An Improved Method for Purifying 2',5'-Oligoadenylate Synthetases*

(Received for publication, June 27, 1983)

James A. Wells†, Elizabeth A. Swyryd, and George R. Stark§
From the Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305

We describe a new, rapid, and convenient procedure for purifying 2',5'-oligoadenylate synthetases, employing precipitation with ammonium sulfate, fractionation by gel filtration, rapid binding to poly(I)-poly(C) cellulose, and elution with 0.35 M KCl. Unlike previously published methods, the procedure does not require sedimentation of the enzyme at 200,000 × g. Therefore, it is more general and more likely to succeed with synthetases extracted from a variety of cells or tissues, or from different subcellular fractions. We have purified the enzymes from two sources to apparent homogeneity, about 2500-fold from the cytoplasm of HeLa cells in 40% yield and more than 400,000-fold from the cytoplasm of rabbit reticulocytes in 25% yield. The specific activity of the HeLa enzyme is about 4 times higher than reported previously. The physical and functional properties of the pure enzymes are very similar to those reported by others for preparations of 2',5'-oligoadenylate synthetase from rabbit reticulocytes, mouse I. cells, and human HeLa cells. A new affinity matrix was prepared by linking periodate-oxidized poly(I)-poly(C) to a hydrazide derivative of finely divided cellulose. Poly(I)-poly(C) cellulose binds about twice as much synthetase as the corresponding amount of poly(I)-poly(C) paper and activates the bound enzyme about three times better.

In interferon-treated cells infected with an RNA virus, viral double-stranded RNA activates an interferon-induced enzyme which synthesizes 2',5'-linked oligoadenylates of chain length 2 to about 15 (Kerr and Brown, 1978; Martin et al., 1979; Dougherty et al., 1980; Justesen et al., 1980a, 1980b). These compounds, collectively referred to as 2-5A,1 activate a specific 2-5A-dependent endoribonuclease which hydrolyzes single-stranded regions of RNA (Sen et al., 1976; Williams et al., 1978; Baglioni et al., 1978; Vaquerio and Clemens, 1979; Slattery et al., 1979; Wreschner et al., 1981). Another enzyme degrades 2-5A specifically by cleaving the 2',5'-phosphodiester linkages (Schmidt et al., 1978).

The 2-5A system (synthetase, ribonuclease, and phosphodiesterase) may also function outside the interferon response, helping to change levels of cellular RNA during differentiation, in response to hormones or in cells that have stopped growing. 2-5A synthetase is found in a wide variety of cells and tissues (Stark et al., 1979; Shimizu and Sokawa, 1979). An increase in the level of the enzyme occurs upon withdrawal of estrogen from chick oviducts (Stark et al., 1979), with glucocorticoid treatment of lymphoblastoid cells (Krishnan and Baglioni, 1980), upon arrest of cell growth, and in certain phases of the cell cycle (Krishnan and Baglioni, 1981). The level of 2-5A synthetase in rat liver also decreases after partial hepatectomy, and the enzyme is found predominantly in nuclei of liver cells (Smekens-Etienne et al., 1983), in contrast to the cytoplasmic location most often found in other cells. In Ehrlich ascites tumor cells, a 2-5A synthetase of approximate molecular weight 20,000 to 30,000 has been found in the nuclei and a synthetase of molecular weight 85,000 to 100,000 has been found in cytoplasmic fractions (St. Laurent et al., 1983 and references therein). Also, two distinctly different mRNA fractions derived from these cells direct synthesis of enzymes of corresponding sizes in oocytes (St. Laurent et al., 1983).

To help understand more fully the roles of the 2-5A system as a part of the interferon response and elsewhere and to elucidate how the system is controlled, it will be important to have pure preparations of the component enzymes and antibodies to these enzymes. To cite just one example, we want to know how 2-5A synthetase is activated in cells that have not been infected by an RNA virus, since the only activator known at present is double-stranded RNA. An antiserum against 2-5A synthetase would be most useful in investigating this point. Human (Yang et al., 1981) and mouse (Dougherty et al., 1980) synthetases have been purified extensively from cytoplasmic fractions by a procedure in which the enzyme is first sedimented into a pellet at 200,000 × g and then purified further by ion exchange chromatography. A method employing differential sedimentation of a soluble enzyme may not be applicable to cytoplasmic fractions from other cells or from tissues, or to the nuclear synthetase, which is probably too small to sediment well. Therefore, we have developed a different and more simple scheme for purifying 2-5A synthetase in which binding to poly(I)-poly(C) cellulose is the major step.

**EXPERIMENTAL PROCEDURES**

Poly(I)-Poly(C)-cellulose—Finely divided cellulose, prepared according to Noyes and Stark (1975), was washed twice with vigorous shaking in 30 volumes of 1.2 M HCl and twice with shaking in 20 volumes of water. The product should be bright white. If any trace of blue color remains from residual Cu(OH)₂, the washes should be repeated. Activation with CNBr was carried out as described by March et al. (1974). Packed finely divided cellulose (500 mg, approximately 15 ml) was suspended in 15 ml of water and 30 ml of 2 M NaNO₃ with vigorous shaking. A solution of CNBr (2 g/ml; Sigma) in acetonitrile (1.5 ml) was added dropwise at 25 °C with vigorous mixing. The cellulose was washed three times with large volumes of 0.1 M NaHCO₃, pH 9.0. CNBr-activated cellulose can be stored for weeks at 4 °C. Aminocaproic acid methyl ester, prepared by dissolving the acid in dry methandlc HCI, was recrystallized from ethanol and stored in a desiccator. To 500 mg of packed, finely divided CNBr-activated cellulose suspended in 15 ml of 0.1 M NaHCO₃, pH 9, at 4 °C was added 1 g of the ester in 8 ml of 0.1 M NaNO₃. The mixture was stirred overnight at 4 °C.
The cellulose was washed once with 20 volumes of water, collected by centrifugation, and suspended in 15 ml of water. After adding 20 ml of 0.1 M stock solution in ethanol, 20 mM potassium acetate, pH 5.6, at 4°C. The reaction was essentially complete after 1 h. Unoxidized ATP did not react with the cellulose, and the 1 mg/ml of ATP in the same buffer, plus 0.35 M KCl. It was important to perform the elution within one day of collection.

2.5-A Synthetase Activity in Subcellular Fractions—Freshly thawed HeLa cells were grown until nearly confluent and treated overnight with 200 units/ml of human leukocyte interferon. Cells were lysed either according to Yang et al. (1981) and Graziadei and Lengyel (1975) or as described in this paper. After removing the nuclei by centrifugation at low speed, the supernatant solutions were centrifuged at 200,000 g for 45 min at 4°C, using a Beckman SW 50.1 rotor at 45,000 rpm. The pellets were resuspended overnight at 4°C in a buffer containing 200 mM potassium acetate, exactly as described by Yang et al. (1981). The suspensions were centrifuged again as above and the pellets were resuspended for 4 h at 4°C in a buffer containing 500 mM potassium acetate (Yang, et al., 1981) and centrifuged again under the same conditions. After dialyzing the solutions were mixed with equal aliquots of a single preparation of oIC-cellulose for 1 h at 25°C and assayed as described above, using [32P]ATP. The supernatant solutions remaining after adsorption of the enzyme to oIC-cellulose were checked for unbound enzyme using a second portion of oIC-cellulose; none was present in any of the samples.

2.5-A Synthetase in subcellular density gradients (20-30%) was performed in 20 mM Hepes buffer, pH 7.8, containing 0.1 M KCl, 5 mM acetate, 3 mM ATP, and 0.35 M NaF. Samples (0.5 ml) were loaded onto preformed gradients (12 ml) and centrifuged at 40,000 rpm for 46 h in a Beckman SW 40 rotor at 4°C. Sedimentation of oIC-cellulose in sucrose density gradients (0.5-1.5 M) was performed in 50 mM Tris hydrochloride buffer, pH 7.6, containing 120 mM KCl and 10 mM MgCl2, or in the same buffer containing 0.5 M KCl. Samples (0.7 ml) were loaded onto preformed gradients (12 ml) and centrifuged at 40,000 rpm for 3 h in a Beckman SW 40 rotor at 4°C.

RESULTS

Use of Poly(I)-Poly(C)-cellulose—Noyes and Stark (1975) showed that DNA reacted with a finely divided, diazotized alylamine derivative of cellulose to give a product with a high ratio of ligand to matrix in which the bound active 2.5-A synthetase was accessible for hybridization. Therefore, the use of poly(I)-poly(C) cellulose to purify and activate 2.5-A synthetase was investigated. Poly(I)-poly(C)-cellulose was attached either to diazotized paper (Stark et al., 1979) or to finely divided diazotized cellulose. A third derivative was also prepared in which peridate-oxidized poly(I)-poly(C)-cellulose was linked to finely divided cellulose through a hydrazide group (oIC-cellulose). Increasing portions of a rabbit reticulocyte extract were incubated at 25°C for 1 h with equal amounts of immobilized poly(I)-poly(C)-cellulose. Each matrix was then washed and assayed (Fig. 1). The relative slopes of the titration curves suggest that poly(I)-poly(C) linked to finely divided cellulose activates 2.5-A synthetase about 3 times better than poly(I)-poly(C) cellulose. All the synthetase was bound when amounts of extracts corre

![Fig. 1. Binding and activation of rabbit reticulocyte 2.5-A synthetase on immobilized poly(I)-poly(C).](http://www.jbc.org/)

5.1-Oligoadenylate Synthetases

The cell was washed three times as described for enzyme assays and the synthetase activity was assayed for 45 min at 0°C with 4 volumes of the same buffer, plus 0.35 M KCl. It was important to perform the elution within one day of collection.

2.5-A Synthetase Activity in Subcellular Fractions—Freshly thawed HeLa cells were grown until nearly confluent and treated overnight with 200 units/ml of human leukocyte interferon. Cells were lysed either according to Yang et al. (1981) and Graziadei and Lengyel (1975) or as described in this paper. After removing the nuclei by centrifugation at low speed, the supernatant solutions were centrifuged at 200,000 g for 45 min at 4°C, using a Beckman SW 50.1 rotor at 45,000 rpm. The pellets were resuspended overnight at 4°C in a buffer containing 200 mM potassium acetate, exactly as described by Yang et al. (1981). The suspensions were centrifuged again as above and the pellets were resuspended for 4 h at 4°C in a buffer containing 500 mM potassium acetate (Yang, et al., 1981) and centrifuged again under the same conditions. After dialyzing the solutions were mixed with equal aliquots of a single preparation of oIC-cellulose for 1 h at 25°C and assayed as described above, using [32P]ATP. The supernatant solutions remaining after adsorption of the enzyme to oIC-cellulose were checked for unbound enzyme using a second portion of oIC-cellulose; none was present in any of the samples.

Sedimentation of 2.5-A synthetase in subcellular density gradients (20-30%) was performed in 20 mM Hepes buffer, pH 7.8, containing 0.1 M KCl, 5 mM acetate, 3 mM ATP, and 0.35 M NaF. Samples (0.5 ml) were loaded onto preformed gradients (12 ml) and centrifuged at 40,000 rpm for 46 h in a Beckman SW 40 rotor at 4°C. Sedimentation of oIC-cellulose in sucrose density gradients (0.5-1.5 M) was performed in 50 mM Tris hydrochloride buffer, pH 7.6, containing 120 mM KCl and 10 mM MgCl2, or in the same buffer containing 0.5 M KCl. Samples (0.7 ml) were loaded onto preformed gradients (12 ml) and centrifuged at 40,000 rpm for 3 h in a Beckman SW 40 rotor at 4°C.

Use of Poly(I)-Poly(C)-cellulose—Noyes and Stark (1975) showed that DNA reacted with a finely divided, diazotized alylamine derivative of cellulose to give a product with a high ratio of ligand to matrix in which the bound active 2.5-A synthetase was accessible for hybridization. Therefore, the use of poly(I)-poly(C) cellulose to purify and activate 2.5-A synthetase was investigated. Poly(I)-poly(C)-cellulose was attached either to diazotized paper (Stark et al., 1979) or to finely divided diazotized cellulose. A third derivative was also prepared in which peridate-oxidized poly(I)-poly(C)-cellulose was linked to finely divided cellulose through a hydrazide group (oIC-cellulose). Increasing portions of a rabbit reticulocyte extract were incubated at 25°C for 1 h with equal amounts of immobilized poly(I)-poly(C)-cellulose. Each matrix was then washed and assayed (Fig. 1). The relative slopes of the titration curves suggest that poly(I)-poly(C) linked to finely divided cellulose activates 2.5-A synthetase about 3 times better than poly(I)-poly(C) cellulose. All the synthetase was bound when amounts of extracts corre
Purification of 2′,5′-Oligoadenylate Synthetases

Table I

Fractionation of 2-5A synthetase by sedimentation

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Hours at 200,000 x g</th>
<th>Specific activity</th>
<th>Yield of activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>S30</td>
<td>0.31</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>S200 I</td>
<td>0.04</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>S200 II</td>
<td>0.008</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>S200 III</td>
<td>0.005</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>

activities for these fractions. The recoveries of activity in the washes with 500 mM salt (fractions S200 III) were 31–37%, somewhat lower than the 66% reported by Yang et al. (1981). Interestingly, the specific activity was highest in the sample which had been sedimented for only 1 h. In an independent experiment, with sedimentation for 2 h, the yields recovered from the pellets were comparable to those shown in Table I but the majority of the activity was recovered in fraction S200 I (data not shown). We do not understand the basis for these somewhat discrepant results and we do not know whether the failure to recover more activity from the pellets is due to failure of some of the enzyme to redissolve, to inactivation, or to some other cause.

Yang et al. (1981) have termed the pellet formed after sedimenting the S30 fraction at 200,000 x g "ribosomal." In order to test more critically the possibility that 2-5A synthetase might be associated with ribosomes, we sedimented NP40 extracts of HeLa cells in sucrose density gradients (0.5–1.5 M) under conditions which favor association (0.13 M KCl) or dissociation (0.5 M KCl) of ribosomes. The profile of ultraviolet absorbancy revealed peaks of monosomes and polysomes in 0.13 M KCl and a single peak of dissociated ribosomal subunits in 0.5 M KCl. In both cases, all the synthetase activity was found at the tops of the gradients, well clear of polysomes, ribosomes, or ribosomal subunits. In both cases, more than half the activity applied to the gradient was recovered 24 h after completing the centrifugation. We conclude that the sedimentation of 2-5A synthetase into a ribosomal pellet at 200,000 x g does not reflect association of this enzyme with ribosomes or polysomes.

Purification of 2-5A Synthetase—Attempts to purify the enzyme from either HeLa or rabbit reticulocyte extracts using diethylaminoethylcellulose (DE-52) led to extensive loss of activity (Fig. 2). ATP or NAD+ at 2.5 mM stabilized the activity somewhat, and poly(I)-poly(C) stabilized it almost completely. Crude extracts from either source could be stored for at least one year at −70°C with less than 15% loss of enzyme activity. Freezing and thawing caused less than 10% loss of activity.

Because the enzyme is so unstable in the absence of poly(I)-poly(C), it was important to develop a rapid procedure for...
These fractions were incubated at 0 °C in the absence (A) or presence of cytops or nuclei. The enzyme was recovered in the void fraction in 80% yield. This protein profile was the only one which correlated with 2-5A synthetase activity.

A representative purification of 2-5A synthetase from HeLa cells is summarized in Table II. The enzyme was consistently recovered in 40% yield in 4 separate experiments. 2-5A Synthetase represents about 0.04% of the protein present in the 250 S30 postnuclear fraction from interferon-treated HeLa cells. The purification of the synthetase from rabbit reticulocytes is summarized in Table III. The ammonium sulfate step is more effective with reticulocytes than with HeLa cells because it removes most of the hemoglobin. Approximately 25% of the enzyme activity was recovered, but the amount of protein was below the limit of the assay, so only a lower estimate of -fold purification is reported. 2-5A Synthetase is more than 100 times less abundant in reticulocytes than in HeLa cells.

In the preparation from HeLa cells, the proteins eluted from oIC-cellulose with 0.35 M KCl in each fraction from the Bio-Gel column were analyzed by SDS-polyacrylamide gel electrophoresis (Fig. 3). A prominent band with an apparent molecular weight of 105,000 was revealed by staining with silver. The purity of this protein was estimated from densitometric scans of the stained gel and is noted above each track. In fractions 56 and 57, the purity exceeds 95%. The amount of protein of molecular weight 105,000 and the amount of 2-5A synthetase activity in each fraction from the Bio-Gel column are shown in Fig. 4. Essentially the same results were obtained with the reticulocyte extract, although only faint bands of protein were visible in the SDS gel (data not shown). A protein with apparent molecular weight 106,000, present at an average purity of 60% across the elution profile, was the only one which correlated with 2-5A synthetase activity.

The oIC-cellulose step alone was not sufficient to purify the HeLa enzyme. SDS-polyacrylamide gel electrophoresis gave some purification as well. (Alternatively, in some cases, it might be possible to sediment the enzyme at 200,000 × g as a first step. However, this procedure is much slower than precipitation with ammonium sulfate and may be unsuitable with enzymes from sources other than HeLa cells or reticulocytes, or with the smaller enzyme from nuclei (St. Laurent et al., 1983). The concentrated enzyme was applied to a column of Bio-Gel 1.5 M. Very little activity was lost in this step because the gel filtration could be performed in the presence of Mg-ATP. To stabilize the activity as rapidly as possible, fractions from the column were collected directly into tubes containing oIC-cellulose. Each portion of oIC-cellulose was washed with a low salt buffer (0.1 M KCl) and the 2-5A synthetase was eluted with a high salt buffer (0.35 M KCl). About 90% of the bound synthetase activity could be eluted within a few hours. However, for reasons that are not clear, much less activity was eluted when the enzyme was left on the oIC-cellulose for a few days at 4 °C. About 80% of the eluted synthetase activity could be recovered by diluting the KCl to 0.1 M and binding the enzyme again to fresh oIC-cellulose.

### Table I

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total Protein</th>
<th>Total Activity</th>
<th>Specific Activity</th>
<th>Purification</th>
<th>Yield of Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postnuclear extract</td>
<td>60</td>
<td>7.7</td>
<td>0.13</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>30–50% Ammonium</td>
<td>18</td>
<td>3.7</td>
<td>0.20</td>
<td>1.5</td>
<td>48</td>
</tr>
<tr>
<td>Bio-Gel (1.5 M)</td>
<td>5.3</td>
<td>3.0</td>
<td>0.57</td>
<td>4.4</td>
<td>40</td>
</tr>
<tr>
<td>oIC-cellulose</td>
<td>0.009</td>
<td>3.0</td>
<td>332</td>
<td>2570</td>
<td>40</td>
</tr>
</tbody>
</table>

* Determined according to Bradford (1976).
Fig. 3. SDS-polyacrylamide gel electrophoresis of purified HeLa 2-5A synthetase (Table II). The proteins released from oIC-cellulose in each fraction from the Bio-Gel column were precipitated quantitatively by adding deoxycholate (60 \( \mu \)g/ml) and 6% trichloroacetic acid (Rensadoun and Weinstein, 1976). The precipitate was washed with acetone, dried, and dissolved in buffer for gel electrophoresis. The proteins were separated by electrophoresis in 8% polyacrylamide gels (Laemmli, 1970) and stained with silver (Oakley et al., 1980). Purity was estimated from densitometric scans of the gels.

Fig. 4. Coincidence of a protein with molecular weight 105,000 and 2-5A synthetase activity on a Bio-Gel column. The relative intensities of the protein bands are shown in Fig. 3. Assays were performed with [\( \gamma \text{-}^{32} \text{P} \)]ATP. The void (V\text{O}) and included volumes (V\text{r}) of the Bio-Gel column are indicated.

Table IV

<table>
<thead>
<tr>
<th>Source of enzyme</th>
<th>Modifying reagent</th>
<th>Competitor</th>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit reticulocytes</td>
<td>2-5A (0.5 mM)</td>
<td>None</td>
<td>35 min</td>
<td>16 %</td>
</tr>
<tr>
<td>2-5A (0.5 mM)</td>
<td>None</td>
<td>30 35</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>oATP (0.5 mM)</td>
<td>None</td>
<td>30 35</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>oATP (0.5 mM)</td>
<td>ATP (1 mM)</td>
<td>30 35</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>oATP (0.5 mM)</td>
<td>ATP (5 mM)</td>
<td>30 35</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>oATP (5 mM)</td>
<td>None</td>
<td>30 35</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>oAMP (0.5 mM)</td>
<td>ATP (10 mM)</td>
<td>None</td>
<td>30 51</td>
<td></td>
</tr>
</tbody>
</table>

HeLa cells

| N-Ethylmaleimide (1 mM) | None | 90 4 |
| NaHSO\text{O} \text{3} (10 mM) | None | 180 90 |
| NaHSO\text{O} \text{4} (10 mM) + CuCl\text{2} (0.1 mM) | None | 180 <1 |
| Diethylpyrocarbonate (1.5 mM) | None | 60 92 |
| Diethylpyrocarbonate (0.7-15 mM) | None | 60 >95 |

* Added to HeLa extract plus oIC-cellulose. After 1 h at 23 °C, the cellulose was washed and assayed.

Chemical and Enzymatic Properties of the Purified Enzyme—The effects of treating 2-5A synthetase bound to oIC-cellulose with some modifying reagents are summarized in Table IV. Since dialdehyde analogs of 2-5A are effective as affinity reagents for the 2-5A-dependent endonuclease (Wreschner et al., 1982), they were evaluated with 2-5A synthetase, using the reticulocyte enzyme. Although both periodate-oxidized ATP and periodate-oxidized 2-5A inactivated the enzyme, loss of activity was blocked only marginally by adding either ATP or 2-5A. Furthermore, periodate-oxidized AMP and even periodate-oxidized glycerol also caused inactivation, indicating that reaction at sites remote from the active site leads to loss of activity indirectly. Various commercial preparations of glycerol also caused loss of enzyme activity, even without periodate oxidation, probably due to aldehyde impurities. As shown in Table IV, 2-5A synthetase was inactivated by N-ethylmaleimide or by CuCl\text{2} plus NaHSO\text{3}, reagents which alkylate or oxidize sulfhydryl groups. The enzyme was not inhibited by diethylpyrocarbonate or 2-mercaptoethanol.

High performance liquid chromatography was used to determine the rate of formation of each 2-5A oligomer by purified HeLa synthetase. Typical profiles of the oligomers derived from [\( \gamma \text{-}^{32} \text{P} \)]ATP are shown in Fig. 5. Peak assignments were made by comparing the ultraviolet absorbancy (amount of adenylylate) with amount of radioactivity (amount of 5'-terminal triphosphate) in each peak. More than 98% of the radioactivity was recovered from the column and more than 95% was found in peaks which also absorbed in the ultraviolet. [\( ^{32} \text{P} \)]Pyrophosphate was eluted in the void volume, and the extent of [\( \gamma \text{-}^{32} \text{P} \)]ATP cleavage calculated from the amount of radioactivity in this part of the chromatogram agreed to within a few per cent with the value determined by the Norite adsorption assay. Only [\( ^{32} \text{P} \)]pyrophosphate and no...
mixtures were quenched with EDTA and applied to Waters (2-18 Bondapak columns. The samples were eluted in 0.1 M ammonium phosphate, pH 7.0, with a linear gradient of 0-7.5% methanol at 0.2 unit for full scale deflection. The enzyme at 200,000 × g (Yang et al., 1981). Cytoplasmic synthetases from cells of species other than those tested or from nuclear origin (St. Laurent et al., 1983) might be difficult to purify if they did not sediment well. The major purification step developed here (i.e. binding to poly(I)-poly(C)-cellulose), should apply generally to 2-5A synthetases.

Poly(I)-poly(C)-cellulose activates bound 2-5A synthetase 3 times better than poly(I)-poly(C) paper and has twice the capacity per equivalent of poly(I)-poly(C). These improved properties may be due to a smaller number of attachment sites to cellulose and also to the greater accessibility of the poly(I)-poly(C) on this finely divided support. Endogenous RNase activity in crude extracts could remove poly(I)-poly(C) and this activity was enhanced by synthesis of a 2-5A from endogenous ATP, with consequent activation of the 2-5A-dependent endonuclease. These problems were solved by inactivating nucleases with diethylpyrocarbonate and by preventing the synthesis of 2-5A by using NAD⁺. It is interesting that EDTA does not inhibit binding of synthetase to poly(I)-poly(C)-cellulose, suggesting that metal ions are not required for this step. However, the enzyme does require Mg²⁺ for catalysis. Because diethylpyrocarbonate also reduces the activity of phosphatases bound to oIC-cellulose from crude extracts, it was possible to assay the synthetase from such extracts by monitoring conversion of [γ-³²P]ATP to [³²P]pyrophosphate using Norite, greatly simplifying the task of performing a large number of assays.

2-5A Synthetase activity from either rabbit reticulocytes or HeLa cells was reasonably stable in crude extracts but lost activity rapidly after passage over diethylaminoethyl-cellulose. We were not able to recover a substantial fraction of enzyme activity in attempts to purify the synthetase directly from postnuclear supernatant solutions by chromatography on diethylaminoethyl- or carboxymethyl-cellulose. The stabilizing factor(s) removed by such chromatography are probably ATP and NAD⁺. In the purification scheme employed, the enzyme is stabilized rapidly by binding to oIC-cellulose, resulting in highly purified enzyme in high yield, either from interferon-treated HeLa cells or from rabbit reticulocytes. A similar procedure was developed by Justesen et al. (1980b) for the rabbit reticulocyte enzyme, using poly(I)-poly(C) agarose.
Although these workers reported no evidence of enzyme purity, in our hands, their procedure gave a preparation less than 10% pure, as judged by SDS-polyacrylamide gel electrophoresis.

Lengyel and co-workers have reported purification of 2-5A synthetase from interferon-treated mouse L cells (Dougherty et al., 1980) and HeLa cells (Yang et al., 1981). In order to compare our results with the previously published data for human 2-5A synthetase, we had to normalize the assays, since the conditions used were quite different (1 mM ATP and incubation at 37 °C by Yang et al., 1981), 5 mM ATP and at 37 °C in the current work). The assays were compared using purified HeLa enzyme, both in solution with soluble poly(I)-poly(C) and on OIC-cellulose. In both cases, the absolute rate was 2.5 times higher using 5 mM ATP and 37 °C. In the purification shown in Table II, we began with an S30 supernatant solution containing 60 mg of protein and a total activity of 7.7 µmol of ATP/h (0.13 units/mg). In other experiments of ours, this value has been as low as 0.1, but it is typically 0.3 or more (see Table I). The differences probably reflect differences in the state of the cells, as noted earlier. In the purification cited by Yang et al. (1981), 1600 mg of protein were present in the S30 fraction and the total activity was 12 units (corrected to our assay conditions), or 0.0075 units/mg. Yang et al. (1981) used mouse interferon to induce the HeLa synthetase and they stated that this treatment was 3 times less effective than when homologous human interferon was used. The final specific activity shown in Table II is 332 µmol of ATP/h/mg of protein, which may be compared with the value 86 calculated from the data of Yang et al. (1981) by correcting for the difference in assay conditions. Since Yang et al. (1981) presented good evidence that the enzyme they prepared was nearly homogeneous, we conclude that some activity was probably lost during purification.

The physical and kinetic properties of 2-5A synthetase preparations from rabbit reticulocytes, HeLa cells, and mouse cells as compared in Table V. Yang et al. (1981) showed by co-electrophoresis that the HeLa and mouse enzymes have slightly different mobilities in SDS gels. Whether the HeLa enzyme isolated by their procedure has the same molecular weight as the enzyme we have isolated has not been determined. An estimate of the molecular weight of the native protein can be made from the sedimentation coefficient and the Stokes radius (Siegel and Monty, 1966). The value obtained, 87,000 for the HeLa enzyme, is consistent with a monomeric native enzyme. Enzymes from all the sources studied have high apparent Kₐ values for ATP. Since the enzyme must have both donor and acceptor sites for ATP, the apparent Kₐ is probably a property of the site with lower affinity.

Justesen et al. (1980a and b) studied the mechanism of partially purified rabbit reticulocyte 2-5A synthetase by characterizing the oligoadenylates formed from [α-³²P]ATP, using thin layer chromatography. Their results and interpretations are very similar to those presented here on the basis of experiments with highly purified HeLa 2-5A synthetase, assayed with [γ-³²P]ATP and high performance liquid chromatography. Both enzymes appear to have distributive rather than processive mechanisms; an oligoadenylate of length n is produced by condensation of ATP with an oligoadenylate of length n - 1, the products then dissociate and the oligoadenylate binds again to be elongated further. Although the physical and mechanistic properties of the rabbit and the HeLa enzymes are very similar, we have observed one interesting difference. The profile of oligoadenylates produced by the two purified synthetases when the same fraction of ATP has been used up (about 78%) revealed that the larger oligoadenylates (pentamer to decamer) were more predominant in the products formed by the reticulocyte enzyme (data not shown). This observation may have some biological significance since Williams et al. (1979) have reported that oligoadenylates shorter than the tetramer triphosphate do not activate the 2-5A-dependent endonuclease from rabbit reticulocytes, whereas the corresponding enzyme from HeLa cells is...
activated by trimer triphosphate as well as by higher oligomers.

Acknowledgments—We thank Barbara Hill for skillful assistance with tissue culture experiments, Carol Hersh, Richard Friedman, Earl Shelton, and Claiborne Glover for helpful advice, Ian Kerr for HeLa cells and critical comments on the manuscript, and Kari Cantell and Genentech, Inc. for samples of human interferon.

REFERENCES


An improved method for purifying 2',5'-oligoadenylate synthetases.
J A Wells, E A Swyryd and G R Stark


Access the most updated version of this article at http://www.jbc.org/content/259/2/1363

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/259/2/1363.full.html#ref-list-1