Allosteric Activation of Rat Liver Cytosolic 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase Kinase by Nucleoside Diphosphates*

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Extensively purified rat liver cytosolic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase kinase was used to examine the role of ADP in inactivation of HMG-CoA reductase (EC 1.1.1.34). Solubilized HMG-CoA reductase was a suitable substrate for HMG-CoA reductase kinase. At sufficiently high concentrations of solubilized HMG-CoA reductase, reductase kinase activity approached that measured using microsomal HMG-CoA reductase as substrate. Inactivation of solubilized HMG-CoA reductase by HMG-CoA reductase kinase required both MgATP and ADP. Other nucleoside diphosphates, including α,β-methylene-ADP, could replace ADP. HMG-CoA reductase kinase catalyzed phosphorylation of bovine serum albumin fraction V by $\gamma^{32P}ATP$. This process also required a nucleoside diphosphate (e.g. α,β-methylene-ADP). Nucleoside diphosphates thus act on HMG-CoA reductase kinase, not on HMG-CoA reductase. For inactivation of HMG-CoA reductase, the ability of nucleoside triphosphates to replace ATP decreased in the order ATP > dATP > GTP > TTP, UTP, TTP and CTP did not replace ATP. Both for inactivation of HMG-CoA reductase and for phosphorylation of bovine serum albumin protein, the ability of nucleoside di- and triphosphates to replace ADP decreased in the order ADP > CDP, GADP > UDP, GDP did not replace ADP. Nucleoside di- and triphosphates thus appear to bind to different sites on HMG-CoA reductase kinase. Nucleoside diphosphates act as allosteric activators of HMG-CoA reductase kinase. For inactivation of HMG-CoA reductase by HMG-CoA reductase kinase, $K_m$ for ATP was 140 μM and the activation constant, $K_a$, for ADP was 1.4 mM. The concentration of ADP required to modulate reductase kinase activity in vitro falls within the physiological range. Modulation of HMG-CoA reductase kinase activity, and hence of HMG-CoA reductase activity, by changes in intracellular ADP concentrations thus may represent a control mechanism of potential physiological significance.

The activity of HMG-CoA reductase (reductase, EC 1.1.1.34), the catalyst for the rate-limiting reaction for biosynthesis of (poly)isoprenoids (1), is modulated both in vitro and in vivo by covalent phosphorylation by the γ-phosphate of ATP (2–5). Inactivation of reductase is catalyzed by HMG-CoA reductase kinase (reductase kinase, EC 2.7.1.1) (5, 6). Reactivation of phosphoreductase is catalyzed by HMG-CoA reductase phosphatase (EC 3.1.3.1) (7, 8). In vitro, these converter enzymes can modulate reductase catalytic activity over an approximately 10-fold range (9–11). While controversy persists (12, 13), emerging evidence favors the interpretation that interconversion of phospho- and dephosphoreductase constitutes a short term control mechanism of true physiological significance (14–18).

Research on reductase kinase was initiated by the discovery of an activity that, in the presence of MgATP, inactivated reductase activity in vitro (2). MgATP-dependent reductase-inactivating activity is present both in microsomes (19) and in cytosol (9) of rat liver. By analogy to better characterized systems, this activity was thought to be a protein kinase. This postulate was, however, complicated by the observation that MgADP was almost as effective as MgATP for inactivation of crude microsomal reductase (9, 20). Brown et al. (20) showed that inclusion of an ADP-regenerating system blocked the ability of MgADP to inactivate reductase activity. While this showed that ATP was required, inclusion of an ATP-regenerating system also abolished the ability of MgATP to inactivate reductase. Reductase-inactivating activity thus was expressed only in the presence of both ADP and ATP. These observations have been confirmed (6, 9), and it was postulated (20) that ATP may act as the phosphoryl donor while ADP may serve as an allosteric activator of reductase kinase activity.

Subsequent investigations have established that the MgATP-dependent reductase-inactivating activity of rat liver tissue is indeed a protein kinase. The microsomal form of reductase kinase has been purified to apparent homogeneity and catalyzes incorporation of radioisotope from $\gamma^{32P}ATP$ into reductase protein (19). Inactivation of reductase is accompanied by its phosphorylation by the γ-phosphate of ATP (21, 22) and the phosphate is covalently attached to seryl residues on reductase (23). To date, however, the explanation for the ADP requirement of cytosolic reductase kinase has not been investigated. We have used extensively purified rat liver cytosolic reductase kinase to show that ADP functions as an allosteric activator of reductase kinase activity.

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Activation of Rat Liver Cytosolic HMG-CoA Reductase Kinase

EXPERIMENTAL PROCEDURES

Chemicals—Chemicals from commercial sources included: diethio- 
threitol, dADP and dATP (U. S. Biochemicals, Cleveland, OH); 
calmodulin, βC-arehynie-6-ADP, cAMP, cGMP and all other nucleo-
tides (Sigma); and [γ-32P]ATP (New England Nuclear). All other 
chemicals were from previously listed sources (6, 24, 25).

Buffered Solutions—Diethiothreitol was added to all solutions just 
prior to use. TEDSF contained 50 mM Tris (pH 7.1), 1.0 mM EDTA, 
5.0 mM diethiothreitol, 250 mM sucrose, and 50 mM NaF. The following 
solutions were used for analysis of reductase kinase activity. Control 
solution contained 50 mM MgCl₂ and 408 mM NaF in half-strength 
TEDSF, ATP solution contained 15 mM ATP in control solution. 
ADP solution contained 15 mM ADP in control solution. ADP/ATP 
solution contained 15 mM ATP plus 15 mM ADP in control solution.

Rats—Rats, 150- to 200-g female Wistar-strain animals from our 
department colony, were fed commercial lab chow and water ad 
libitum. Rats used for preparation of reductase kinase were housed 
under normal lighting conditions. For preparation of microsomal 
reductase, rats were housed in a room darkened from 1500 to 0300 
and illuminated from 0300 to 1500 h. For preparation of solubilized 
reductase, rats were fed ground commercial lab chow that contained 
3% cholestyramine for 5 days prior to use.

Enzymes—The heated microsomal reductase used as substrate for 
analysis of reductase kinase activity was prepared as previously 
described (6). Blue Sepharose fraction (specific activity, 60-84 nano-
units/mg) or hydrolyzate fraction (specific activity, 2,300-2,500 
nanounits/mg) reductase kinase were prepared as described in the 
Miniprint.

To prepare solubilized reductase, microsomal reductase was solu-
ibilized and purified through the heat fraction as described by Rogers 
et al. (26) and subsequently through the blue Sepharose fraction as 
described by Harwood (27) to a specific activity of 10,900 nanounits/ 
mg. The reductase subunit of this fraction migrated as a protein of 
Mr = 56,000 on sodium dodecyl sulfate-polyacrylamide gels.

Analysis of Enzyme Activities—Reductase activity (11, 28) and 
reductase kinase activity (6) were assayed as previously described. 
One picounit of reductase activity is that which converts 1 pmol of 
HMG-CoA to mevalonate in 1 min at 37 °C. One picounit of reductase 
kinase activity is that amount which inactivates 1 picounit of reduc-
tase activity in 30 min at 30 °C. General protein kinase activity was 
determined by measuring the incorporation of 32P from [γ-32P]ATP 
into trichloroacetic acid-insoluble material as follows (28). Portions, 
50 to 100 μl, containing phosphorylated protein, were applied to 
dry 2.0-cm diameter Whatman 3MM filter paper disks previously 
impregnated with 100 μl of 200 μM ATP (pH 7.5). The disks were then 
placed in ice-cold 10% trichloroacetic acid (10 ml/disk), washed as 
described by Corbin and Reimann (29), and counted in 4.0 ml of 
Beckman Redi-Solv fluor.

Analysis of Protein—Protein was determined by the method of 
Bradford (30) using bovine serum albumin fraction V as standard.

RESULTS

Inactivation of Solubilized Reductase by Reductase Kinase— 
Reductase kinase readily catalyzed inactivation of solubilized 
reductase and inactivation was a function both of incubation 
time and of reductase kinase concentration. Relative to 
microsomal reductase, solubilized reductase was a poor substrate 
for reductase kinase. We used equal quantities (150 picounits) of 
solubilized and of microsomal reductase to assay reductase 
kinase fractions of widely differing specific activity. Apparent 
reductase kinase activity using solubilized reductase as sub-
strate was in all cases only 4.3 ± 0.5% of that measured using 
microsomal reductase as substrate. Either form of reductase 
may, however, be used to assay reductase kinase activity. Fold purification and recovery of activity measured through-
out the course of a reductase kinase purification were identical 
using either microsomal or solubilized reductase as substrate. 
When higher concentrations of solubilized reductase were 
used, reductase kinase activity approached that measured 
using microsomal reductase as substrate (Fig. 1). Reductase 
kinase activity exhibited a Michaelis-Menten dependence on 
the concentration of solubilized reductase (substrate) (Fig. 1). 
Under the conditions studied, 885 picounits of solubilized 
reductase gave half-maximal reductase kinase activity (Fig. 1, inset).

ADP Is Required for Reductase Kinase Activity—Inactiva-
tion of solubilized reductase by reductase kinase occurred only 
when both ATP and ADP were present simultaneously (Table I). 
In the presence of a saturating concentration of ATP, the 
activation constant, Kₐ, for ADP was 1.4 mM (Fig. 2). In the 
presence of 2.0 mM ADP, the Kₐ for ATP was 140 μM (Fig. 3).

ADP Does Not Act As a Second Phosphate Donor—We 
asked whether βC-arehynie-ADP, an ADP analog incapable 

2 Portions of this paper (including part of "Experimental Pro-
dedures") are presented in miniprint at the end of this paper. Miniprint 
is easily read with the aid of a standard magnifying glass. Full size 
photographs are available from the Journal of Biological Chemistry, 
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**Table II**

**α,β-Methylene-ADP can replace ADP for inactivation of solubilized reductase by reductase kinase**

Complete incubation mixtures contained, in 70 μl of TESDSF, 10 μl of control solution, 2.6 μg of solubilized reductase protein, 0.66 μg of reductase kinase protein (hydroxylapatite fraction), and the indicated nucleoside di- and triphosphates. Four control incubations were included. These contained (a) no nucleotides, (b) 2.0 mM ATP alone, (c) 2.0 mM ADP alone, or (d) 4.0 mM α,β-methylene-ADP alone. Incubation was at 30 °C for 30 min. Portions, 35 μl, were then assayed for remaining reductase activity. Data are average values ± S.E. for quadruplicate determinations.

<table>
<thead>
<tr>
<th>Nucleotides added</th>
<th>Reductase activity neat units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control incubations</td>
<td>540 ± 22</td>
</tr>
<tr>
<td>2.0 mM ATP + 2.0 mM ADP</td>
<td>310 ± 10</td>
</tr>
<tr>
<td>2.0 mM ATP + 4.0 mM ADP</td>
<td>440 ± 14</td>
</tr>
<tr>
<td>α,β-methylene ADP</td>
<td></td>
</tr>
</tbody>
</table>

*a* Average value ± S.E. for all four control incubations.

**Table III**

**α,β-Methylene-ADP can replace ADP for phosphorylation of nonreductase proteins by reductase kinase**

Complete incubation mixtures contained, in 80 μl of TESDSF, 10 μl of control solution, 1.8 mM [γ-32P]ATP (specific activity, 76.4 cpm/pmol), 0.75 μg of reductase kinase protein (hydroxylapatite fraction) and 500 μg of bovine serum albumin fraction V protein. One tube received no further additions. The second received 1.8 mM ADP, and the third, 1.8 mM α,β-methylene-ADP. Controls paired with each of the above lacked either reductase kinase or bovine serum albumin fraction V protein. Incubation was at 30 °C for 30 min. Portions, 70 μl, were then assayed for trichloroacetic acid-precipitable radioactivity. Data are corrected for background levels of radioactivity present in control incubations (2,400 ± 130 cpm).

<table>
<thead>
<tr>
<th>Additions</th>
<th>Incorporation of 32P into trichloroacetic acid-insoluble material of bovine serum albumin fraction V</th>
</tr>
</thead>
<tbody>
<tr>
<td>[32P]ATP</td>
<td>2,400</td>
</tr>
<tr>
<td>[32P]ATP + ADP</td>
<td>2,900</td>
</tr>
<tr>
<td>α,β-methylene-ADP</td>
<td>2,500</td>
</tr>
</tbody>
</table>

Adapted from "Rat Liver Cytosolic HMG-CoA Reductase Kinase" by guest on August 29, 2017.

ADP is required by reductase kinase for phosphorylation of Bovine Serum Albumin Protein—To determine whether ADP acted on reductase or on reductase kinase, we sought an alternate substrate for the kinase. We detected no incorporation of radioactivity from [γ-32P]ATP into cytochrome c, phosvitin, or protamine and only minimal incorporation into casein, phosphorylase kinase, or histone 2A. Significant radioactivity was, however, incorporated into protein of bovine serum albumin fraction V. Significant incorporation of 32P from [γ-32P]ATP occurred even in the absence of reductase kinase, indicating the presence in bovine serum albumin fraction V of endogenous protein kinase activity. Phosphorylation catalyzed by endogenous protein kinase was, however, not significantly affected by addition of ADP. By contrast, and by analogy to inactivation of reductase activity (Table I), that catalyzed by reductase kinase required ADP or α,β-methylene-ADP (Table III).

ADP is an allosteric activator of reductase kinase—We reasoned that, if ADP activates reductase kinase by binding at a site distinct from the ATP-binding (phosphorylation group transfer site) site, the structural specificity of the two nucleotide binding sites might differ. We therefore examined the ability of various nucleoside di- and triphosphates to replace ADP or ATP. In the presence of 2.0 mM ADP, the phosphoryl group transfer site was specific for a purine nucleoside triphosphate.
TABLE IV
Nucleotide specificities of the phosphoryl transfer site and of the allosteric site of reductase kinase

<table>
<thead>
<tr>
<th>Additions</th>
<th>Reductase kinase activity</th>
<th>Fraction of activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With ADP</td>
<td>With ATP</td>
</tr>
<tr>
<td>Experiment I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>ATP</td>
<td>200 (100)</td>
<td>200</td>
</tr>
<tr>
<td>dATP</td>
<td>182</td>
<td>182</td>
</tr>
<tr>
<td>GTP</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>TTP</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>UTP</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>CTP</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>GDP</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Experiment II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ADP</td>
<td>134 (100)</td>
<td>134</td>
</tr>
<tr>
<td>CDP</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>dADP</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>UDP</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>GDP</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The nucleoside diphosphate specificity for phosphorylation of nonreductase protein qualitatively resembles that for inactivation of solubilized reductase

Complete incubations contained, in 70 µl of TEDSF, 10 µl of control solution, 10.4 µg of reductase kinase protein (blue Sepharose fraction), 2.6 µg of solubilized reductase protein, and either 2.0 mM ADP (Experiment I) or 2.0 mM ATP (Experiment II). Each tube contained, in addition, the indicated nucleoside d- or triphosphate, present at a final concentration of 2.0 mM. Incubation was at 30 °C for 30 min. Portions, 35 µl, were then assayed for remaining reductase activity.

<table>
<thead>
<tr>
<th>Nucleoside diphosphate added</th>
<th>Trsp incorporated</th>
<th>Phosphorylation of albumin</th>
<th>Inactivation of reductase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pmol</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>None</td>
<td>37</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>ADP</td>
<td>234 (100)</td>
<td>234</td>
<td>100</td>
</tr>
<tr>
<td>α,β-Methylene-ADP</td>
<td>155</td>
<td>66</td>
<td>42</td>
</tr>
<tr>
<td>dADP</td>
<td>127</td>
<td>54</td>
<td>22</td>
</tr>
<tr>
<td>CDP</td>
<td>113</td>
<td>48</td>
<td>36</td>
</tr>
<tr>
<td>UDP</td>
<td>64</td>
<td>27</td>
<td>12</td>
</tr>
<tr>
<td>GDP</td>
<td>5</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Discussion

Inactivation of microsomal reductase by reductase kinase and Mg2+-nucleotides has been reported by many laboratories (1, 2, 4-6, 11, 19-20). There appear to be at least two forms of reductase kinase in rat liver present in microsomes (9, 20) and cytosol, respectively. As was previously shown using microsomal reductase (6, 9, 20) inactivation of solubilized reductase by cytosolic reductase kinase requires both a nucleoside diphosphate and a nucleoside triphosphate. Since microsomes interconvert ATP and ADP (20), this requirement previously was demonstrable for microsomal reductase only after inclusion of an ATP-regenerating system (20). Using purified cytosolic reductase kinase as catalyst and solubilized reductase as substrate, the dinucleotide requirement is readily demonstrable even without this precaution. While ADP was the most effective dinucleotide we tested, α,β-methylene-ADP, CDP, dADP, and UDP (but not GDP) replaced ADP with an effectiveness that decreased in the order listed. To our knowledge, the nucleoside diphosphate requirement of cytosolic reductase kinase is unique among ATP-phosphotransferases. Possible exceptions might include nucleoside diphosphate kinases (EC 2.7.4.X), although for these enzymes the nucleoside diphosphate is itself a substrate. We confirm the observation of Brown et al. (20) that reductase kinase activity was not substantially affected by addition of CAMP. In addition, reductase kinase activity was unaffected by addition of cGMP, Ca2+, calmodulin, or any combination of these factors. Finally, no combination of the above compounds relieved the requirement of reductase kinase for ADP. Since α,β-methylene-ADP serves as an acceptable nucleoside diphosphate, nucleoside diphosphates cannot act as second or alternate phosphate donors. Nucleoside diphosphates might bind to reductase (e.g. to make it a more effective substrate), to reductase kinase (e.g. to make it a more effective catalyst), or to both. We observed that 1) addition of ADP subsequent to the reductase kinase reaction had no discernible effect on reductase inactivation, 2) the ability of reductase kinase to catalyze phosphorylation of nonreductase proteins also required ADP, and 3) the nucleoside diphosphate specificity for protein phosphorylation resembled that for inactivation of reductase. Based on these observations, we therefore consider interaction of ADP with reductase as an unlikely explanation and conclude that nucleoside diphosphates act directly on reductase kinase.

If nucleoside diphosphates bind at the same site as ATP they should inhibit rather than activate reductase kinase activity. This was not observed. It thus seemed likely that nucleoside di- and triphosphates bound at distinct sites on cytosolic reductase kinase. This inference was supported by the differential ability of various nucleoside di- and triphosphate derivatives to replace ADP and ATP. While several analogs replaced either nucleotide, those that replaced ADP differed significantly from those that replaced ATP. We interpret these observations to suggest that nucleoside di- and triphosphates bind at distinct sites on reductase kinase and that, as suggested by Brown et al. (20), nucleoside diphosphates act as allosteric activators of cytosolic reductase kinase activity.

Does allosteric activation of reductase kinase by nucleoside diphosphates occur in vivo, and if so, might this fulfill a regulatory role? The activation constant, Ka, of reductase kinase for ADP was 1.4 mM, a concentration approximately twice that of the reported intracellular concentration of ADP.
in hepatocytes (approximately 0.8 nM) (31). At an ADP concentration of 0.8 mM, cytosolic reductase kinase thus may act in vivo at about one-third maximal velocity. Small fluctuations in intracellular ADP concentration thus might significantly alter in vivo reductase kinase activity. Allosteric regulation of reductase kinase by small changes in intracellular concentrations of ADP or other nucleoside diphosphates thus may be of physiological significance.

Acknowledgments—We thank John Gill, Willis Brown, and Peter Kennelly for many helpful suggestions.

REFERENCES
Activation of Rat Liver Cytosolic HMG-CoA Reductase Kinase

Material Supplementary to
ALLOSTERIC ACTIVATION OF RAT LIVER CYTOSOLIC HMG-COA REDUCTASE KINASE BY NUCLEOSIDE DIPHOSPHATES

A. James Marwood, Jr., Ralph C. Brandt and Victor W. Rodwell

Gives below is the purification protocol for rat liver cytosolic HMG-CoA reductase kinase.

Chemicals - Those included: protein molecular weight standards, DEAE-Sephadex, Sephacryl S-200 and Sephacryl G-50 (Pharmacia Fine Chemicals, Uppsala, Sweden); hydroxylapatite (Bio-Rad) (Bio-Rad Laboratories, Richmond, CA); 

Buffer Solutions - Dimethylsulfoxide was added to all solutions final prior to use. Tyrode contained 40 mm Tris (pH 7.5), 1.9 mm EDTA, 1.0 mm dithiothreitol, 75 mm KCl and 50 mm NaCl. Buffer I contained 2% glycerol, but was otherwise identical in composition to Sephacryl buffer but contained 1% sodium azide. 

Preparation of Rat Cytosol - Two rats were killed at 0600 hours by cervical dislocation. The livers were removed and placed in a chilled, perfused tissue grinder and homogenized at 4°C in a total volume of 10 ml of buffer I and then centrifuged at 105,000 × g, 20 min 4°C and the sediment discarded. The supernatant liquid was decanted and centrifuged for 10 min at 105,000 × g, and the supernatant liquid was retained as cytosol.

First DEAE Fraction - DEAE-Sephadex was mixed with excess buffer I and allowed to settle to a packed volume of 15 ml. Enzyme buffer was decanted and discarded. The supernatant was then added to the cytosol, and the mixture was transferred to a 2.5 × 40 cm DEAE column. The column was washed with Tyrode buffer to remove excess salt and finally eluted with a linear gradient of 100 ml each of buffer I and II. The entire column was decanted and the wash was retained as the First DEAE Fraction. 

Second DEAE Fraction - The Second DEAE Fraction was applied to a 2.5 × 40 cm column of DEAE-Sephadex in buffer I. All the applied protein bound to this support. The peak was then eluted with an 80 ml gradient of 0 to 0.2 M KCl in buffer II (4 ml fractions). Fractions with conductivity of 15 to 14 were combined (pool size 28 to 30 ml) to give the Second DEAE Fraction.

Hydroxylapatite Fraction - The Second DEAE Fraction was applied on 3.5 × 15 cm column of hydroxylapatite in buffer II. The eluate was washed with 10 ml of buffer II, then eluted with 10 ml of 0.4 M K2HPO4 buffer II. The entire 10 ml eluate was retained as the Hydroxylapatite Fraction. The hydroxylapatite fraction retained HMG-CoA reductase kinase activity was stored in liquid N2 for up to one month. Table 1 summarizes a typical purification.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total reduction</th>
<th>Total specific purification</th>
<th>Recovery of activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytosol</td>
<td>9,360</td>
<td>3,040</td>
<td>93</td>
</tr>
<tr>
<td>First DEAE</td>
<td>596</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>Second DEAE</td>
<td>1,940</td>
<td>44</td>
<td>24</td>
</tr>
<tr>
<td>Sephacryl S-200</td>
<td>2,670</td>
<td>90</td>
<td>28</td>
</tr>
<tr>
<td>Second Hydroxylapatite</td>
<td>473</td>
<td>95</td>
<td>16</td>
</tr>
</tbody>
</table>

The above procedure is sufficiently reproducible to permit omission of time-consuming assays of reductase kinase activity at each stage of purification. For optimal recovery of enzyme, we recommend carrying purification through the Blue Sephacryl Fraction in a single day, freezing the fraction in liquid N2, and completing the purification the following day. This is possible only if analysts for fractions for protein and reductase kinase activity are available before completion of the purification. Freeze-thawing of the Blue Sephacryl Fraction is incompatible with storage of reductase kinase activity. (Table 1). However, we have found that the reductase kinase activity of Blue Sephacryl and Hydroxylapatite fractions stored in liquid N2 was stable for several months.
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