Reductase for Aromatic Aldehydes and Ketones

THE PARTIAL PURIFICATION AND PROPERTIES OF A REDUCED TRIPHOSPHOPYRIDINE NUCLEOTIDE-DEPENDENT REDUCTASE FROM RABBIT KIDNEY CORTEX

HILMAN W. CULP AND ROBERT E. MCMAHON
From the Lilly Research Laboratories, Indianapolis, Indiana 46206

(Received for publication, August 18, 1967)

SUMMARY

The partial purification of the enzyme, aromatic aldehyde-ketone reductase (aromatic A-K reductase), from rabbit kidney cortex is described. Aromatic A-K reductase utilizes TPNH but not DPNH as a cosubstrate. Substituted benzaldehydes and acetophenones are readily reduced to carbons. Aromatic A-K reductase does not, however, catalyze the reverse reaction, the dehydrogenation of carbons under the experimental conditions used. Aromatic A-K reductase utilizes the β hydrogen atom at C-4 of TPNH.

EXPERIMENTAL PROCEDURE

Materials

The enzymes yeast glucose 6-phosphate dehydrogenase (EC 1.1.1.49) and liver alcohol dehydrogenase (EC 1.1.1.1), were obtained from Nutritional Biochemicals. The inhibitors, 1,10-phenanthroline, 2,2'-dipyridyl, N-ethylmaleimide, iodoacetamide, and p-chloromercuribenzoate, were obtained from the same source. Pig heart isocitric acid dehydrogenase (EC 1.1.1.42) was obtained from C. F. Boehringer und Soehne, Mannheim. Substrates were obtained from other commercial chemical supply houses. The purity of the aldehydes was at least 95%, while the ketones were better than 98% pure, as determined by gas-liquid chromatography procedures. For the gas-liquid chromatography work, an F and M model 402 was utilized. Two columns, SE-30 (nonpolar) and XE-60 (polar), both manufactured by General Electric, Schenectady, New York, were used at temperatures near 100°. L-Lactaldehyde was prepared from L-threonine by the procedure of Huff and Rudney (4). Dihydro-19-nortestosterone was kindly supplied by Dr. Eugene Farkas, while adamantanone was obtained through the courtesy of Dr. Koert Gerzon.

Enzyme Preparation

Tissue Extraction—Frozen kidneys from mature rabbits were obtained from Pel-Freez, Rogers, Arkansas. The kidneys were partially thawed, and the medulla and any fatty tissue were removed manually. The kidney cortex was minced and then homogenized in an equal volume of ice-cold 0.10 M phosphate buffer, pH 7.4, in a Potter-Elvehjem tissue grinder. The homogenate was centrifuged at 300 x g for 5 min in a refrigerated centrifuge to remove gross tissue debris. The supernatant was then recentrifuged at 30,000 x g for 30 min, and the supernatant was used for the next purification step.

Ammonium Sulfate Fractionation—The 30,000 x g supernatant was placed in an ice bath and stirred with a magnetic stirring bar. Crystalline ammonium sulfate was added slowly until a concentration of 1.4 M (0.35 saturation) was reached. Stirring was continued for 30 min, and the precipitated protein was then removed by centrifugation. The clear supernatant was treated with additional ammonium sulfate until a concentration of 2.4 M (0.60 saturation) was attained. Stirring was continued for 30 min. The precipitated crude enzyme was then recovered by centrifugation and redissolved in 0.10 M phosphate buffer, pH 7.4. The enzyme solution was dialyzed overnight at 6° against...
of the alumina fractions was extracted once with 2 to 4 ml of 0.10 M sodium phosphate buffer at pH 7.4. After the pH of the effluent stabilized, the buffer was changed to 0.01 M phosphate, pH 7.4, containing 0.01 M Versene. All buffers beyond this point contained 0.01 M Versene. Columns (3.5 x 35 cm) were packed by pouring in a slurry of the washed DEAE-cellulose and allowing it to settle, and then washing with 300 ml of 0.01 M phosphate buffer. Dialyzed ammonium sulfate precipitate was diluted to 20 mg per ml, and 100 ml of protein solution were put on the column. Development was started with 0.01 M phosphate buffer for about the first 30 fractions (6 to 8 mg each). This contained a "pink" protein band, which was probably identical with that described by Gupta and Robinson as 1-lactaldehyde reductase (2). The column was further developed by setting up a two-compartment gradient elution in which the first compartment contained 300 ml of 0.010 M phosphate buffer, pH 7.4, and the second, 600 ml of 0.010 M phosphate buffer, pH 7.4, plus 9.25 g of ammonium sulfate per liter. The column flow was adjusted to about 1 ml per min. The progresse of the column was followed by absorbance determinations at 280 m\mu. Aliquots from each protein-containing fraction were assayed for enzyme activity with p-chloroacetophenone as substrate. The active fractions were pooled and were dialyzed against distilled water for about 4 hours. This solution was freeze dried. Enzyme prepared in this manner remained active for several weeks in the refrigerator.

Alumina Gel Adsorption—The freeze dried material from the DEAE-cellulose column was dissolved in 0.010 M sodium phosphate buffer, pH 7.4, to a concentration of 10 mg per ml. This solution was dialyzed for 3 to 4 hours against distilled water containing 0.06 m\mu Versene. The dialyzed solution was cooled in an ice bath and stirred while moist Alumina Gel C (Nutritional Biochemicals) was added directly from the bottle in amounts equal to one half the weight of starting protein. This was stirred for 30 min and the gel was then removed by centrifugation. The supernatant was treated repeatedly until all the enzyme activity was removed (three to four treatments). Each of the alumina fractions was extracted once with 2 to 4 ml of 0.10 M phosphate buffer, pH 7.4, in the centrifuge tubes. After standing for 10 min, the tubes were centrifuged, and the supernatant was removed with a dropper. The gel was re-extracted progressively with 0.15, 0.20, 0.30, and 0.50 M sodium phosphate buffer, pH 7.4. This treatment usually removed most of the enzyme activity. Each of the fractions was kept separate until specific enzyme activities had been determined, and those with similar values were combined. The 0.15 and 0.20 M fractions contained most of the aromatic A-K reductase activity. This preparation, which was stable for about 1 week when stored at 0\degree, was used to determine the properties of aromatic A-K reductase.

Enzyme Assay Procedure

All enzyme solutions to be assayed were analyzed for total protein content by the method of Lowry et al. (5). A Gilford multiple sample absorbance recorder was used to follow the oxidation of TPNH at 25\degree, as determined by the rate of decrease in optical density at 340 m\mu. The standard system consisted of enzyme (0.05 to 5.0 mg of protein in 0.10 ml), TPNH (0.48 \mu mole in 0.10 ml), and 0.10 M sodium phosphate buffer, pH 7.0, added to make the volume 2.9 ml. The rate of decrease in optical density at 340 m\mu was recorded for 4 to 8 min to establish the blank for each sample. The blank was appreciable in the more crude enzyme preparations but was negligible on material beyond the (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} precipitate. Many of the substrates used were soluble in water to a limited extent. It was found that up to 0.20 ml of absolute methanol could be added to the 3.0-ml reaction medium without effect on the enzyme reaction rate. All substrates were dissolved in absolute methanol or a water-methanol mixture. After the blank was measured, 3 \mu moles of substrate (0.10 ml) were added to each sample, and the resultant rate of change in optical density was recorded. The slope of the initial, linear part of the curve was used to calculate enzyme activity.

For the determination of the pH optimum, a series of 0.1 M sodium phosphate buffers was prepared, from pH 5.5 to 8.5. Each was used under standard assay conditions. After the run, the pH of each solution was rechecked with a pH meter. Carbonate buffers (0.10 M) were used for experiments in the pH range, 8 to 10.5.

In the experiments with inhibitors, the inhibitor in water solution was added prior to the addition of substrate.

Preparation in Vitro of S-(−)-Methylphenylcarbinol—For studies on a preparative scale, a 100,000 x g supernatant from rabbit kidney homogenate was used as an enzyme source. This preparation contained glucose 6-phosphate dehydrogenase, which served for TPNH regeneration. In a typical run, 2 \mu moles of glucose 6-phosphate, 2 \mu moles of acetophenone, and 15 mg of TPN\textsuperscript{+} were added to 20 ml of 100,000 x g supernatant (from 10 g of kidney) at pH 7. The solution was incubated at 37\degree for 3 hours.

The reaction product was recovered by direct extraction of the incubation mixture with 3 volumes of butanone-2. The solvent was then removed under reduced pressure, and the residue was taken up in a small volume of benzene and applied to fluorescent silica gel preparative thin layer plates (Brinkmann Silica Gel F\textsubscript{254}). The plates were developed in a benzene-ethyl acetate (19:1) system. The band corresponding to known methylphenylcarbinol was located by viewing the plates under short wave length ultraviolet light. Methylphenylcarbinol was then recovered by elution with butanol 2. In order to obtain material that was pure, as determined by gas-liquid chromatography, the preparative thin layer chromatography procedure was repeated. Since losses in purification were substantial, a 100,000 x g supernatant containing glucose 6-phosphate dehydrogenase, which served for TPNH regeneration in a preparative scale, a 100,000 x g supernatant (from 10 g of kidney) at pH 7. The solution was incubated at 37\degree for 3 hours.

The reaction product was recovered by direct extraction of the incubation mixture with 3 volumes of butanone-2. The solvent was then removed under reduced pressure, and the residue was taken up in a small volume of benzene and applied to fluorescent silica gel preparative thin layer plates (Brinkmann Silica Gel F\textsubscript{254}). The plates were developed in a benzene-ethyl acetate (19:1) system. The band corresponding to known methylphenylcarbinol was located by viewing the plates under short wave length ultraviolet light. Methylphenylcarbinol was then recovered by elution with butanol 2. In order to obtain material that was pure, as determined by gas-liquid chromatography, the preparative thin layer chromatography procedure was repeated. Since losses in purification were substantial, a typical yield was 10 to 30 mg of methylphenylcarbinol, as determined by quantitative gas-liquid chromatography. Rotations were determined in methanol in a 1-dm micropolarimeter tube.

Stereochemistry of Transfer of Hydride from TPNH—TPN\textsuperscript{+} labeled with tritium in position 4 of the pyridine ring was obtained from New England Nuclear Corporation. Their figure of >95% purity was accepted, and no further purification was undertaken. This material, which had a specific activity of 112 \mu C per mmole, was diluted to 18 \mu C per mmole with unlabeled TPN\textsuperscript{+}.

For the determination of the stereochemistry of the aromatic A-K reductase-catalyzed hydride transfer, the following procedure was used. A mixture of 0.10 mg of isocitric dehydrogenase, 0.1 mg of MnCl\textsubscript{2}, 0.48 \mu mole of labeled TPN\textsuperscript{+}, and 30
TABLE I
Purification of aromatic A-K reductase from rabbit kidney

The assay procedure is described in "Experimental Procedure."

Substrate | 30,000 × g Supernatant | (NH₄)₂SO₄ precipitate | DEAE-cellulose | Alumina gel
--- | --- | --- | --- | ---
 | Total enzyme* | Specific activity | Recovery | Specific activity | Recovery | Specific activity | Recovery | Specific activity | Recovery
Acetohexamide | 2,360 | 0.27 | 100 | 1.1 | 53 | 2 | 28 | 0 | 0
Phenylpyruvic acid | 26,200 | 2.90 | 100 | 18.0 | 80 | 90 | 46 | 1 | 0
L-Lactaldehyde | 7,700 | 1.08 | 100 | 14.4 | 90 | 0 | 0 | 0 | 0
p-Chlorobenzaldehyde | 60,900 | 7.20 | 100 | 38.3 | 70 | 246 | 44 | 144 | 8
Propiophenone | 3,770 | 0.42 | 100 | 2.0 | 60 | 16 | 50 | 123 | 11
p-Chloroacetophenone | 10,800 | 1.28 | 100 | 5.9 | 63 | 41 | 51 | 280 | 9

* One unit of enzyme activity is its capacity to oxidize 1 nmole of TPNH per min with the indicated substrate. Amount shown is amount present in the 30,000 × g supernatant from 500 g of kidney.

--

RESULTS

Enzyme Preparation

Initially it was found that activity was present in rat, rabbit, and hog kidneys. Since the rabbit kidney was the most active, it was used as an enzyme source for these studies. The activity was found only in cortex tissue.

In the first step a supernatant fraction was prepared by centrifugation of a homogenate of rabbit kidney cortex at 30,000 × g. The preparation was assayed with a selection of six substrates with TPNH as a cofactor. The results are summarized in Table I.

The second step in purification was ammonium sulfate fractionation. The protein fraction that precipitated between 0.35 and 0.60 saturation contained most of the reductase activity. After removal of ammonium sulfate by dialysis, this fraction was again assayed against the six selected substrates. It was found that the specific activity had increased from 4- to 7-fold for the various substrates (Table I).

DEAE-cellulose column chromatography, the next step, led to a separation of activity. Two active protein fractions were obtained (see Fig. 1). The first active peak (Fractions 8 to 12) was pink in color and catalyzed the TPNH-dependent reduction of L-lactaldehyde. This fraction also readily reduced o-hydroxyacetophenone.

The activity of major interest in this study appeared later as a part of a broad protein peak (Fractions 70 through 90). Those fractions active in the reduction of p-chlorobenzaldehyde and p-chloroacetophenone were combined and saved for further studies. The activity of this fraction with the six test substrates is shown in Table I. The only activity completely lost in this step was the lactaldehyde reductase.

Alumina gel adsorption was the final purification step used. The most active fraction was that recovered by elution with 0.15 to 0.20 M sodium phosphate (pH 7.4). The activity of this fraction is shown in Table I. Although this procedure resulted in substantial losses of total activity, the specific activity against p-chlorobenzaldehyde, p-chloroacetophenone, and propiophenone...
Enzyme Properties

Cofactor Requirement—Only TPNH, and not DPNH, served as a cofactor for the reduction of benzaldehyde, p-chlorobenzaldehyde, acetophenone, p-chloroacetophenone, and propiophenone. The apparent $K_m$ for TPNH with p-chlorobenzaldehyde as substrate was estimated to be 0.013 mm.

Substrate Specificity—A very large number of aldehydes and ketones were found to serve as substrates for this enzyme. Table II lists the activities of a series of representative substrates of purified aromatic A-K reductase (alumina gel material). In the case of pyruvic acid and acetaldehyde, no activity was observed with either TPNH or DPNH; this indicates that neither lactic acid dehydrogenase nor alcohol dehydrogenase activity is present.

Tissue Distribution—Aromatic A-K reductase activity was found to be present in rabbit liver but was not detectable in heart, lung, or smooth muscle.

Table II

<table>
<thead>
<tr>
<th>Substrate</th>
<th>TPNH oxidized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pmoles/min/mg protein</td>
</tr>
<tr>
<td>p-Chlorobenzaldehyde</td>
<td>1263</td>
</tr>
<tr>
<td>p-Nitrobenzaldehyde</td>
<td>1277</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>453</td>
</tr>
<tr>
<td>p-Methoxybenzaldehyde</td>
<td>984</td>
</tr>
<tr>
<td>p-Nitrocateophenone</td>
<td>635</td>
</tr>
<tr>
<td>p-Chloroacetophenone</td>
<td>280</td>
</tr>
<tr>
<td>Acetophenone</td>
<td>42</td>
</tr>
<tr>
<td>Propiophenone</td>
<td>108</td>
</tr>
<tr>
<td>p-Methoxyacetophenone</td>
<td>20</td>
</tr>
<tr>
<td>o-Nitrocateophenone</td>
<td>0</td>
</tr>
<tr>
<td>Benzyl methyl ketone</td>
<td>8</td>
</tr>
<tr>
<td>Benzalacetone</td>
<td>100</td>
</tr>
<tr>
<td>Pentanone-3</td>
<td>24</td>
</tr>
<tr>
<td>Cyclopentanone</td>
<td>3</td>
</tr>
<tr>
<td>Cyclohexanone</td>
<td>452</td>
</tr>
<tr>
<td>2-Decalone (cis- and trans-mixture)</td>
<td>696</td>
</tr>
<tr>
<td>Adamanantone</td>
<td>140</td>
</tr>
<tr>
<td>Dihydro-19-nortestosterone</td>
<td>75</td>
</tr>
<tr>
<td>Tetralone-1</td>
<td>78</td>
</tr>
<tr>
<td>Indanone-1</td>
<td>0</td>
</tr>
<tr>
<td>Benzoylformic acid</td>
<td>0*</td>
</tr>
<tr>
<td>Pyruvic acid</td>
<td>0*</td>
</tr>
<tr>
<td>Acetone</td>
<td>0</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>0*</td>
</tr>
</tbody>
</table>

* Also no activity when DPNH was substituted for TPNH.

Effect of inhibitors on aromatic A-K reductase activity

Activity was measured by adding 0.10 ml (0.10 mg) of enzyme to 2.6 ml of sodium phosphate buffer, pH 7.0, followed by 450 nmoles of TPNH, 3 nmoles of inhibitor, and 3 nmoles of substrate. The change in absorbance at 340 nm per min was compared to a sample without inhibitor.

Table III

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration</th>
<th>$%$</th>
<th>$%$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,10-Phenanthroline</td>
<td>$10^{-5}$</td>
<td>51</td>
<td>15</td>
</tr>
<tr>
<td>2,2'-Diprydyl</td>
<td>$10^{-4}$</td>
<td>38</td>
<td>29</td>
</tr>
<tr>
<td>$N$-Ethylmaleimide</td>
<td>$10^{-3}$</td>
<td>25</td>
<td>16</td>
</tr>
<tr>
<td>Iodoacetamide</td>
<td>$10^{-3}$</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>p-Chloromercurobenzoate</td>
<td>$10^{-3}$</td>
<td>12</td>
<td>18</td>
</tr>
</tbody>
</table>

Table IV

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>Specific activity of p-chlorobenzyl alcohol formed$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol dehydrogenase</td>
<td>1.01 $\times 10^6$</td>
</tr>
<tr>
<td>$\alpha$-TPNH</td>
<td>1.31 $\times 10^6$</td>
</tr>
<tr>
<td>$\beta$-TPNH</td>
<td>1.33 $\times 10^6$</td>
</tr>
<tr>
<td>Aromatic A-K reductase</td>
<td>6.18 $\times 10^4$</td>
</tr>
<tr>
<td>$\alpha$-TPNH</td>
<td>1.31 $\times 10^6$</td>
</tr>
<tr>
<td>$\beta$-TPNH</td>
<td>1.33 $\times 10^6$</td>
</tr>
</tbody>
</table>

$^a$ The ratios of activity with $\alpha$-TPNH to activity with $\beta$-TPNH are, for alcohol dehydrogenase, 77, and for aromatic A-K reductase, 0.046.

pH Dependence—With p-chloroacetophenone, maximum rates were observed at pH 6.0, while with p-chlorobenzaldehyde as substrate the maximum was pH 5.5.

Effect of Inhibitors

Table III summarizes the results of a survey of the effects of inhibitors on the kidney enzyme. The metal-binding agents were in general more effective than the sulfhydryl reagents. However, in both cases inhibitory activity was observed only when the inhibitor was present in relatively high concentration (0.001 M).

Reverse Reaction

When crude enzyme (30,000 x g supernatant) was used, it was found that p-methoxybenzyl alcohol could be oxidized slowly. TPN$^+$ was used as the cofactor, and the rate was followed by the increase in absorbance at 340 nm. The reaction occurred only at high pH (10 to 11). Other carbinols, such as p-chlorobenzyl alcohol and methyl phenyl carbainol, were not oxidized.

With purified aromatic A-K reductase and either DPN$^+$ or TPN$^+$ as cosubstrate, no oxidation of p-methoxybenzyl alcohol, p-nitrobenzyl alcohol, p-chlorobenzyl alcohol, or methylphenylcarbinol was observed at pH 10. An attempt to force the reac-
tion by adding semicarbazide to trap any aldehyde formed was also unsuccessful.

Stereochemistry of Reduction

The stereochemistry of the reduction was explored with the use of the conversion of acetophenone to methylphenylcarbinol as the test system. In two runs the methylphenylcarbinol formed had $[\alpha]_D = -25.5^\circ$ in one case and $[\alpha]_D = -22.5^\circ$ in the other, an average of $-23.9^\circ$. Since the rotation of optically pure material is $46^\circ$, the value of $23.9^\circ$ represents a mixture of $76\%$ S(-) and $24\%$ R(+) carbinol, or $52\%$ excess of S(-) isomer.

Stereochemistry of Transfer of Hydride from TPNH

The results obtained when p-chlorobenzaldehyde was reduced to labeled p-chlorobenzyl alcohol in the presence of stereo specifically labeled TPNH are tabulated in Table IV.

These results show clearly that alcohol dehydrogenase utilizes the $\alpha$ hydrogen at carbon atom 4 of TPNH, as it is known to do in the case of DPNH (8-10). Contrariwise the data show conclusively that aromatic aldehyde-ketone reductase utilizes the $\beta$ hydrogen.

DISCUSSION

The reduction in vivo of a number of aromatic ketones in mammals has been described (11). The results of the present study suggest that aromatic A-K reductase may be one of the enzymes responsible for these reductions. Apparently, however, it is not the enzyme responsible for the reduction of acetohexamide, N-cyclohexyl-N'-(4-acetylanisylsulfonyl)-urea, which is readily reduced to the carbinol in the intact species (12).

The ability of aromatic A-K reductase to mediate the reduction of substituted benzaldehydes is difficult to evaluate. In the intact animal benzaldehydes are converted almost exclusively to the corresponding benzoic acid. Also, unlike the case of aromatic ketones, aromatic aldehydes are known to be reduced by liver alcohol dehydrogenase (13, 14).

The effect of ring substitution on the rate of reduction was of interest (cf. Table II). Electron-donating groups, such as $p$-chloro and $p$-nitro, greatly enhance the rate, while the electron-donating group $p$-methoxy enforces a reduced rate. These results are quite consistent with a mechanism involving direct transfer of hydride ion from TPNH to carbonyl carbon of substrate. These results are also consistent with those reported by Blomquist (14) for the relative rates of reduction of $p$-substituted benzaldehydes by liver alcohol dehydrogenase. The fact that aromatic carbonyl compounds serve as admirable substrates for aromatic A-K reductase will greatly facilitate further studies of substituent effects.

The reduction of cyclic ketones by aromatic A-K reductase was also investigated (Table II). In previous work Elliott, Tao, and Williams (15), Elliott, Robertson, and Williams (16), and Cheo, Elliott, and Tao (17) have reported on extensive studies on the reduction of a number of methylcyclohexanones and of the isomeric decaones in the rabbit, while Graves, Clark, and Ringold (18) have investigated the stereochemical aspects of the reduction of cyclic ketones by liver alcohol dehydrogenase.

There were a number of differences between aromatic A-K reductase and alcohol dehydrogenase with respect to the reduction of various cyclic ketones. For example, 2-decalone is a better substrate than cyclohexanone for aromatic A-K reductase, whereas the reverse is true for alcohol dehydrogenase (18). Furthermore, the rigid ketone adamantanone is one-third as active as cyclohexanone in the case of aromatic A-K reductase, while for alcohol dehydrogenase the ratio is 1:500 (19). As is true for alcohol dehydrogenase, cyclohexanone is essentially inactive as a substrate for aromatic A-K reductase. The related five-membered ring compound indanone is completely inactive. The steroid, dihydro-19-nortestosterone, an excellent substrate for 3a-hydroxysteroid dehydrogenase (20), is reduced only slowly by aromatic A-K reductase.

Alcohol dehydrogenase is known to utilize the $\alpha$ hydrogen atom at carbon atom 4 of TPNH (8-10). Dalziel and Dickinson (21) have recently confirmed that TPNH is also utilized, although slowly, as a cofactor for alcohol dehydrogenase. The data obtained in the present study (Table IV) show that when TPNH does serve, it is the $\alpha$ hydrogen atom that is used, as would be anticipated. The finding (Table IV) that aromatic A-K reductase, on the other hand, utilized the $\beta$ hydrogen atom of TPNH clearly differentiates the two enzymes. Indeed, aromatic A-K reductase appears to be more closely related to the reductase from Curculicula falcatu described by Prelog (22), which also utilizes the $\beta$ hydrogen atom of TPNH.

REFERENCES

Reductase for Aromatic Aldehydes and Ketones: THE PARTIAL PURIFICATION AND PROPERTIES OF A REDUCED TRIPHOSPHOPYRIDINE NUCLEOTIDE-DEPENDENT REDUCTASE FROM RABBIT KIDNEY CORTEX
Hilman W. Culp and Robert E. McMahon


Access the most updated version of this article at http://www.jbc.org/content/243/4/848

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/243/4/848.full.html#ref-list-1