Binding of tRNA nucleotidyltransferase to Affi-Gel Blue: rapid purification of the enzyme and binding studies*

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ABSTRACT

Rabbit liver tRNA nucleotidyltransferase bound to columns of Affi-Gel Blue and could be specifically eluted with tRNA. This observation led to development of a rapid purification procedure for the enzyme. The adsorbent was also used to assess interaction of tRNA nucleotidyltransferase with various polynucleotides and substrates. The enzyme could be efficiently desorbed from Affi-Gel Blue by low concentrations of tRNA-C-C, less well by tRNA-C-C-A, and not at all by poly(A), poly(C), ATP or CTP.

INTRODUCTION

The enzyme ATP(CTP) tRNA nucleotidyltransferase catalyzes the synthesis of the -C-C-A terminus of tRNA in vitro (1). Recent evidence indicates that the enzyme plays a similar role in vivo, both in the biosynthesis of some tRNA molecules (2-4), and in the repair of others (5,6). tRNA nucleotidyltransferase has also found use as a reagent for modifying the -C-C-A terminus of tRNA since the enzyme can incorporate a variety of nucleotide analogs into this sequence (see 1,7). One impediment to this type of study is the requirement for substantial amounts of enzyme. Since cells contain relatively low quantities of this protein, cumbersome purification procedures are required (8-10). Studies currently in progress in our laboratory dealing with model acceptor substrates (11) similarly require large amounts of enzyme.

To circumvent this problem we have developed a rapid purification procedure for the rabbit liver enzyme which makes use of binding of this protein to Affi-Gel Blue. The chromophore of this adsorbent, Cibacron blue F3GA, is known to bind a variety of nucleotide-requiring enzymes, which can be eluted with specific ligands

(12,13). Recently, it has been shown that this type of adsorbent (Blue-dextran Sepharose) can bind interferon, and that the protein can be eluted with some polynucleotides or tRNA (14,15). In this paper we show that tRNA nucleotidyltransferase also binds to Affi-Gel Blue and that the protein can be eluted specifically with tRNA. This finding has proved useful for development of a rapid purification procedure, as well as for studies of polynucleotide binding to tRNA nucleotidyltransferase.

MATERIALS AND METHODS

Affi-Gel Blue (100-200 mesh) was purchased from Bio-Rad Laboratories. In this adsorbent the chromophore is attached directly to cross-linked agarose. Labeled and unlabeled nucleoside triphosphates and yeast tRNA were obtained from Schwarz/Mann. Poly(A) and poly(C) were products of Miles Laboratories. Whatman DEAE cellulose (DE52) and phosphocellulose (P11) were obtained from Reeve-Angel.

tRNA nucleotidyltransferase was purified from frozen rabbit livers obtained from Pel-Freez Biologicals. Enzyme assays determined the incorporation of [^{14}C] ATP into tRNA-C-C as described earlier (8). Buffer B contained the indicated amount of potassium phosphate, pH 7.5, 1 mM MgCl_2 , 0.1 mM EDTA, 0.1 mM dithiothreitol, 5% glycerol. tRNA-C-C was prepared as previously described (15).

RESULTS AND DISCUSSION

1. Rapid purification of tRNA nucleotidyltransferase

Purification of the enzyme through the DEAE cellulose step was essentially as described earlier (16) except that the alumina C_γ fractionation was eliminated. This change necessitated minor modifications in the procedure. The $(\text{NH}_4)_2\text{SO}_4$ pellet was dialyzed against buffer B (8 mM potassium phosphate) lacking glycerol, rather than Buffer A to prepare the sample for DEAE cellulose chromatography. Since the enzyme was less pure than previously at this stage,

a larger DEAE cellulose column was required to prevent overloading. In the preparation described here, which used 3 pounds of liver, the column dimensions were 9 x 27 cm. The column was equilibrated with buffer B containing 8 mM potassium phosphate, pH 7.5. After washing extensively to remove unbound protein, tRNA nucleotidyltransferase was eluted with buffer B containing 30 mM potassium phosphate. Active fractions were combined for further purification. At this stage of purification the enzyme was free of nucleic acid as determined by the A₂₆₀/A₂₈₀ ratio.

The DEAE cellulose fraction was applied to a column of Affi-Gel Blue equilibrated with buffer B containing 30 mM potassium phosphate. After addition of the sample the column was washed with this buffer until the A₂₈₀ was between 0.1 and 0.2. tRNA nucleotidyltransferase was eluted with the same buffer containing 20 µg per ml of yeast tRNA (Fig. 1). Before the next purification step, tRNA was first removed by passing the enzyme solution through a small column of DEAE cellulose (1.5 x 7 cm) in buffer B containing 30 mM potassium phosphate. At this point in some preparations the enzyme solution was also concentrated on an Amicon filter (PM-10). The enzyme was then applied to a column of phosphocellulose (0.9 x 13.5 cm) equilibrated with the same buffer lacking Mg²⁺. After washing with the same buffer, tRNA nucleotidyltransferase was eluted with a 230 ml gradient from the starting buffer to the same buffer containing 0.2 M KCl. Activity was eluted in two peaks, in a ratio of about 1:2, as shown in earlier studies (8).

By this procedure it was possible to prepare about 2 mg of highly-purified enzyme in one week. A summary of the purification is shown in Table I. At this stage of purification peak I was about 60% pure and peak II about 80% pure as judged by the specific activity and SDS acrylamide gel electrophoresis. It probably would be possible to prepare homogeneous enzyme by this procedure if the fractions with highest specific activity were used at each step.

Development of this rapid purification procedure was due mainly to the use

Fig. 1. Purification of tRNA nucleotidyltransferase on Affi-Gel Blue. Approximately 400 ml of the combined DEAE cellulose fractions were applied to a column (3 x 9 cm) of Affi-Gel Blue equilibrated with buffer B containing 30 mM potassium phosphate. The column was washed with this buffer to remove unbound protein, and the enzyme was eluted with the same buffer containing 20 μ g/ml of yeast tRNA beginning at tube 160. The first 30 fractions were 16 ml each; subsequent fractions were 5 ml. The A₂₈₀ after the addition of yeast tRNA has been approximated by subtraction of the contribution due to the tRNA.

of Affi-Gel Blue. The adsorbent bound only a very small percentage of the total protein in the DEAE cellulose fraction (see Fig. 1), and in addition, elution with tRNA removed only a portion of the bound proteins. Purifications of 100-200 fold were routinely obtained in this step. tRNA nucleotidyltransferase also could be eluted with buffers of increased ionic strength, but at a considerably lower specific activity. We have not yet attempted to increase the specificity of elution, perhaps by the use of a tRNA gradient. Binding of tRNA nucleotidyltransferase to Affi-Gel Blue was dependent on the presence of the chromophore, Cibacron F3GA, since the enzyme was not bound by the agarose matrix alone (data not shown). This observation, together with the recent studies of interferon binding to Blue dextran-Sepharose (14,15), suggests that these types of adsorbents may be generally useful for purification of polynucleo-

TABLE I
Summary of Purification of tRNA Nucleotidyltransferase

Step	Total activity ^a (units)	Specific activity (units/mg)	Relative purification (- fold)	Yield ^a (%)
1a. Homogenate	4,500	0.017	----	100
1b. 15,000 g supernatant	4,200	----	----	93
2. Ammonium sulfate	2,800	0.052	3.1	62
3. DEAE cellulose	1,800	0.38	22	40
4. Affi-Gel Blue	1,180	56	3300	26
5. Phosphocellulose				
Peak I	330	~ 300	~17,500	7
Peak II	700	~ 410	~24,100	16

^a The total activity is based on three pounds of liver. The total activity and yield have been corrected for samples removed at each step. Re-extraction of the pellet from step 1b was eliminated in this procedure (16).

tide-requiring enzymes. It has already been suggested that proteins bind to these adsorbents because the chromophore resembles a nucleotide (12,13). Presumably, the high concentration of dye residues on the matrix leads to a structure that is recognized as a polynucleotide. Enzymes binding to the adsorbent because of this "polynucleotide-type" structure would be eluted only with polynucleotides, and not by mononucleotides, in contrast to the proteins thought to possess the dinucleotide fold (12). This type of behavior was observed with tRNA nucleotidyltransferase (see below).

2. Use of Affi-Gel Blue for tRNA nucleotidyltransferase binding studies

The rapid elution of tRNA nucleotidyltransferase from Affi-Gel Blue by yeast tRNA suggested that this system might be used to measure the relative interaction of various compounds with the enzyme. A similar approach has been used to study binding of interferon to polynucleotides (14,15). Since commercial yeast tRNA was mainly a mixture of tRNA-C-C-A and tRNA-C-C, we first compared

purified liver tRNA-C-C and intact tRNA-C-C-A for their relative efficiencies desorbing tRNA nucleotidyltransferase from an Affi-Gel Blue column. As shown in Fig. 2, tRNA-C-C caused a more rapid and sharper desorption of the enzyme than tRNA-C-C-A, as expected from the greater affinity of tRNA nucleotidyltransferase for tRNA-C-C (J.A. Evans and M.P. Deutscher, unpublished results). The amount of enzyme eluted was the same in both cases. These results confirm that elution from Affi-Gel Blue columns can be used to measure relative interactions with the enzyme.

In a second experiment, the interaction of poly(A) and poly(C) with tRNA nucleotidyltransferase was examined (Fig. 3). Neither of these polynucleotides at 100 μ g per ml was able to desorb the enzyme, whereas addition of yeast tRNA led to immediate removal. Poly(A) and poly(C) have previously been shown to be very poor substrates for tRNA nucleotidyltransferase (17,18). In a similar type of experiment, 5 mM CTP or 10 mM ATP (which are at least 10x and 5x K_m , respectively) were unable to remove tRNA nucleotidyltransferase from Affi-Gel Blue (data not shown), confirming that the enzyme probably binds the adsorbent

Fig. 2. Comparison of liver tRNA-C-C-A and tRNA-C-C for desorption of tRNA nucleotidyltransferase from Affi-Gel Blue. Enzyme (35 units) was applied to a column of Affi-Gel Blue (0.9 x 3.5 cm) as in Fig. 1, and eluted at the indicated point with either tRNA-C-C or tRNA-C-C-A, at 20 μ g/ml.

Fig. 3 Test for desorption of tRNA nucleotidyltransferase from Affi-Gel Blue by poly (A) and poly(C). Enzyme (12 units) was applied to the Affi-Gel Blue column as in Fig. 2 and eluted with poly(A) or poly(C) at 100 μ g/ml. Yeast tRNA was added, as indicated, at 50 μ g/ml.

via its tRNA attachment site.

The ability to easily purify large amounts of liver tRNA nucleotidyltransferase should permit experiments which were not previously possible because of the limited amount of pure protein available. Studies are now in progress to determine whether the rapid purification procedure can be used to purify the *Escherichia coli* enzyme, and its mutant counterpart (5).

Since this work was submitted for publication, similar results have been obtained with polynucleotide phosphorylase (19). This enzyme could be eluted from Blue dextran-Sepharose columns by its polymeric substrates, but not by nucleoside diphosphates. In addition, these workers showed that tryptophanyl-tRNA synthetase could be eluted from such columns by tRNA^{Trp}, and in agreement with earlier work (20) on other synthetases, that it could also be displaced by ATP.

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REFERENCES

1. Deutscher, M.P. (1973) Prog. Nucl. Acid. Res. 13, 51-92.
2. Deutscher, M.P., Foulds, J. and McClain, W.H. (1974) J. Biol. Chem. 249, 6696-6699.
3. McClain, W.H., Seidman, J.G. and Schmidt, F. (1978) J. Mol. Biol., in press.
4. Goodman, H.M., Olson, M.V. and Hall, B.D. (1977) Proc. Nat. Acad. Sci. USA 74, 5453-5457.
5. Deutscher, M.P. and Hilderman, R.H. (1974) J. Bacteriol. 118, 621-627.
6. Deutscher, M.P., Lin, J.J.-C. and Evans, J.A. (1977) J. Mol. Biol. 117, 1081-1094.
7. Sprinzl, M., Sternbach, H., Von Der Haar, F. and Cramer, F. (1977) Eur. J. Biochem. 81, 579-589.
8. Deutscher, M.P. (1972) J. Biol. Chem. 247, 450-458.
9. Sternbach, H., Von Der Haar, F., Schlimme, E., Gaertner, E. and Cramer, F. (1971) Eur. J. Biochem. 22, 166-172.
10. Schofield, P. and Williams, K.R. (1977) J. Biol. Chem. 252, 5584-5588.
11. Masiakowski, P. and Deutscher, M.P. (1977) FEBS Lett. 77, 261-264.
12. Thompson, S.T., Cass, K.H. and Stellwagen, E. (1975) Proc. Nat. Acad. Sci. USA 72, 669-672.
13. Wilson, J.E. (1976) Biochem. Biophys. Res. Commun. 72, 816-823.
14. De Maeyer-Guignard, J., Thang, M.N. and De Maeyer, E. (1977) Proc. Nat. Acad. Sci. USA 74, 3787-3790.
15. De Maeyer-Guignard, J. and De Maeyer, E. (1977) FEBS Lett. 80, 365-370.
16. Deutscher, M.P. (1974) Methods Enzymol. 29, 706-716.
17. Deutscher, M.P. (1972) J. Biol. Chem. 247, 459-468.
18. Deutscher, M.P. (1973) J. Biol. Chem. 248, 3108-3115.
19. Drocourt, J.-L., Thang, D.-C. and Thang, M.-N. (1978) Eur. J. Biochem. 82, 355-362.
20. Moe, J.G. and Piskiewicz, D. (1976) FEBS Lett. 72, 147-150.

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