(K_m 6.0 mM). Both ATP and Mg^{2+} were inhibitory above their respective optimal concentrations of 2 and 10 mM. Citrate activated the enzyme 10-fold at a concentration of 10 mM; preincubation was not necessary for full activation.

During the determination of optimal substrate concentrations it was observed that preincubation of the enzyme with ATP/Mg^{2+} in the absence of acetyl-CoA resulted in a marked inactivation of the enzyme. Incubation with 2 mM-ATP for 1 h brought about a loss of 80% of the initial activity. No loss of activity was observed on incubation in the absence of ATP. Similar results were reported by Carlson & Kim (1974a) using a partially purified acetyl-CoA carboxylase from rat liver. A series of experiments, essentially similar to those described by these workers, was performed to investigate the inactivation phenomenon further. Samples of the enzyme preparation were incubated at 37°C in the presence and absence of ATP, as described in the legend to Fig. 1. Over a 1 h period, enzyme activity in the incubation containing ATP decreased to less than 20% of the original. In the absence of ATP no inactivation was observed. Binding of radioactivity from [y-32P]ATP increased throughout incubation, whereas very little 32P was acid-precipitable when the incubation mixture contained heat-inactivated enzyme. Very little binding of radioactivity from [U-14C]ATP was noted. It would appear that inactivation of the enzyme is associated with the phosphorylation of one or more components of the enzyme preparation.

In further experiments, 32P-labelled proteins were isolated by (NH_4)_2SO_4 precipitation after incubation of the enzyme preparation with [y-32P]ATP. The labelled proteins were redissolved in appropriate buffers and subjected to chromatography on columns of Sephadex G-25, DEAE-cellulose and Sepharose 6B. In each case, 32P radioactivity was eluted coincident with acetyl-CoA carboxylase activity.

These results strongly imply the presence in our partially purified enzyme preparation of a protein kinase that catalyses the phosphorylation, and consequent inactivation, of acetyl-CoA carboxylase. Although the occurrence of such a kinase remains to be more exhaustively confirmed, and the existence of an appropriately specific protein phosphatases in extracts of bovine mammary tissue has not yet been demonstrated, our findings are consistent with the phosphorylation–dephosphorylation cycle proposed by Carlson & Kim (1974b) for the regulation of rat liver acetyl-CoA carboxylase activity in vivo. Such mechanisms are well established for other enzyme systems (Cohen, 1976) and serve to render the enzyme open to control by hormone-sensitive kinases and phosphatases.

We are grateful to the Agricultural Research Council for support.


Calf Thymus Ribonucleotide Reductase: Purification and Properties

S. ERIKSSON, M. ÅKERMAN and L. THELANDER

Medical Nobel Institute, Department of Biochemistry I, Karolinska Institute,
S-104 01 Stockholm, Sweden

Ribonucleotide reductase, the enzyme responsible for the production of the deoxyribonucleotides, has been isolated and carefully studied in Escherichia coli and Lactobacillus leichmannii (Hogenkamp & Sando, 1974). Ribonucleotide reductase from regenerating rat liver (Larsson, 1969), Novikoff hepatoma cells (Moore & Hurlbert, 1966) and rabbit bone marrow (Hopper, 1972) has also been studied, but only after partial purification.
Table 1. Purification of calf thymus ribonucleotide reductase.

Data refer to 1 kg of thymus. A unit of enzyme activity is defined as 1 nmol of dCDP formed/30 min at 25°C under conditions described by Larsson (1969).

<table>
<thead>
<tr>
<th>Protein (mg)</th>
<th>Total activity (units)</th>
<th>Specific activity (units/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>23000</td>
<td>2300</td>
</tr>
<tr>
<td>Streptomycin supernatant</td>
<td>21000</td>
<td>3700</td>
</tr>
<tr>
<td>(NH₄)₂SO₄ ppt.</td>
<td>6400</td>
<td>2900</td>
</tr>
<tr>
<td>DEAE-cellulose</td>
<td>950</td>
<td>2200</td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>300</td>
<td>1900</td>
</tr>
<tr>
<td>dATP-Sepharose</td>
<td>3</td>
<td>1200*</td>
</tr>
</tbody>
</table>

* Assayed in the presence of 'stimulating factor'.

A major problem has been the low activity of enzyme found in eukaryotic cells. We have chosen to use calf thymus as a source for the purification of ribonucleotide reductase, since it contains a large number of proliferating cells and it is easily available in large quantities.

Thymuses from young animals were purchased frozen, and after thawing were homogenized in a Waring blender in 0.05 M-Tris/HCl, pH 7.6 (1 kg of tissue/3 litres of buffer). After centrifugation (2.10 g for 30 min) the nucleic acids were removed from the suspension with streptomycin sulphate (0.5 %) and then proteins were precipitated with (NH₄)₂SO₄ (0-40 % satn.). The proteins were dissolved and dialysed overnight against 0.01 M-Tris/HCl, pH 7.6, and applied to a DEAE-cellulose column (6 cm x 23 cm). A gradient of KCl from 0 to 0.25 M in the same buffer was used and the ribonucleotide reductase activity was eluted in the fractions between 0.1 and 0.18 M-KCl. These fractions were directly applied to a hydroxapatite column (4 cm x 23 cm), which was eluted with 0.001-0.2 M-potassium phosphate buffer, pH 7.0. The active fractions, around 0.09 M-potassium phosphate, were concentrated by precipitation with (NH₄)₂SO₄ (80 % satn.), followed by passage through a Sephadex G-25 column (2.5 cm x 40 cm). The last purification step was affinity chromatography on dATP-Sepharose (1 cm x 1.6 cm).

dATP is a potent inhibitor of ribonucleotide reductase (Moore & Hurlbert, 1966) and therefore the enzyme was strongly bound to a column of dATP-Sepharose (Berglund & Eckstein, 1972). After the column had been washed with 50 mM-Tris/HCl (pH 7.6)/15 mM-MgCl₂/2 mM-dithiothreitol containing 1 mM-ATP to elute contaminating proteins, the enzyme could be desorbed by using 50 mM-ATP and a slower flow rate. The ATP eluate was then concentrated by ultra-dialysis and stored at -70°C.

A summary of the purification is presented in Table 1. The specific activity in the crude extract is similar to the activity found in other rapidly proliferating cells (Larsson, 1969; Moore & Hurlbert, 1966; Hopper, 1972), and it was increased 4000-fold during the purification. The specific activity of the enzyme under optimal conditions was 400 units/mg of protein and the total yield was 3 mg of protein from 1 kg of tissue, with 30 % overall recovery.

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of the ATP eluate showed one major band (85 % of the total protein), with mol.wt. 80000. Both polyacrylamide-gel electrophoresis of the native enzyme and isoelectric focusing in urea showed one major compound and also some smaller components, representing less than 10 % of the total protein. When analysed by sucrose-gradient centrifugation, enzyme activity and protein in the ATP eluate co-sedimented as one peak at 11S. In the presence of 1 mM-ATP the activity instead sedimented at 19S, indicating aggregation.

The activity of the ATP eluate alone was low, and addition of a crude fraction (see below) stimulated the activity 3-5-fold. The stimulating factor was present in
excess in crude extracts, i.e. 1 µl of extract saturated an amount of ATP eluate corresponding to 50 µl of crude extract. The stimulating factor was purified 10-fold by heat treatment for 2 min at 80°C in the presence of 1 M-potassium phosphate, pH 7. It was sensitive to trypsin digestion and thiol-blocking reagents. However, on gel filtration the stimulating activity was eluted in multiple positions in the chromatogram.

No stimulation of the ATP eluate was found on addition of E. coli thioredoxin or calf liver thioredoxin. Neither could dithiothreitol be replaced by thioredoxin reductase and NADPH as reducing agent.


Preliminary Characterization of the Deoxyribonucleases of Chlamydomonas reinhardii

GEORGE C. L. TAIT* and WILLIAM J. HARRIS

Department of Biochemistry, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, Scotland, U.K.

DNAases† have been studied in a wide variety of viruses, bacteria, fungi and mammalian cells, but relatively few studies of these enzymes in protozoan species have been described. The present work describes the initial characterization of the DNAases from the unicellular alga Chlamydomonas reinhardii, undertaken as part of a general study of DNA metabolism in this organism. Very high DNAase activity within this organism has been described (Schonherr & Keir, 1972), and an endonuclease activity has been partially purified and studied (Small & Sparks, 1972).

At least five DNAases have been identified in extracts of Chlamydomonas reinhardii [strain wild-type (+), provided by the Culture Centre of Algae and Protozoa, Cambridge, U.K.] grown under photosynthesizing conditions (Kates & Jones, 1964). Algae were grown synchronously and harvested at the time of nuclear DNA synthesis. Cell extracts were prepared by sonication of the cells, and DNA was removed with DEAE-cellulose. Subsequent chromatography of extracts on DEAE-cellulose resolved the DNAase activities into unbound (DNAases 1, 4 and 5) and bound fractions (DNAases 2 and 3). The unbound activity was further resolved by phosphocellulose chromatography into bound (DNAase 1) and unbound (DNAases 4 and 5) fractions. DNAases 4 and 5 were purified by CM-cellulose chromatography and finally resolved into separate enzymic activities by isoelectric focusing (Fig. 1).

The enzyme activity retained by phosphocellulose (DNAase 1) was subsequently extensively purified and studied in depth (G. C. L. Tait & W. L. Harris, unpublished work). The activity retained by DEAE-cellulose (DNAases 2 and 3) was further purified by phosphocellulose chromatography and finally resolved into two separate activities by isoelectric focusing. Table 1 summarizes the properties of these various enzyme species. DNAases 2 and 3 were active in the presence of 2 mM-Ca²⁺, but in contrast with DNAase 1, were stimulated to about the same extent by 10 mM-MgCl₂. Whereas DNAase 1, even after extensive purification, digested native DNA to 10% of the activity measured with denatured DNA as substrate, DNAases 2 and 3 were inactive with native DNA. DNAase 1 gave a single peak in isoelectric-focusing studies. DNAases 4 and 5 were resolved by isoelectric focusing into two activities, both activated by 2 mM-CaCl₂. The fraction with pI 9.0 was active only with denatured DNA as substrate, whereas the

* Present address: Centre de Geneticque Moleculaire, CNRS, 91190 Gif-sur-Yvette, France.
† Abbreviation: DNAase, deoxyribonuclease.