Purification and Characterization of a Nuclear Protein Kinase from Rat Liver and a Hepatoma That Is Capable of Activating Poly(A) Polymerase*

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The cyclic nucleotide-independent protein kinase which is separated from poly(A) polymerase during its purification from nuclei of rat liver and Morris hepatoma 3924A was purified essentially to homogeneity. Liver nuclear poly(A) polymerase was dissociated from protein kinase by phosphocellulose column chromatography. In contrast, protein kinase copurified with the hepatoma poly(A) polymerase on the phosphocellulose column. Neither liver nor hepatoma kinase was stimulated by spermine or inhibited by heparin. These enzymes did not utilize GTP as phosphoryl donor, or histones or tyrosine-containing [Val-1]-angiotensin II as phosphoryl acceptors. The apparent $K_m$ with respect to ATP was similar for the liver (4.7 $\mu$M) and hepatoma (11 $\mu$M) kinases, and the apparent $K_m$ with respect to casein was identical (0.6 $\mu$g/µl) for these enzymes. Both enzymes were capable of phosphorylating poly(A) polymerase and stimulating both tumor and liver poly(A) polymerase activity. However, in addition to their different chromatographic properties, the two kinases differed in molecular weight (liver, 37,000; hepatoma, 56,000), in their response to various divalent metal ions, and in their ability to phosphorylate hepatoma poly(A) polymerase ($K_m$ 7.9 and 30 $\mu$g/µl for liver and hepatoma enzymes, respectively). These latter characteristics distinguished the liver and hepatoma protein kinases from each other as well as from the previously described NI protein kinase.

The phosphorylation of nonhistone nuclear proteins has been implicated as a regulatory mechanism for transcription in eucaryotic cells (Allfrey et al., 1973; Kleinsmith, 1975). Further, the association of protein kinase activities with nuclear and cytoplasmic messenger ribonucleoprotein particles (Egely et al., 1976; Blanchard et al., 1977) suggests that phosphorylation may also have a role in the control of posttranscriptional events. Two major cyclic nucleotide-independent nuclear protein kinases, designated NI and NII, have been described (Desjardins et al., 1972). Protein kinase NI has been purified to homogeneity from rat liver (Thornburg et al., 1978) and porcine liver (Baydoun et al., 1982). Protein kinase NII has been purified from normal rat liver (Thornburg and Lindell, 1977) and a rat hepatoma (Rose et al., 1981a). Although protein kinases NI and NII resemble the cytoplasmic casein kinases I and II, respectively, a structural and/or functional relationship between the nuclear and extranuclear kinases has not been established. (For recent reviews of this subject see Rose and Jacob (1984) and Hathaway and Traugh (1982).) The existence of additional nuclear protein kinases has been reported (Thornburg et al., 1979), but these enzymes have not been characterized.

Because both NI and NII protein kinases are fully capable of phosphorylating protein substrates such as casein and phosvitin, neither of which are nuclear proteins, criterion other than mere phosphorylation must be used to identify physiological substrates. Our laboratory has extensively purified protein kinase NII from a rat hepatoma (Rose et al., 1981a) and has demonstrated its ability to phosphorylate RNA polymerases I (Rose et al., 1981b) and II (Rose et al., 1983) with concomitant stimulation of RNA synthesis (Duchen et al., 1981; Rose et al., 1983). In an analogous situation, an NI-type protein kinase, partially purified from the same hepatoma, was capable of phosphorylating and activating poly(A) polymerase (Rose and Jacob, 1979). These effects on polymerase activities strongly indicate that these polymerases are natural substrates for the respective kinases.

Poly(A) polymerase is initially associated with protein kinase activity during its purification from isolated nuclei (Rose and Jacob, 1979). In the course of this purification, we noted that the chromatographic profile of the hepatoma poly(A) polymerase-associated protein kinase was different from that of the liver enzyme. It was, therefore, of considerable interest to investigate whether the hepatoma protein kinase was distinct from the liver enzyme and, if so, whether they have distinct functions. As the first step toward achieving this goal, we have purified the protein kinase from hepatoma following its dissociation from poly(A) polymerase and have compared it to the enzyme from normal liver.

**EXPERIMENTAL PROCEDURES**

**RESULTS**

Purification of a Poly(A) Polymerase-associated Protein Kinase—The purification scheme for the isolation of the poly(A) polymerase-associated kinase is described in detail under

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* Portions of this paper (including "Experimental Procedures" and Figs. 1-3) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 83M-3076, cite the authors, and include a check or money order for $2.00 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
Purification of poly(A) polymerase-associated protein kinase from rat liver and Morris hepatoma 3924A

Nuclear extracts from 260 g of hepatoma or liver were subjected to chromatography, and kinase activity was determined as described under "Experimental Procedures." Protein concentration was determined by the method of Schaffner and Weissman (1973) except at the last stage of purification. Protein obtained after ATP-Sepharose chromatography was estimated by silver staining after polyacrylamide gel electrophoresis as described under "Experimental Procedures." Because determination of the protein concentration of highly purified liver protein kinase was not technically feasible (see legend to Fig. 5), the values for this enzyme were conservatively estimated from the limit of sensitivity of the silver stain.

Table I

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total protein</th>
<th>Total activity</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hepatoma</td>
<td>Liver</td>
<td>Hepatoma</td>
</tr>
<tr>
<td></td>
<td>mg</td>
<td>units</td>
<td>units/mg protein</td>
</tr>
<tr>
<td>DEAE</td>
<td>600.0</td>
<td>1040.0</td>
<td>1.7</td>
</tr>
<tr>
<td>QAE</td>
<td>457.0</td>
<td>2027.0</td>
<td>4.6</td>
</tr>
<tr>
<td>Phosphocellulose</td>
<td>120.4</td>
<td>282.0</td>
<td>2.3</td>
</tr>
<tr>
<td>Hydroxypatite</td>
<td>6.2</td>
<td>100.9</td>
<td>16.3</td>
</tr>
<tr>
<td>Casein-Sepharose</td>
<td>0.4</td>
<td>71.9</td>
<td>171</td>
</tr>
<tr>
<td>ATP-Sepharose</td>
<td>0.0023</td>
<td>85.5</td>
<td>37,000</td>
</tr>
<tr>
<td></td>
<td>(&lt;0.0005)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

"Experimental Procedures" in the Miniprint Section provided at the end of this report. Briefly, the purification involved sequential chromatography of nuclear extracts on DEAE-Sephadex, QAE (quaternary aminoethyl)-Sephadex, and phosphocellulose columns. Liver poly(A) polymerase was separated from protein kinase by phosphocellulose chromatography (Fig. 1, panel A); the polymerase eluted from the column at 0.23 (major species) and 0.33 M (minor species) KCl and the kinase at 0.5 M. In contrast, kinase activity remained closely associated with hepatoma poly(A) polymerase; both kinase and poly(A) polymerase eluted from the phosphocellulose column at a salt concentration of about 0.33 M (Fig. 1, panel B). The apparent low recovery of the hepatoma enzyme from this column was primarily due to the fact that only the protein kinase activity associated with the peak of poly(A) polymerase activity was subjected to further purification (see Fig. 1). A recovery of approximately 40% could have been achieved if the entire fractions containing the protein kinase activity had been pooled. A partial separation of hepatoma poly(A) polymerase and the kinase was achieved in some experiments by phosphocellulose chromatography, but the majority of the kinase was dissociated from poly(A) polymerase only by subsequent chromatography on a hydroxypatite column; the kinase eluted in the wash fractions while poly(A) polymerase was bound to the column and eluted at 0.2 M salt. The tumor protein kinase was then subjected to further purification by sequential chromatography on casein-Sepharose (Fig. 2) and ATP-Sepharose (Fig. 3) affinity columns.

The liver protein kinase was subjected to the same procedure with similar results (not shown). A summary of the purifications is given in Table I. Over 12,000- and 20,000-fold purifications were achieved with yields of 0.5- and 2-3 μg of protein from 200–300 g of liver and hepatoma tissue, respectively. The specific activities of pure liver and hepatoma enzyme were over 100,000 and 30,000 nmol/mg of protein/30 min at 30 °C, respectively.

Molecular-weight Determination—The native molecular weights of the hepatoma and liver kinases were approximately 56,000 and 37,000, respectively, as determined by chromatography on Sephacryl S-200 (Fig. 4). One major protein band with a molecular weight of 56,000 was detected when the hepatoma enzyme was subjected to polyacrylamide gel electrophoresis under denaturing conditions (Fig. 5). It was, therefore, concluded that the hepatoma enzyme is monomeric, which distinguishes this enzyme from the NI kinase described by Thornburg et al. (1978). Due to the limited quantity of highly purified liver kinase, it was technically difficult to obtain a protein band on a polyacrylamide gel even by using the silver stain. Nevertheless, the absence of a band in the track containing the liver kinase at the position of a protein of M, 56,000 (not shown) demonstrated that the band observed in Track B (hepatoma enzyme) was not merely an artifact of the gel system (see legend to Fig. 5).

Characteristics of the Kinase Reaction—As for most casein kinases, magnesium stimulated both kinase activities, with optimum activity being expressed at magnesium concentrations of 5 mM. The ability of the two enzymes to utilize other divalent cations in place of magnesium distinguished them from each other (Table II). Specifically, with casein as substrate, the liver protein kinase was stimulated by molybdate to an extent even greater than that observed with magnesium; molybdate had no effect on the hepatoma enzyme. Conversely, manganese and cobalt stimulated the hepatoma kinase but were not utilized by the liver enzyme. The ability of both kinases to utilize calcium distinguished them from the major liver NI kinase (Thornburg et al., 1979) and the NII kinase of hepatoma (Rose et al., 1981a). The inability of manganese to stimulate the liver kinase further differentiated this enzyme from the major NI kinase. Interestingly, the ability of several
Protein Kinases Capable of Activating Poly(A) Polymerase

**TABLE II**

<table>
<thead>
<tr>
<th>Metal ion</th>
<th>Hepatoma*</th>
<th>Liver*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein kinase activity</td>
<td></td>
</tr>
<tr>
<td>Mg2+</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Ag+</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Sn4+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Zn2+</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Mn2+</td>
<td>18</td>
<td>33</td>
</tr>
<tr>
<td>Cu2+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Co2+</td>
<td>24</td>
<td>11</td>
</tr>
<tr>
<td>Mo2+</td>
<td>0</td>
<td>120</td>
</tr>
<tr>
<td>Ca2+</td>
<td>13</td>
<td>40</td>
</tr>
</tbody>
</table>

* Source of kinase.

**TABLE III**

<table>
<thead>
<tr>
<th>Protein substrate</th>
<th>Hepatoma protein kinase</th>
<th>Liver protein kinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Poly(A) polymerase</td>
<td>30.0</td>
<td>7.9</td>
</tr>
</tbody>
</table>

**TABLE IV**

<table>
<thead>
<tr>
<th>Source of poly(A) polymerase</th>
<th>Addition</th>
<th>Poly(A) polymerase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>None</td>
<td>16 ± 0.5</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>Hepatoma protein kinase</td>
<td>258 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>Liver protein kinase</td>
<td>233 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>49 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>Hepatoma protein kinase</td>
<td>148 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>Liver protein kinase</td>
<td>97 ± 0.9</td>
</tr>
</tbody>
</table>

Finally, the substrate saturation curves for the protein kinases were determined by measuring the reaction velocities at different ATP concentrations. The $K_m$ for ATP as determined by double-reciprocal plot was 11 and 4.7 μM for the hepatoma and liver enzymes, respectively. These are similar to the value for NI kinase from porcine liver (7 μM) (Baydoun et al., 1982) but are significantly less than that reported for the major NI kinase from rat liver nuclei (50 μM) (Thornburg et al., 1979). As is typical for NI-type casein kinases, the enzymes described in the present studies did not utilize GTP.
as a phosphate donor (data not shown).  

**Reaction with Poly(A) Polymerase**—Since the kinases described in this report copurify with poly(A) polymerase during the early stages, it would seem reasonable to expect that the polymerase would be a natural substrate for the kinases. As indicated in Table III, both the hepatoma and liver kinases could phosphorylate poly(A) polymerase. The \( K_a \) values with respect to hepatoma poly(A) polymerase as phosphoryl acceptor were rather high for both kinases compared to that with casein substrate. However, both kinases were capable of stimulating poly(A) polymerase activity 2–16-fold (Table IV) which indicates that phosphorylation of the polymerase by these kinases is of biological significance.

**DISCUSSION**

The present studies have described the purification and characterization of a nuclear protein kinase from a rat hepatoma and normal rat liver. A unique feature of these enzymes is their association with poly(A) polymerase prior to extensive purification. The higher \( K_a \) with respect to poly(A) polymerase relative to casein as phosphoryl acceptor could be due to a limited number of sites available for phosphorylation on each molecule of poly(A) polymerase. In addition, the poly(A) polymerase had most likely been phosphorylated to some extent in vivo, which would render it a relatively poor substrate for exogenous protein kinase. Hence, despite the relatively higher \( K_a \), poly(A) polymerase could conceivably be a physiological substrate for these kinases. This contention is substantiated by the observations that (a) the hepatoma kinase does not phosphorylate RNA polymerases I and II (Ducman et al., 1981; Rose et al., 1983) and (b) both kinases activate poly(A) polymerase (Table IV) which leads to an increased rate of polyadenylation in vitro (Rose and Jacob, 1980).

Although the protein kinases described here resemble in some respects the major protein kinase NI purified from rat liver (Thornburg et al., 1978; Thornburg et al., 1979) and porcine liver (Baydoun et al., 1982), they do not appear to be identical to this enzyme. The protocol used by other investigators who have purified protein kinase NI to homogeneity does not include phosphocellulose chromatography, which makes comparison difficult. However, there has been one report (Yutani et al., 1982) where rat liver protein kinase NI was subjected to DEAE-Sephadex followed by phosphocellulose chromatography. In this case, the NI kinase was eluted at the equivalent of 0.22 M KCl, a significantly lower salt concentration than that required in the present study for elution of the hepatoma (0.33 M KCl) and liver (0.5 M KCl) kinases. Further, other investigators used a different nuclear extraction technique and the kinase activity eluted from a DEAE-Sephadex column with 40–50 mM ammonium sulfate as the starting material for purification. In the present study, poly(A) polymerase and associated protein kinase activity were eluted from DEAE-Sephadex with 10 mM ammonium sulfate. This serves to separate poly(A) polymerase from RNA polymerase I; a small fraction of the latter enzyme is eluted with 50 mM salt. Subsequent elution of the column with 50 mM salt results in the release of additional protein kinase activity with NI-type characteristics (data not shown). Whether this enzyme is the NI protein kinase described by others is not known. Regardless, the two protein kinases purified here differ from the NI kinase in molecular weight and in response to various metal ions. Nevertheless, these enzymes are related to NI kinase in that they are eluted from DEAE-Sephadex with relatively low salt concentrations, are not sensitive to heparin, and are not significantly activated by polyamines. The liver enzyme characterized in the present study is most likely one of the other protein kinases noted by Thornburg et al. (1979) during purification of NI from this tissue.

Despite some similarities between the hepatoma and liver kinases, they appear to be different enzymes. Thus, whereas the liver kinase is always separated from poly(A) polymerase by chromatography on phosphocellulose, the peak of hepatoma kinase elutes with the major poly(A) polymerase at 0.33 M salt and is separated from the polymerase only after subsequent chromatography on hydroxylapatite. The liver and hepatoma kinases also differ in their native molecular weights (37,000 and 56,000, respectively), in their affinity for hepatoma poly(A) polymerase (\( K_a \), 7.9 and 30 \( \mu \)g/\( \mu \)l, respectively), and in their response to various ions. Whether these latter differences are significant with respect to the role of these enzymes in the polyadenylation reaction is under investigation.

The purity of these kinases deserves comment. Due to the relatively small amount of the purified enzymes, the hepatoma protein could be visualized in polyacrylamide gels only by silver staining following concentration of the enzyme. Using this technique, only one protein band was evident which indicates a high degree of purity. The liver enzyme protein could not be detected even with this highly sensitive technique. ATP-Sepharose chromatography, the last step of purification, was essential to achieve this level of purity. In fact, this step alone provided more than a 200-fold purification.

In every respect, the protein kinases purified in the present study differ from protein kinase NII, a major nuclear protein kinase purified from the same tumor (Rose et al., 1981a). Thus, the latter enzyme utilizes both ATP and GTP, is stimulated by physiological concentrations of spermine or spermidine, and is inhibited by very low concentrations of heparin (Thornburg et al., 1979; Rose et al., 1981a). Protein kinase NII exhibits a molecular weight of 140,000 consisting of two polypeptides (M, = 42,000 and 24,000) in equimolar amounts. In addition, unlike protein kinase NII, which phosphorylates and dramatically activates RNA polymerase I (Ducman et al., 1981), the hepatoma kinase purified in the present studies had a relatively small effect on this polymerase (Ducman et al., 1981). Conversely, poly(A) polymerase is relatively unaffected by protein kinase NII (data not shown).

Finally, although the exact physiological role of these kinases has not been elucidated, their ability to phosphorylate and activate poly(A) polymerase has important functional implications. Whether phosphorylation of poly(A) polymerase also plays a key role in regulating polyadenylation in vivo and whether the differences in the liver and tumor kinase are significant with respect to neoplasia remain to be elucidated.

**Acknowledgments**—We are grateful to Yohan Park for excellent technical assistance.

**REFERENCES**


Protein Kinases Capable of Activating Poly(A) Polymerase

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Experimenal Procedures

Materials

The proteins (long chain), enzymes (essential protease, protein and chloramphenicol) and (enzymes) (35S, and 32P) were obtained from Sigma Chemical Company, 30, Ind., Ml. 35S-Sepharose (7.5 ml, 3.0 ml, and 1.5 ml), were obtained from the same company (Arthurton, England). Sepharose and diethyldithiothreitol were purchased from Calbiochem (La Jolla, CA.) and high molecular weight agarose-polyacrylamide) were obtained from Bio-Rad Laboratories (Richmond, CA.)

The following buffers were used during the purification: Buffer A: 50 mM Tris-Cl (pH 7.5), 100 mM NaCl, 5 mM MgCl2, 0.1% BSA. 20 mM GDP, 5 mM dithiothreitol, 0.1% BSA. Buffer C: 10 mM Tris-Cl, 100 mM NaCl, 5 mM MgCl2, 0.1% BSA. Buffer D: 10 mM Tris-Cl, 100 mM NaCl, 5 mM dithiothreitol, 0.1% BSA. dithiothreitol, 0.1% BSA. Buffer D: 10 mM Tris-Cl, 100 mM NaCl, 5 mM dithiothreitol, 0.1% BSA. Buffer D: 10 mM Tris-Cl, 100 mM NaCl, 5 mM dithiothreitol, 0.1% BSA.

Extraction and Initial Purification of the Enzyme

Poly(A) polymerase was purified from tissue isolated from liver and liver nuclei purified using a protocol described previously (Hathaway and Jacob, 1982). Rose et al. (1981). The initial stage of protein kinase purification was performed essentially as previously described for poly(A) polymerase. Briefly, nuclear extracts were applied to a 35S-Sepharose column (3.0 ml) and proteinase K was added to the mixture. The column was washed with 2 x 5 ml volumes of the same buffer, and fractions were collected. The protein kinase activity was pooled and negatively charged applied to a 35S-Sepharose column. The 35S-Sepharose column (1.0 ml of 10/10) was eluted with buffer A containing 20 mM Cl and fractions containing poly(A) polymerase activity were pooled. The enzyme was eluted from both 35S-Sepharose and 35S-Sepharose columns.

Phosphatase-Chromatography

In summary, the 35S-Sepharose fractions were applied to a phosphatase column (0.5 ml) containing 35S-Sepharose. The column was washed with 2 x 5 ml volumes of buffer B containing 10 mM Cl, a linear gradient from 10 mM to 100 mM Cl in buffer B was applied and 3.0 ml fractions collected. Fractions were assayed for poly(A) polymerase and kinase activity. Enzyme units given in Figure 1 have been standardized to that reported for 10% of liver at optimum concentrations for protein kinase activity. Enzyme units given in Figure 1 have been standardized to that reported for 10% of liver at optimum concentrations for protein kinase activity.

Other Methods

Separation-200 Chromatography

Figure 2. Chromatography of protein kinase on phosphorylase b.

Figure 3. Chromatography of protein kinase on phosphorylase b.

Figure 4. Chromatography of protein kinase on phosphorylase b.

Figure 5. Chromatography of protein kinase on phosphorylase b.

Figure 6. Chromatography of protein kinase on phosphorylase b.

Figure 7. Chromatography of protein kinase on phosphorylase b.