Purification and Characterization of Catabolic Dehydroquinase, an Enzyme in the Inducible Quinic Acid Catabolic Pathway of *Neurospora crassa*

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Catabolic dehydroquinase which functions in the inducible quinic acid catabolic pathway in *Neurospora crassa* has been purified 8000-fold. The enzyme was purified by two methods. One used heat denaturation of contaminating proteins; the other used antibody affinity chromatography. The preparations obtained by these two methods were identical by all criteria. The purified enzyme is extremely resistant to thermal denaturation as well as denaturation by urea and guanidine hydrochloride at 25°. It is irreversibly inactivated, although not efficiently dissociated, by sodium dodecyl sulfate and guanidine hydrochloride at 55°. At pH 3.0, the enzyme is reversibly dissociated into inactive subunits. At high concentrations catabolic dehydroquinase aggregates into an inactive, high molecular weight complex. The native enzyme, which has a very high specific activity, has a molecular weight of approximately 220,000 and is composed of identical subunits of 8,000 to 12,000 molecular weight each. The native enzyme and the subunit are both asymmetric.

The first three reactions in the inducible quinic acid catabolic pathway of *Neurospora crassa* are controlled by a tightly linked cluster of four genes—the qa cluster (1). Three of these four genes are the structural genes for the individual enzymes: qa-2, catabolic dehydroquinase (5-dehydroquinate hydrolyase, EC 4.2.1.10); qa-3, quinate dehydrogenase (quinate. NAD oxidoreductase, EC 1.1.1.24); and qa-4, dehydroshikimate dehydrase. The fourth gene, qa-1, encodes a regulatory protein which in the presence of inducer (quinic acid) acts in a positive fashion to initiate synthesis of the qa enzymes. The genetic characteristics of this operon-like regulatory system have been extensively investigated and the system is quite well defined at the genetic level.

As a first step in the biochemical analysis of this regulatory mechanism, one of the qa gene products, catabolic dehydroquinase, has been isolated and extensively characterized. This enzyme has not previously been isolated from *N. crassa* or any other source. Catabolic dehydroquinase was selected because it possesses two characteristics which facilitate purification; i.e., extreme resistance to thermal denaturation and precipitation at low ammonium sulfate concentrations.

Characterization of purified catabolic dehydroquinase by a variety of techniques has revealed that the enzyme possesses several unusual structural, physical, and biochemical properties; e.g., a very high specific activity, a high degree of stability in the presence of a variety of denaturing conditions and reagents, and a polymeric structure composed of approximately 22 identical subunits.

**MATERIALS AND METHODS**

**Strain**

A qa-3 mutant strain, M-16,† which lacks quinate dehydrogenase activity was employed for all extractions. This strain was chosen because it does not metabolize the inducer, quinic acid, and therefore possesses higher levels of induced catabolic dehydroquinase activity than wild type.

**Growth Conditions**

Cultures were started by conidial inoculation of six 2000-ml Erlenmeyer flasks containing 500 ml of Fries minimal media plus 1.5% sucrose. The cultures were shaken at 25° for 16 hours at 300 rpm on a rotary shaker. These cultures were transferred into a barrel containing 200 liters of Fries minimal media plus 1.5% sucrose and grown under forced aeration. After 24 hours the mycelia were harvested by filtration through cheesecloth, washed with water, and resuspended in 100 liters of Fries minimal media plus 1 mM quinic acid. Growth under forced aeration was allowed to proceed for 6 hours and resulted in maximal induction of the qa enzymes. The mycelia were then harvested as above, squeezed dry, and stored at -70° until used.

**Reagents**

Urea, guanidine hydrochloride, and sucrose were Ultra-Pure grade from Schwarz/Mann. Sodium dodecyl sulfate was Sequanol grade from Pierce Chemical Company. Hydrochloric acid for amino acid hydrolyses was Aristar grade from British Drug Houses. Acrylamide monomer was purchased from Eastman and N,N'-methylenebisacrylamide monomer from Canalco. DNase I and RNase B (type III-B) were from Sigma. Fluorescamine (Fluram) was a product of Roche Diagnostics. All other reagents were reagent grade.

Strain M-16 was isolated by Dr. Mary Case.

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‡Postdoctoral Fellow supported by National Institutes of Health Fellowship No. 1 FO2 GM55828 from the Institute of General Medical Sciences.
Catabolic dehydroquinase was purified by two different methods. All procedures were performed at 4°C unless a different temperature is specified. All centrifugations were run at 12,000 × g for 15 min.

**Scheme 1**

**Step 1** - One hundred grams of lyophilized mycelia were ground in a Wiley mill. The resulting powder was suspended at a concentration of 1 g per 20 ml in Buffer A (100 mM potassium phosphate (pH 7.5), 0.4 mM diithiothreitol, 0.1 mM EDTA, and 0.1 mM phenylmethylsulfonyl fluoride (a protease inhibitor)) containing 2.5 mM magnesium chloride. The resulting suspension was stirred for 30 min and the cell debris was removed by centrifugation.

**Step 2** - DNase and RNase were added to the supernatant from Step 1 at a final concentration of 5 μg per ml. The solution was incubated at 37°C for 2 hours.

**Step 3** - The solution from Step 2 was heated at 71°C for 15 min. The denatured proteins were removed by centrifugation.

**Step 4** - Solid ammonium sulfate was added to the supernatant from Step 3 to yield a final concentration of 32% and the suspension was stirred for 30 min. The resulting precipitate was collected by centrifugation. The pellet was gently resuspended in Buffer B (10 mM potassium phosphate (pH 7.5), 0.4 mM diithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride) to yield a final volume of approximately 15 ml. The resuspended pellet was centrifuged to remove insoluble material.

**Step 5** - The supernatant was loaded on a Sephadex G-200 column (2.5 × 80 cm) which had been equilibrated with Buffer B. The column was run at a flow rate of 20 ml per hour collecting 4-ml fractions.

**Step 6** - The active fractions from Step 5 were combined and bound to a DEAE-cellulose column (Whatman DE52, 2.5 × 35 cm) which had been equilibrated with Buffer B. The column was then washed with 75 ml of 1.0 M potassium chloride in Buffer A. The column was then washed with 75 ml of 1.0 M potassium chloride in Buffer A. The catabolic dehydroquinase sample recovered from Step 4 was chromatographed on DEAE-cellulose as described in Scheme 1, Step 6.

**Step 7** - The active fractions were combined, concentrated 10 fold against solid sucrose, and stored at -20°C. Purity was determined by analytical polyacrylamide gel electrophoresis.

**Scheme 2**

**Step 1** - Twenty grams of lyophilized mycelia were ground in a Wiley mill and extracted as in Scheme 1, Step 1.

**Step 2** - The resulting supernatant was made 0.14% in protamine G-200 in the presence of 6 M guanidine hydrochloride according to the method of Martin and Ames (10). Catalase (240,000) and alkaline phosphatase (86,000) were used as internal standard proteins.

**Step 3** - Solid ammonium sulfate was added to the supernatant from Step 2 to yield a final concentration of 32%. The solution was stirred for 30 min and the precipitated proteins were removed by centrifugation.

**Step 4** - The resulting pellet was gently resuspended in Buffer A to a final volume of approximately 15 ml and dialyzed overnight against 1 liter of Buffer A. The dialyzed sample was centrifuged to remove insoluble material.

**Step 5** - The supernatant from Step 3 was loaded on an affinity column (1.9 x 20 cm) of anti-catabolic dehydroquinase immunoglobulin which had been equilibrated with Buffer A. The column was run at a flow rate of 20 ml per hour collecting 4-ml fractions.

**Step 6** - The catabolic dehydroquinase sample recovered from Step 5 was chromatographed on DEAE-cellulose as described in Scheme 1, Step 6.

**Step 7** - The active fractions were combined, concentrated 10 fold against solid sucrose, and stored at -20°C. Purity was determined by analytical polyacrylamide gel electrophoresis.

**Enzyme Assay**

The assay employed for catabolic dehydroquinase has been reported previously (2). Specific activity is expressed as nanomoles dehydroshikimate produced per min mg of protein at 37°C.

**Purification of Catabolic Dehydroquinase**

Catabolic dehydroquinase was purified by two different methods. All procedures were performed at 4°C unless a different temperature is specified. All centrifugations were run at 12,000 × g for 15 min.

**Step 1** - One hundred grams of lyophilized mycelia were ground in a Wiley mill. The resulting powder was suspended at a concentration of 1 g per 20 ml in Buffer A (100 mM potassium phosphate (pH 7.5), 0.4 mM diithiothreitol, 0.1 mM EDTA, and 0.1 mM phenylmethylsulfonyl fluoride (a protease inhibitor)) containing 2.5 mM magnesium chloride. The resulting suspension was stirred for 30 min and the cell debris was removed by centrifugation.

**Step 2** - The resulting supernatant was made 0.14% in protamine G-200 in the presence of 6 M guanidine hydrochloride according to the method of Martin and Ames (10). Catalase (240,000) and alkaline phosphatase (86,000) were used as internal standard proteins.

**Step 3** - Solid ammonium sulfate was added to the supernatant from Step 2 to yield a final concentration of 32% and the suspension was stirred for 30 min. The resulting precipitate was collected by centrifugation. The pellet was gently resuspended in Buffer B (10 mM potassium phosphate (pH 7.5), 0.4 mM diithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride) to yield a final volume of approximately 15 ml. The resuspended pellet was centrifuged to remove insoluble material.

**Step 5** - The supernatant was loaded on a Sephadex G-200 column (2.5 × 80 cm) which had been equilibrated with Buffer B. The column was run at a flow rate of 20 ml per hour collecting 4-ml fractions.

**Step 6** - The active fractions from Step 5 were combined and bound to a DEAE-cellulose column (Whatman DE52, 2.5 × 35 cm) which had been equilibrated with Buffer B. The column was then washed with 75 ml of 1.0 M potassium chloride in Buffer A. The column was then washed with 75 ml of 1.0 M potassium chloride in Buffer A. The catabolic dehydroquinase sample recovered from Step 4 was chromatographed on DEAE-cellulose as described in Scheme 1, Step 6.

**Step 7** - The active fractions were combined, concentrated 10 fold against solid sucrose, and stored at -20°C. Purity was determined by analytical polyacrylamide gel electrophoresis.
Amino Acid Analyses

Amino acid analyses were run on enzyme samples hydrolyzed in vacuo with 6 M hydrochloric acid at 105° for 24 hours. The hydrolysates were analyzed on a Beckman 120-C analyzer.

RESULTS

Purification of Catabolic Dehydroquinase

Using Scheme 1, catabolic dehydroquinase has been purified approximately 8000-fold (Table I). DEAE-cellulose chromatography yielded three or four peaks of catabolic dehydroquinase activity (Fig. 1). Polyacrylamide gel electrophoresis demonstrated that each of the three major activity peaks contained a single homogeneous protein although the fourth peak contained several proteins. The proteins from the three major peaks differ in electrophoretic mobility (Fig. 1) and in the wavelength of their tyrosine fluorescence maximum (Fig. 2). They are, however, identical by several other criteria; i.e. subunit band pattern on sodium dodecyl sulfate-polyacrylamide gels, sedimentation behavior in sucrose density gradients, immunologic cross-reactivity, and amino acid content. Therefore, the differential binding to DEAE-cellulose, the differences in electrophoretic mobility, and the variations in the fluorescence emission spectra probably reflect charge differences resulting from either altered conformational states of the enzyme or differential binding of small charged molecules.

Catabolic dehydroquinase purified using the antibody affinity chromatography procedure (Scheme 2) was identical with the enzyme purified by the standard method by all criteria examined; i.e. electrophoretic behavior, sedimentation behavior in sucrose density gradients, immunologic cross-reactivity, and amino acid content.

Stability of Catabolic Dehydroquinase

Catabolic dehydroquinase is extremely resistant to denaturation. The enzyme can be heated at 80° for 80 min without any loss of activity although it does rapidly inactivate at 90° (13). The enzyme is fully active in 8 M urea whereas guanidine hydrochloride at 25° exerts a reversible denaturing effect. An enzyme sample in 6 M guanidine hydrochloride assayed in the presence of 8 M urea whereas guanidine hydrochloride. However, if an enzyme sample in 6 M guanidine hydrochloride is heated to 55°, inactivation is complete and irreversible. Catabolic dehydroquinase is also irreversibly inactivated by treatment with 2% sodium dodecyl sulfate.

Catabolic dehydroquinase is resistant to cleavage by either trypsin or cyanogen bromide. A lyophilized enzyme sample incubated with 6 M urea for 30 min and digested with 0.02% trypsin in 2 M urea at 37° for 24 hours retained 23% of its original activity. Samples treated with a 100-fold weight excess of cyanogen bromide in 70% formic acid at 37° for 20 hours have up to 25% of their polypeptide chains intact.

Catabolic dehydroquinase is completely, but reversibly, inactivated below pH 5.0. An enzyme sample in pH 3.0 buffer exhibits exponentially increasing activity when diluted 100-fold in a pH 7.2 assay mixture and recovers 100% of its original activity when dialyzed into a pH 7.5 buffer. Enzyme activity is unaffected by alkaline pH.

Determination of Native Molecular Weight of Catabolic Dehydroquinase

Sucrose Density Gradient Centrifugation—The native molecular weight of catabolic dehydroquinase was initially determined by sucrose density gradient centrifugation to be 150,000 (14). In the present studies, an average molecular weight of 167,000 ± 25,000 was calculated from 15 individual determinations. In addition, four sucrose density gradients containing either 2 or 6 M urea revealed an average molecular weight for the enzyme of 166,000 ± 21,500.

Sedimentation Equilibrium Centrifugation—In catabolic dehydroquinase samples concentrated enough to give an absorbance of 0.4 at 280 nm (the limit of sensitivity of the ultraviolet optical system used), the protein aggregated to very high molecular weight species which rapidly pelleted. The addition of 8 M urea did not affect this aggregation, but it could be eliminated by treatment with 6 M guanidine hydrochloride. An enzyme sample brought to equilibrium at 10,000 rpm yielded a nonlinear plot of log c versus r² which could be described by a two-component system with apparent molecular weights of 56,600 and 112,000. Therefore, the addition of 6 M guanidine hydrochloride not only prevented aggregation but caused dissociation of catabolic dehydroquinase.

Light Scattering—The refractive increment of catabolic dehydroquinase was measured on a sample whose absolute protein concentration was determined in duplicate by amino acid analysis. Each of these concentration values was used with the measured refractive increment to calculate two values for the machine constant H according to Equation 1. Turbidity measurements were made on serial dilutions of a second catabolic dehydroquinase sample. Absolute protein concentrations of the two most concentrated samples were determined by amino acid analysis and the absolute concentrations of the other dilutions were calculated. These data were used with each of the H values for two graphic estimations of the molecular weight according to Equation 2.

As shown in Fig. 3, the Hc/r versus c plots are nonlinear and the best fit of the data is two independent lines with the break from linearity occurring at a protein concentration of approximately 2 × 10⁻⁴ g per ml. The two lines extrapolate to give molecular weights of 450,000 ± 70,000 and 210,000 ± 30,000.

Sedimentation Velocity Centrifugation and Gel Filtration—Siegel and Monty (15) have described a technique for determining the molecular weight of a protein on the basis of its sedimentation coefficient and Stokes radius. The rela-

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Total protein mg</th>
<th>Total activity units*</th>
<th>Specific activity units/mg</th>
<th>Recovery %</th>
</tr>
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<tbody>
<tr>
<td>Crude supernatant</td>
<td>76,500</td>
<td>2400</td>
<td>0.03</td>
<td>100</td>
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<tr>
<td>71% Supernatant</td>
<td>11,000</td>
<td>2370</td>
<td>0.22</td>
<td>99</td>
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<td>Ammonium sulfate</td>
<td>480</td>
<td>1880</td>
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<td>78</td>
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<tr>
<td>(0 to 32%)</td>
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</tr>
<tr>
<td>Sephadex G-200</td>
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<td>1510</td>
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<td>63</td>
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<tr>
<td>DEAE-cellulose</td>
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<td></td>
</tr>
<tr>
<td>Peak A</td>
<td>2.2</td>
<td>560</td>
<td>255</td>
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<tr>
<td>Peak B</td>
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<td>610</td>
<td>244</td>
<td>25</td>
</tr>
<tr>
<td>Peak C</td>
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<td>128</td>
<td>9.6</td>
</tr>
<tr>
<td>Peak D</td>
<td>1.4</td>
<td>135</td>
<td>96</td>
<td>5.6</td>
</tr>
</tbody>
</table>

* Nanomoles of dehydroshikimate produced per min at 37°.

Per Ströman, unpublished experiments.
relationship between these parameters and the molecular weight are given in Equation 3. The sedimentation coefficient for catabolic dehydroquinase was determined by sedimentation velocity analysis at 20° and 52,000 rpm. Using protein concentrations low enough to minimize aggregation, a value of 8.69 x 10^{-12} was calculated for the sedimentation coefficient (s_{eq}). The Stokes radius for catabolic dehydroquinase was determined by the method of Porath (16) on the basis of its partitioning on a gel filtration column by application of Equation 4. A Sephadex G-200 column (1.5 x 100 cm) equilibrated with Buffer A was calibrated using several proteins of known Stokes radius. The Kd for catabolic dehydroquinase was measured and a Stokes radius of 62 A was determined graphically (Fig. 4).

Substituting the sedimentation coefficient, Stokes radius, and partial specific volume of 0.726 (calculated from the amino acid content) into Equation 3, a molecular weight of 223,000 was calculated for catabolic dehydroquinase.

**Determination of Subunit Structure of Catabolic Dehydroquinase**

**Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis**—Although catabolic dehydroquinase is inactivated by sodium dodecyl sulfate, initial analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed that the denaturation of the enzyme was incomplete. Several protein bands ranging in molecular weight from approximately 10,000 to 75,000 were present. The actual number of bands observed depended on the method of sample preparation. The more gentle the preparation technique, the more bands were present. Complete dissociation by sodium dodecyl sulfate could be achieved only by prolonged heating at 100° (Table II). The minimum molecular weight for the catabolic dehydroquinase subunit obtained from 43 different sodium dodecyl sulfate-polyacrylamide gel analyses is 10,200 ± 1,100.

**Chromatography on Sephadex G-200 in Presence of Guanidine Hydrochloride**—The subunit molecular weight of catabolic dehydroquinase was determined by chromatography on Sephadex G-200 at 55° in the presence of 0 M guanidine hydrochloride on a column calibrated with pepsin and cytochrome c. Elution of the catabolic dehydroquinase subunit was monitored using the Schaffner-Weissmann protein determination technique (5). The three protein peaks detected had molecular weights of 7,600, 14,000, and 25,000. These probably are the monomer, dimer, and trimer of the subunit.

**Sucrose Density Gradient Centrifugation**—Since catabolic dehydroquinase is reversibly inactivated at low pH, sucrose density gradient centrifugation was performed in phosphate-citrate buffer at pH 3.0 in order to ascertain if this inactivation results from dissociation. The results of one such experiment are shown in Fig. 5A. The enzyme activity (assayed at pH 7.2) appears as a single peak which sediments slightly slower than cytochrome c. A molecular weight of 9,000 ± 1,000 was calculated from the three internal standards.

If the active fractions from a pH 3.0 gradient are combined, dialyzed into pH 7.5 buffer, and centrifuged in a sucrose density gradient at pH 7.5, the activity returns to the position of the native enzyme with a molecular weight of 167,000. Therefore the inactivation of the enzyme which occurs at pH 3.0 results from reversible dissociation to subunits.

Subsequent analysis by fluorescamine protein assay of sucrose gradients of pure catabolic dehydroquinase at pH 7.5 detected a low molecular weight species present in all enzyme preparations (Fig. 5B). Unlike the pH 3.0 dissociated material, this low molecular weight species is incapable of reassociating to give an active enzyme. If the active peak from one gradient is recentrifuged, the low molecular weight species is still present indicating that it is a dissociation product.

**Sedimentation Velocity Centrifugation and Gel Filtration**—The molecular weight of the catabolic dehydroquinase subunit observed at pH 3.0 was calculated from the Stokes radius and sedimentation coefficient according to the method of Siegel and Monty (15) described earlier. The Sephadex G-200 column used to determine the Stokes radius was equilibrated with phosphate-citrate buffer at pH 3.0. Centrifugation was performed at 47,000 rpm for 24 hours. Enzyme activity in the fractions was measured by the standard assay procedure, see “Materials and Methods.” The internal standards were: A, ovalbumin (43,000); B, chymotrypsinogen A (25,000); and C, cytochrome c (12,400). Panel B, the enzyme sample and the gradient were in 100 mM potassium phosphate buffer (pH 7.5) and centrifugation was at 37,000 rpm for 15 hours. Following measurement of enzyme activity by the standard assay procedure, the pH of the fractions was adjusted to approximately 9.0. They were then reacted with fluorescamine and the protein content measured by fluorescence emission.

**Sedimentation Equilibrium**—The subunit molecular weight was determined by sedimentation equilibrium centrifugation.
at 20° and 14,000 rpm in phosphate-citrate buffer (pH 3.0). The average molecular weight calculated from four scans of a single run was 12,500 ± 1,700.

Amino Acid Composition of Catabolic Dehydroquinase—The amino acid composition of catabolic dehydroquinase is given in Table III. The enzyme contains neither cysteine nor tryptophan. No cysteine degradation products were observed and hydrolysis in the presence of thioglycolate verified the absence of tryptophan. The absence of tryptophan is further supported by the fact that no fluorescence is observed when the protein is excited at 295 nm, the wavelength of tryptophan excitation. The minimum molecular weight calculated from the amino acid content is 9,600.

Frictional Ratios of Catabolic Dehydroquinase

The frictional ratios (f/f₀) of catabolic dehydroquinase and its subunit were calculated from Equation 5. Based on a Stokes radius of 62 A and a molecular weight of 223,000, the frictional ratio for native catabolic dehydroquinase is 1.55. The subunit with a Stokes radius of 30 A and a molecular weight of 17,000 has a frictional ratio of 1.78. The frictional ratios indicate that both the native enzyme and the subunit deviate significantly from an ideal globular protein.

Discussion

Catabolic dehydroquinase was purified by two procedures. In one method the key step was a heat treatment at 71° which denatured a large fraction of the contaminating proteins. The enzyme could then be purified to homogeneity by subjecting the 32% ammonium sulfate fraction to gel exclusion chromatography on Sephadex G-200 and ion exchange chromatography on DEAE-cellulose. The principal technique employed in the second method was affinity chromatography on immobilized anti-catabolic dehydroquinase immunoglobulin. Pure enzyme was obtained by subjecting the 32% ammonium sulfate fraction to chromatography on the antibody affinity column followed by ion exchange chromatography on DEAE-cellulose. This procedure gave a significantly lower yield than the original procedure and was not used routinely for purification. It was developed in order to establish that the characteristics of purified catabolic dehydroquinase were not due to alteration of the enzyme during the drastic 71° heat treatment. Catabolic dehydroquinase purified by the two procedures is identical by all criteria; i.e., behavior on ion exchange chromatography and electrophoresis, molecular weight, subunit structure, immunologic cross-reactivity, and amino acid content. Therefore, the 71° heat treatment had no apparent effect on catabolic dehydroquinase and the small subunit size cannot be attributed to the action of heat activated proteases.

The stability of catabolic dehydroquinase is remarkable. The enzyme is extremely stable to thermal denaturation below 90°. It retains 100% of its activity and its native multimeric structure in 8 M urea. In 6 M guanidine hydrochloride at 25°, the enzyme retains 25% of its activity and recovers full enzymatic activity when diluted 1:100 in the standard pH 7.2 assay mixture. Six molar guanidine hydrochloride at 55° or 2% sodium dodecyl sulfate do completely and irreversibly inactivate the enzyme, but the dissociation into monomeric subunits is incomplete and several low molecular weight multimers remain. The enzyme is inactivated rapidly and completely at low pH. This inactivation is reversible since a catabolic dehydroquinase sample in phosphate-citrate buffer (pH 3.0) which has no activity when assayed at pH 3.0, exhibits an exponentially increasing activity approaching the original when assayed in the standard pH 7.2 buffer. Full activity is restored by dialysis into a pH 7.5 buffer. In all cases, inactivation of catabolic dehydroquinase appears to result from dissociation of the active complex rather than from a simple disruption of the active site. The enzyme is also remarkably resistant to proteolytic digestion and cyanogen bromide cleavage.

Measurement of the native molecular weight and the subunit molecular weight for catabolic dehydroquinase was complicated by the tendency of the native enzyme to aggregate at even relatively dilute protein concentrations and its resistance to dissociation into monomeric subunits. Consequently, the molecular weights of the active species and the monomeric subunit were determined by several independent techniques. The results of these determinations are shown in Table IV.

### Table III

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<tr>
<th>Amino acid</th>
<th>Residues/subunit</th>
<th>Molecular weight contribution</th>
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<tbody>
<tr>
<td>Lysine</td>
<td>3.1</td>
<td>397</td>
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<tr>
<td>Histidine</td>
<td>3.9</td>
<td>533</td>
</tr>
<tr>
<td>Arginine</td>
<td>4.3</td>
<td>672</td>
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<tr>
<td>Aspartic acid</td>
<td>7.0</td>
<td>891</td>
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<tr>
<td>Threonine</td>
<td>6.1</td>
<td>617</td>
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<tr>
<td>Serine</td>
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<tr>
<td>Glutamic acid</td>
<td>9.7</td>
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<tr>
<td>Proline</td>
<td>7.9</td>
<td>767</td>
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<tr>
<td>Glycine</td>
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<tr>
<td>Alanine</td>
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<td>Valine</td>
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<tr>
<td>Methionine</td>
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<tr>
<td>Tyrosine</td>
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<tr>
<td>Phenylalanine</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>91.2</strong></td>
<td><strong>9621</strong></td>
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### Table IV

<table>
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<tr>
<th>Method of determination</th>
<th>Native molecular weight</th>
<th>Subunit molecular weight</th>
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<tbody>
<tr>
<td>Sucrose density gradient centrifugation</td>
<td>167,000</td>
<td>9,000</td>
</tr>
<tr>
<td>Light scattering</td>
<td>185,000</td>
<td></td>
</tr>
<tr>
<td>Determination 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Determination 2</td>
<td>232,000</td>
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</tr>
<tr>
<td>Siegel and Monty (15) tech nique</td>
<td>223,000</td>
<td>17,000</td>
</tr>
<tr>
<td>Sodium dodecyl sulfate gel electrophoresis</td>
<td>10,000</td>
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<tr>
<td>Gel exclusion chromatography in 6 M guanidine hydrochloride</td>
<td>7,600</td>
<td></td>
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<tr>
<td>Amino acid analysis</td>
<td>9,600</td>
<td></td>
</tr>
<tr>
<td>Sedimentation equilibrium centrifugation</td>
<td>12,500</td>
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</table>
The molecular weight values obtained from sucrose density gradient centrifugation are erroneous because these calculations are based on the assumption that the protein is globular. Both native catabolic dehydroquinase and its subunit have frictional ratios greater than 1.5 which indicates a considerable degree of asymmetry. The technique of Siegel and Monty (15) corrects the sedimentation data to take into account this asymmetry and yields a native molecular weight for catabolic dehydroquinase of 223,000. Data from the light scattering experiments gave a slightly lower average value, but the difficulties in accurately measuring the absolute protein concentrations make this value subject to a large experimental error. All of the techniques employed (except the Siegel and Monty calculation at pH 3.0) gave a subunit molecular weight in the range of 8,000 to 12,000. The most reliable value is probably 9,600 based on the amino acid content. Based on a subunit molecular weight of 10,000, active catabolic dehydroquinase is a multimer composed of 22 identical subunits. However, because of the uncertainty concerning the exact subunit molecular weight, this number could be as low as 18 or as high as 28. The final determination of the exact subunit molecular weight must await the completion of peptide mapping studies and the sequencing of the polypeptide. These experiments are currently underway.

Acknowledgments—We would like to thank Fred Lewis for his excellent technical assistance. We would also like to thank Dr. Francis J. Johnston for the use of his light scattering instruments and Dr. John M. Brewer for the sedimentation equilibrium analyses.

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Purification and characterization of catabolic dehydroquinase, an enzyme in the inducible quinic acid catabolic pathway of Neurospora crassa.
J A Hautala, J W Jacobson, M E Case and N H Giles


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