mL, 0.72 mmol) and stirred at room temperature until a clear solution was obtained (approximately 1 h). The solvent was evaporated under reduced pressure and the residue dried thoroughly by repeated evaporation under vacuum with dry DMF (3 × 1 mL). The dried product was dissolved in anhydrous methanol–DMF (1:9 v/v, 7 mL), treated with dicyclohexylcarbodiimide (0.302 g, 1.44 mmol), and stirred overnight at room temperature in a desiccator. The solvent was evaporated under reduced pressure at the end of the reaction.

m^7GTP p-Nitrophenyl γ-Ester. The activated m^7GTP was dissolved in dry DMF (7 mL) and treated with *p*-nitrophenol (0.68 g, 4.8 mmol) and triethylamine (0.65 mL, 4.8 mmol) as previously described (Knorre et al., 1976). The reaction flask was tightly stoppered and stirred for 24 h at room temperature. The reaction mixture was then diluted with water (20 mL) and cooled, and the pH was adjusted to 3.5 with HCl. The mixture was extracted with ether (2 × 25 mL) to remove unreacted *p*-nitrophenol. The aqueous layer was neutralized and chromatographed on a column (1.8 × 45 cm) of DEAE-Sephadex in the HCO_3^- form that was extensively washed with water before loading of the sample. After the sample was loaded, the column was washed with water and eluted with a linear gradient of 0–0.6 M TEAB, pH 7.8 (1.2-L total volume). Fractions were assayed spectrophotometrically at 260 and 340 nm. Two closely spaced peaks eluted at 0.33 and 0.37 M, respectively. The location of the desired *p*-nitrophenyl γ-ester peak was determined by enzymatic assay of individual fractions (Berglund & Eckstein, 1972). Alkaline phosphatase (5 units) and phosphodiesterase I (5 units) were incubated with aliquots of 250 μL diluted to 1 mL with a buffer containing 0.11 M Tris-HCl, pH 8.8, 0.11 M NaCl, and 15 mM $MgCl_2$. The release of *p*-nitrophenolate ion (ϵ_{405} = 18 500 at pH 9.5) was monitored by the increase in absorbance at 405 nm. The first peak, which had a high A_{340} reading, gave a negative test and was discarded. The purity of the fractions was determined by HPLC analysis. The *p*-nitrophenyl γ-ester of m^7GTP had a retention time of 4.2 min. The peak eluting at 0.37 M was pooled and repeatedly evaporated to dryness with water under reduced pressure to give the *p*-nitrophenyl γ-ester of m^7GTP (713 A_{260} units, 0.071 mmol).

Reduction of p-Nitrophenyl γ-Ester of m^7GTP. The *p*-nitrophenyl γ-ester of m^7GTP (700 A_{260} units, 0.07 mmol) was dissolved in H_2O (10 mL) and reduced by the method of Berglund & Eckstein (1972). Argon was bubbled through the solution, after which palladium on charcoal (100 mg) was added. Hydrogen gas was bubbled through the suspension for 3 h. The reaction mixture was then filtered and the catalyst washed repeatedly with water. The filtrate and combined washings were loaded on a DEAE-Sephadex column (1 × 40 cm). The column was washed with H_2O and the product eluted with a linear gradient of 0–0.8 M TEAB buffer (800-mL total volume). The product eluted at 0.49 M. The peak was pooled and evaporated under vacuum to give the *p*-aminophenyl γ-ester of m^7GTP (287 A_{260} units, 0.029 mmol). The product was found to be essentially pure by HPLC analysis and had a retention time of 2.8 min.

Coupling to Sepharose 4B. The coupling reaction was performed by established procedures (Cuatrecasas et al., 1968). H_2O (15 mL) was added to settled Sepharose 4B (15 mL). The suspension was stirred and cooled to 5 °C. A solution of cyanogen bromide (1.8 mL of a 1 g/mL solution) in acetonitrile was added, and the pH was maintained between 11 and 11.5 by addition of 1 N NaOH for 20 min. The resin was then filtered and washed with H_2O (1 L) and cold 0.1 M $NaHCO_3$–Na_2CO_3 buffer (pH 9.0, 1 L). The washed resin in buffer (30-mL total volume) was treated with the *p*-aminophenyl γ-ester of m^7GTP (287 A_{260} units). The slurry was stirred overnight at 4 °C after which the resin was filtered and washed with water. An aliquot of this resin (300-μL settled volume) was assayed as described under m^7GTP *p*-Nitrophenyl γ-Ester, except that the change in absorbance of the supernatant fluid was followed at 260 nm. The amount of bound *p*-aminophenyl γ-ester of m^7GTP was found to be 0.18 μmol/mL (1.8 A_{260} units/mL) of settled Sepharose. This is subsequently referred to as m^7GTP–Sepharose (Figure 1, resin **3**).

Synthesis of GTP-Substituted Sepharose. The *p*-aminophenyl γ-ester of GTP was synthesized by the procedure outlined above. The final ester bound to Sepharose 4B was 0.29 μmol/mL of resin.

Affinity Purification of CBP. Ribosomes were pelleted from rabbit reticulocyte lysate, and a 0.5 M KCl extract was prepared as previously described (Hellmann et al., 1982). The extract was precipitated by using 70% saturation of ammonium sulfate, dialyzed against buffer A containing 100 mM KCl, and passed over a column of m^7GTP–Sepharose (1 mL of resin per 70 mg of extract). The column was washed with 30–50 volumes of buffer A containing 100 mM KCl, and CBP was

eluted with 700 μM m^{7}GTP and 100 mM KCl in buffer A. In some cases, the column was stripped with 1 M KCl in buffer A. Finally, any residual protein was removed with 0.2% sodium dodecyl sulfate before the affinity material was reused. Generally, it was necessary to repass the bound fraction to obtain pure CBP.

CBP was also purified from the postribosomal supernatant fraction of rabbit reticulocyte lysate (Hellmann et al., 1982) by essentially the same procedure. In this case, 1 mL of resin was used per 100 mL of supernatant fluid.

Assay of CBP. Activity was detected by the binding of oligonucleotides of the form m^{7}Gppp(Np)$_{6-8}$[^{32}P]pCp to protein samples and applying them to nitrocellulose filters (Hellmann et al., 1982). Aliquots of 5 μL, eluted from the affinity column, were incubated in a total reaction volume of 10 μL with 1 pmol of oligonucleotide in buffer A containing 1 mM ATP, 0.2 mM GTP, 1 mM $MgCl_2$, and 100 mM KCl for 10 min at room temperature. The reaction mixture was applied to Millipore HAWP filters, the filters were washed 3 times with 100-μL portions of the assay buffer, and radioactivity was determined by liquid scintillation spectrometry.

Other Methods. Protein was estimated in crude fractions (ribosomal salt wash, unbound fraction) by the absorption of 280 nm and in purified fractions (bound) by the method of Bradford (1976) using the protein assay dye reagent from Bio-Rad Laboratories. Gel electrophoresis of protein fractions was by the method of Laemmli & Favre (1973) and detection by staining with silver (Morrissey, 1981). Sucrose gradients were run in buffer A containing 0.1 M KCl as described previously (Hellmann et al., 1982).

Results

Synthesis of m^{7}GTP–Sepharose. In the preparation of m^{7}GTP *p*-nitrophenyl γ-ester described here, m^{7}GTP was activated by conversion to the cyclic trimetaphosphate by dicyclohexylcarbodiimide, and this intermediate was reacted with *p*-nitrophenolate ion to produce m^{7}GTP *p*-nitrophenyl γ-ester. The nitro group was reduced to the amine and the resulting compound coupled to Sepharose 4B by using cyanogen bromide. The nucleotide–Sepharose linkage is stable for months at 4 °C as determined by the enzymatic release of bound nucleotide (see Materials and Methods).

Binding of CBP to m^{7}GTP–Sepharose. A crude salt wash of reticulocyte ribosomes was applied to the m^{7}GTP–Sepharose affinity medium. After being extensively washed, the column was eluted with 70 μM m^{7}GTP. As shown in Figure 2, very little of the protein was retained on the column. The protein concentration of the m^{7}GTP-eluted fraction (first arrow) could not be measured by the absorption at 280 nm, but by the method of Bradford (1976), it was shown to be in the range of 20–30 μg/mL. By gel analysis, the major protein species detected was 24 kDa. The presence of other minor polypeptides was also detected, but these were removed upon a second pass over the affinity column. The prominant bands at 50 and 65 kDa are artifacts of the silver staining method. When the column was washed with less buffer before elution with m^{7}GTP (e.g., 10 vs. 50 volumes), other polypeptides were retained and eluted with m^{7}GTP (data not shown). The specificity of this binding is currently under investigation.

The 24-kDa polypeptide corresponds in mobility to a protein previously identified as CPB by its cross-linking to the 5′-terminus of mRNA (Sonenberg et al., 1978), by its ability to restore protein synthesis to poliovirus-infected cell lysates (Trachsel et al., 1980), and by its ability to reverse inhibition by cap analogues of cell-free protein synthesis (Hellmann et al., 1982). The identity of this polypeptide as CBP was further

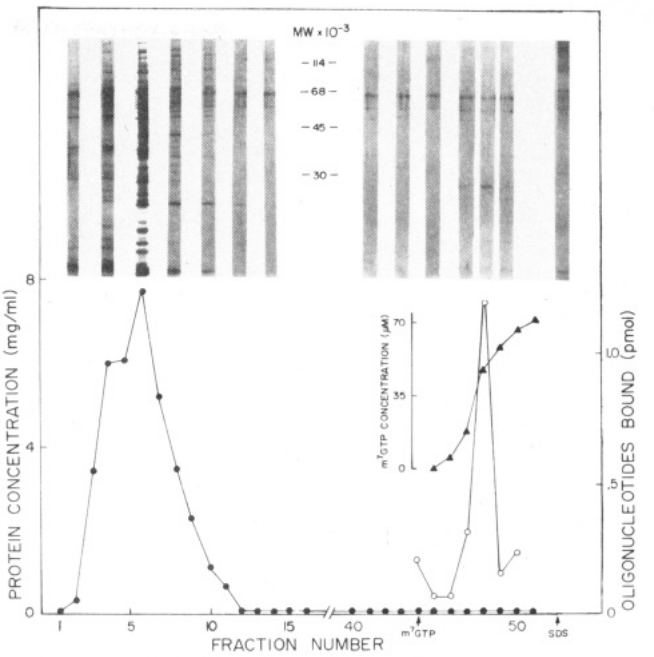

FIGURE 2: Elution of CBP from m^{7}GTP–Sepharose. The high-salt ribosomal wash fraction derived from 87 mL of lysate (30.5 mg of protein) was applied to a 1-mL column of m^{7}GTP–Sepharose as described under Materials and Methods, and fractions of 320 μL were collected. At fraction 44 (first arrow), elution was begun with 70 μM m^{7}GTP, and fractions were assayed for CBP by the oligonucleotide-binding assay. Finally, residual protein was stripped with 0.2% sodium dodecyl sulfate (second arrow). (○) Oligonucleotides bound; (▲) m^{7}GTP concentration used for elution; (●) protein concentration. (Inset) Aliquots of selected fractions were analyzed by electrophoresis, with detection of protein by silver staining. For unbound fractions, 1 μL was analyzed. For bound fractions, 6-μL aliquots were used. The staining of protein in the lane representing fraction 5 was so intense that a lighter photographic exposure was used compared with the other lanes. The two prominent bands at approximately 50 and 65 kDa are artifacts of the staining method and do not correspond to polypeptides.

confirmed by assay with labeled, capped oligonucleotides (Figure 2). The peak in oligonucleotide binding was coincident with the appearance of the 24-kDa polypeptide and occurred as the eluting m^{7}GTP was reaching a maximum concentration. The specificity of binding was confirmed in a separate experiment. An equal quantity of ribosomal salt wash was applied to a control column of GTP–Sepharose, in which the nucleotide–Sepharose linkage was identical with that of m^{7}GTP–Sepharose. No polypeptide of 24 kDa was eluted with m^{7}GTP, although several larger polypeptides were observed (data not shown). Thus, the binding of CBP to m^{7}GTP–Sepharose appears to be by specific recognition of the m^{7}G moiety as opposed to the purine ring, triphosphates, etc.

To determine whether the m^{7}GTP completely eluted CBP, the column was stripped with sodium dodecyl sulfate (second arrow). While it is apparent that a considerable amount of protein was nonspecifically bound, no CBP was eluted by this treatment.

Previously, it was shown that binding of capped oligonucleotides to CBP is improved in the presence of nucleotides such as ATP or GTP and Mg^{2+} (Hellmann et al., 1982). Also, Sonenberg (1981) has shown that in the presence of ATP and Mg^{2+}, cross-linking of the 5′-terminus of mRNA to the 24-kDa CBP is reduced in favor of cross-linking to other polypeptides of 28, 50, and 80 kDa. It was therefore of interest to repeat the experiment shown in Figure 2 but in the presence of 1 mM ATP, 0.2 mM GTP, and 1 mM $MgCl_2$. The pattern of retained protein was essentially the same as in the absence of

Table I: Assay of Affinity-Purified CBP by Oligonucleotide Binding[a]

fraction	total protein (mg)	oligonucleotide-binding activity (pmol bound)			sp act. (pmol/mg of protein)	normalized total activity (pmol/mL of lysate)
		$-m^7GTP$	$+m^7GTP$	net		
expt 1						
ribosomal salt wash	30.5	192	147	45	1.48	0.52
m^7GTP-Sepharose unbound	17.6	95	109	0	0	0
m^7GTP-Sepharose bound	0.071	22	2	20	282	0.23
expt 2						
ribosomal salt wash, GTP-Sepharose unbound	17.4	62	43	19	1.09	0.22
expt 3						
postribosomal supernatant, m^7GTP-Sepharose bound	0.065	8.3	1.6	6.7	103	0.33

[a] Various fractions from rabbit reticulocyte lysate were passed over either m^7GTP-Sepharose or GTP-Sepharose, and protein was eluted with m^7GTP, as described under Materials and Methods. In experiment 1, a ribosomal salt wash was prepared from 87 mL of lysate, precipitated with 70% saturated ammonium sulfate, dialyzed, and passed over a 1-mL column of m^7GTP-Sepharose. Fractions equivalent to 0.1–0.2 mL of original lysate were assayed for binding of ^{32}P-labeled capped oligonucleotides, in either the absence or the presence of 200 μM m^7GTP to control for nonspecific binding. In experiment 2, a GTP-Sepharose column was used instead. In experiment 3, the postribosomal supernatant fraction derived from 20 mL of lysate was passed over a 1-mL column of m^7GTP-Sepharose.

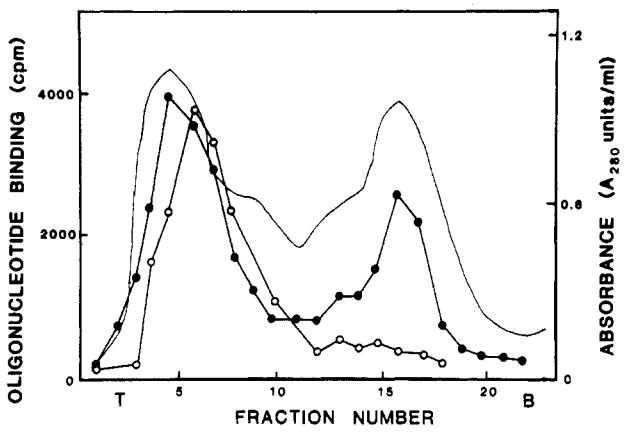

FIGURE 3: Oligonucleotide-binding activity of the reticulocyte ribosomal salt wash purified through step 3 in Hellmann et al. (1982). The protein fraction was sedimented on a 12-mL 15–30% linear sucrose gradient in a Beckman SW-40 rotor for 24 h at 35 000 rpm at 4 °C. Fractions of 40 μL were assayed for binding of ^{32}P-labeled capped oligonucleotides in the absence (●) or presence (○) of competing m^7GTP (200 μM).

nucleotides (data not shown). However, the volume of 70 μM m^7GTP required to elute all the CBP was roughly 3-fold greater than in the absence of nucleotides, suggesting a tighter binding.

Quantitation of Affinity-Purified CBP. In order to estimate the yield and specific enrichment of CBP by affinity purification, column fractions were tested by the oligonucleotide-binding assay. However, a considerable amount of nonspecific binding of capped oligonucleotides occurs with crude fractions. This is illustrated in Figure 3. A reticulocyte high-salt ribosomal wash was purified by precipitation with 40% saturated ammonium sulfate, adsorption to DEAE-cellulose, and sedimentation on a sucrose gradient in 100 mM KCl [step 4 in Hellmann et al. (1982)]. Previously, it has been shown that most of the CBP cosediments with eIF-3, the faster of the two peaks (Trachsel et al., 1980; Hellmann et al., 1982). As shown in the figure, oligonucleotide binding occurred with both the slowly sedimenting protein and eIF-3, but only binding to the latter was blocked by the competitor m^7GTP. Thus, in testing crude fractions, it is essential that m^7GTP be employed as a competitor to distinguish between specific and nonspecific binding.

The affinity-purified material was tested by oligonucleotide binding in the presence and absence of m^7GTP (Table I). While the activity of the crude starting material could be inhibited only 23%, that of the purified fraction could be inhibited 91%. The unbound fraction exhibited no specific binding, indicating that all of the CBP was removed in one pass through the column. This was confirmed by passing the unbound fraction through a second affinity column and showing, by electrophoresis, that no CBP was retained (data not shown). By contrast, the unbound fraction of a GTP–Sepharose column retained oligonucleotide-binding activity (experiment 2).

By computing the specific binding per milligram of protein assayed, it was shown that the purified material was enriched 191-fold over the starting material (Table I). The recovery of activity was 44%. This may be due to the instability of CBP when extensively purified (Trachsel et al., 1980; Hellmann et al., 1982). Also, due to the elution conditions, there was some competing m^7GTP present in the reaction mixtures (10–35 μM) when purified fractions were assayed; this would be expected to give an underestimate of the activity. The purified protein retained activity through one freeze–thaw cycle but lost it upon repeated freezing and thawing. Attempts to stabilize it with bovine serum albumin were unsuccessful. Similarly, storage at 4 °C resulted in loss of activity, as did dialysis to remove the m^7GTP.

CBP in the Postribosomal Supernatant Fraction. Previous purification schemes for CBP have generally started from the ribosomal salt wash of reticulocyte ribosomes (Sonenberg et al., 1979; Trachsel et al., 1980; Hellmann et al., 1982). In Table I, experiment 3, the results of an isolation of CBP from the postribosomal supernatant fraction are shown. Oligonucleotide-binding activity was observed in the eluted fraction, and it could be inhibited 81% by m^7GTP, indicating it was specific. Quantitatively, there was slightly more activity than in the ribosomal salt wash. By electrophoretic analysis, the eluted material consisted primarily of the 24-kDa CBP (data not shown). A second affinity column purification was sufficient to remove contaminating polypeptides and leave only CBP.

Discussion

CBP has previously been purified by conventional methods of protein fractionation (Trachsel et al., 1980, Hellmann et al., 1982). Affinity chromatography, however, offers the advantages of speed and simplicity. The affinity-purified preparation appeared by gel analysis (Figure 2) to be comparable in purity to conventionally purified material and re-

tained the ability to bind capped oligonucleotide (Table I). In addition, it was possible to test a different cell fraction than the ribosomal wash for the presence of CBP by using this affinity material. Surprisingly, the postribosomal supernatant fluid contained a considerable amount of CBP, even more than the ribosomal salt wash. Previously, it had not been possible to test this fraction by using the cap analogue reversal assay (Hellmann et al., 1982) because of interferences with the assay. Hansen et al. (1983) have also reported detection of the CBP in the supernatant fraction of HeLa cells by a cross-linking assay. They found approximately twice as much activity as in the ribosomal pellet.

Two previous reports have described the synthesis of different affinity media for the purification of CBP. Sonenberg et al. (1979) coupled the levulinic acid acetal of m^7GDP to Sepharose (Figure 1, resin **1**), and Rupprecht et al. (1981), a 7-carboxypentyl derivative of m^7GDP (Figure 1, resin **2**). Both were capable of specifically purifying the 24-kDa CBP. In a comparison of these two resins with the *p*-nitrophenyl ester described here (Figure 1, resin **3**), there is some indication that the binding of CBP to the latter may be tighter. Sonenberg et al. (1979) reported that application of a 0–40% ammonium sulfate fraction of the ribosomal salt wash to resin **1** did not yield any polypeptides upon elution with m^7GDP. It was necessary to first remove eIF-3 by sucrose gradient centrifugation before application of extracts to the affinity material if the CBP was to be obtained. This is not the case with resin **3**, as shown in the present report. Presumably there is competition between eIF-3 and the affinity resin for binding of CBP (since CBP is known to bind to eIF-3). In the case of resin **3**, the affinity may be great enough to allow complete retention of CBP, even in the presence of eIF-3. Rupprecht et al. (1981) also reported that not all of the CBP could be eluted with m^7GDP from resin **2**; a considerable amount was present in the 1.0 M KCl wash which followed. We demonstrate here that no CPB was left on resin **3** after elution with m^7GTP, since sodium dodecyl sulfate failed to elute it (Figure 2). Thus, resin **3** may provide more specific binding of CBP than resin **2**. Some of these improved characteristics of binding may be due to the fact that resin **3** more closely resembles the natural linkage of cap to mRNA than resins **1** or **2**.

Finally, the m^7GTP affinity resin **3** may be prepared by a much simpler synthetic scheme than that of resin **1** or **2**. The intermediate steps are more easily monitored and less ambiguous. For instance, the preparation of **1** involves the formation of two diastereomeric ketals of levulinic acid which could bind differentially to CBP. Furthermore, one might expect the 5′-diphosphates of **1** and **2** to be both chemically and enzymatically less stable than the caplike triphosphate structure of **3**.

Registry No. GTP, 86-01-1; m^7GTP triethylammonium salt, 88106-66-5; m^7GTP tri-*n*-octylammonium salt, 88106-67-6; activated m^7GTP, 88106-68-7; *p*-nitrophenol, 100-02-7; m^7GTP *p*-nitrophenyl γ-ester, 88106-69-8; m^7GTP *p*-aminophenyl γ-ester, 88106-70-1.

References

Banerjee, A. K. (1980) *Microbiol. Rev. 44*, 175.

Berglund, O., & Eckstein, F. (1972) *Eur. J. Biochem. 28*, 492.

Bradford, M. (1976) *Anal. Biochem. 72*, 248.

Cuatrecases, P., Wilchek, M., & Anfinsen, C. B. (1968) *Proc. Natl. Acad. Sci. U.S.A. 61*, 536.

Ehrenfeld, E. (1982) *Cell (Cambridge, Mass.) 28*, 435.

Grifo, J. A., Tahara, S. M., Morgan, M. A., Shatkin, A. J., & Merrick, W. C. (1983) *J. Biol. Chem. 258*, 5804.

Hansen, J. L., Etchinson, D. O., Hershey, J. W. B., & Ehrenfeld, E. (1983) *Mol. Cell. Biol. 2*, 1639.

Hellmann, G. M., Chu, L.-Y., & Rhoads, R. E. (1982) *J. Biol. Chem. 257*, 4056.

Hendler, S., Furer, E., & Srinivasan, P. R. (1970) *Biochemistry 9*, 414.

Knorre, D. G., Kurbatov, V. A., & Samukov, V. V. (1976) *FEBS Lett. 70*, 105.

Laemmli, U. K., & Favre, M. (1973) *J. Mol. Biol. 80*, 575.

Morrissey, J. H. (1981) *Anal. Biochem. 117*, 307.

Rhoads, R. E. (1984) in *Progress in Molecular and Subcellular Biology* (Hahn, F. E., Kopecko, D. J., & Muller, W. E. G., Eds.) Springer-Verlag, Berlin, Heidelberg, New York, and Tokyo (in press).

Rupprecht, K. M., Sonenberg, N., Shatkin, A. J., & Hecht, S. M. (1981) *Biochemistry 20*, 6570.

Sonenberg, N. (1981) *Nucleic Acids Res. 9*, 1643.

Sonenberg, N., Morgan, M. A., Merrick, W. C., & Shatkin, A. J. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 4843.

Sonenberg, N., Rupprecht, K. M., Hecht, S. M., & Shatkin, A. J. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4345.

Tahara, S. M., Morgan, M. A., & Shatkin, A. J. (1981) *J. Biol. Chem. 257*, 7691.

Trachsel, H., Sonenberg, N., Shatkin, A. J., Rose, J. K., Leong, K., Bergmann, J. E., Gordon, J., & Baltimore, D. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 770.