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# PURIFICATION AND CHARACTERIZATION OF QUINATE (SHIKIMATE) DEHYDROGENASE, AN ENZYME IN THE INDUCIBLE QUINIC ACID CATABOLIC PATHWAY OF *NEUROSPORA CRASSA*

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## Summary

The bifunctional enzyme quinate (shikimate) dehydrogenase (quinate:$NAD^+$ oxidoreductase, EC 1.1.1.24), which catalyzes the first reaction in the inducible quinic acid catabolic pathway of *Neurospora crassa*, has been purified to homogeneity. The enzyme is a monomer of 41000 daltons with an $s_{20,w}$ = 2.94 S. However, electrophoresis under non-denaturing conditions revealed three protein species, which have both quinate and shikimate dehydrogenase activities. The enzyme, with a single binding site for both substrates, has a $K_m$ of 0.37 mM for quinate and of 1.18 mM for shikimate, although the $V$ is about 3-fold higher with shikimate. Essential sulphydryl groups which were not localized in the active site were detected. Thermal stability of the enzyme was greatly enhanced by low concentrations of quinate, shikimate, NADH, or by high ionic strength.

## Introduction

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-3*, quinate (shikimate) dehydrogenase (quinate:$NAD^+$ oxidoreductase, EC 1.1.1.24); *qa-2*, catabolic dehydroquinase (5-dehydroquinate hydrolyase, EC 4.2.1.10); 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 the synthesis of the *qa* enzymes. The system has been extensively characterized genetically, and the second

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enzyme of the pathway, catabolic dehydroquinase, has been purified and thoroughly studied [2].

Since the discovery of quinate dehydrogenase in *N. crassa* [3], genetic and biochemical evidence has indicated that this same enzyme is responsible for the oxidation of shikimate to dehydroshikimate [3,4]. This bifunctional enzyme has now been purified and found to be a monomer of 41000 daltons. The enzyme has only one binding site for both substrates and exists as three differently charged species. The enzyme has been found to be specifically protected from thermal inactivation by substrates and cofactors, as well as by high ionic strength. Kinetic properties of the enzyme are also reported.

## Materials and Methods

*Strain and growth conditions.* A *qa-4* mutant strain, M18, which lacks dehydroshikimate dehydrase, was used in all extractions since blocking the third reaction in the quinic acid catabolic pathway results in a higher induced level of quinate (shikimate) dehydrogenase [4]. Conditions for growth and induction were the same as previously described [2], except that 200 l of Fries minimal medium plus 1 mM quinic acid were used for enzyme induction.

*Reagents.* Quinic acid, NADH, $NAD^+$, EDTA, α-thioglycerol, phenylmethyl sulfonyl fluoride, dinitrothreitol, nitro blue tetrazolium, phenazine methosulfate, cetyltrimethyl ammonium bromide, β-mercaptoethanol, amido black, Coomasie Blue G-250, Sepharose 4B, Sephadex G-100 and G-200, DNAase, RNAase, bovine serum albumin, myoglobin and cytochrome *c* were purchased from Sigma Chemical Co. Shikimic acid was purchased from Calbiochemical Co., acrylamide from Eastman Chemical Co., bis-acrylamide from Canalco, *p*-chloromercuribenzoate from K and K Labs (Plainview, N.Y.), bromophenol phenol blue from Fisher Scientific Co., sodium dodecyl sulfate from Pierce Chemical Co., and urea AR from Mallinckrodt Chemical Co. 5′-AMP-Sepharose was purchased from Pharmacia Fine Chemicals. The cibacron blue F3G-A was a gift from Ciba-Geigy. Ovalbumin, yeast alcohol dehydrogenase, α-chymotripsinogen A, lysozyme, and horseradish peroxidase were purchased from Worthington Biochemocal Corp. All other compounds were reagent grade.

*Enzyme assays.* Quinate dehydrogenase activity and shikimate dehydrogenase activity were assayed as previously reported [4]. One unit of enzyme is defined as the amount of enzyme which reduces 1 μmol of $NAD^+$ per min. Specific activities are expressed as units per mg of protein.

*Purification of quinate (shikimate) dehydrogenase.* All procedures were performed at 4°C unless otherwise specified. All centrifugations were run at 10900 × *g* for 15 min unless otherwise specified.

Lyophilized mycelia were ground in a Wiley mill. The resulting powder was suspended at 1 g/20 ml Buffer A (20 mM potassium phosphate (pH 7.1)/1 mM EDTA/1 mM α-thioglycerol/0.1 mM phenylmethyl sulfonyl fluoride). The suspension was stirred for 30 min and the cell debris removed by centrifugation. DNAase and RNAase were added to the supernatant at a final concentration of 10 μg/ml. The solution was incubated for 2 h at 25°C and then brought to 40% saturation by addition of 0.67 vol. cold, saturated $(NH_4)_2SO_4$ (pH 7.1). This suspension was stirred for 30 min and the precipitate removed by centrifuga-

tion. The resulting supernatant was brought to 50% saturation with $(NH_4)_2SO_4$, stirred for 30 min and centrifuged. The pellet was resuspended in 10% of the initial volume of Buffer A, and the suspension dialyzed overnight against 10 vols. of Buffer A with two changes.

The dialyzate was cleared by centrifugation for 15 min at 27000 × *g* and loaded on a DEAE-cellulose (Whatman DE-52) column (5 × 50 cm) equilibrated with Buffer A. The column was washed with 2 l of Buffer A plus 0.1 M NaCl and the enzyme eluted with 4 l linear gradient of 0.1—0.3 M NaCl. The active fractions were pooled, concentrated by ultrafiltration and dialyzed overnight against Buffer B (5 mM potassium phosphate (pH 7.1)/0.25 mM EDTA/ 0.25 mM α-thioglycerol/0.25 mM phenylmethyl sulfonyl fluoride). The dialyzed preparation was loaded on a Cibacron Blue-Sepharose 4B column (2.5 × 35 cm) equilibrated with Buffer B. The enzyme was eluted with 0.1 mM NADH in Buffer B. The active fractions were pooled, concentrated by ultrafiltration and subjected to gel filtration on a Sephadex G-100 (superfine) column (2.5 × 90 cm) equilibrated with Buffer A plus 0.1 M NaCl. The active fractions were pooled, concentrated, and analyzed by polyacrylamide gel electrophoresis. The pure enzyme was stored at —20°C.

*Preparation of Cibacron Blue-Sepharose 4B.* Cibacron Blue F3G-A was coupled to the Sepharose 4B by heating at 60°C in the presence of NaCl, and at 71°C in the presence of $Na_2CO_3$ followed by slow cooling at room temperature [5]. The affinity support was recycled by extensive washing with 1 M potassium phosphate buffer (pH 7.1).

*Protein determination.* Protein content in the eluates was monitored at 280 nm using a Uvicord 8301-A in connection with an Ultrorac-7000 fraction collector (LKB Produktor, Sweden). Absolute protein concentrations were determined by the biuretphenol method using bovine serum albumin as a standard [6].

*Thermolability of quinate (shikimate) dehydrogenase.* Crude extracts, dialyzed overnight against Buffer A, were used in these experiments. The samples were supplemented with quinate, shikimate, or NaCl at the indicated concentrations and incubated for 2 h at 40°C. In the time-course experiments, the extracts were made 0.3 M in quinate, shikimate of NaCl, or 0.01 M in NADH and incubated at 40°C for the times indicated.

*Analytical techniques.* Polyacrylamide gel electrophoresis: Analytical gel electrophoresis was performed by the method of Davis [7], using 7.5% acrylamide and 0.2% bisacrylamide at pH 8.9 for the lower gel and 1.25% acrylamide and 0.31% bisacrylamide at pH 6.9 for the stacking gel. Samples of 50—200 μg were electrophoresed at 2 mA/tube. Protein staining was done with 1% amido black in 7% acetic acid. The gel was placed at room temperature in 800 μmol glycine buffer (pH 7.1)/29 μmol $NAD^+$/4 μmol EDTA/4 μmol dithiothreitol/ 4.5 mg nitro blue tetrazolium/0.1 mg phenazine methosulfate/100 μmol quinate or shikimate (pH 7.1) per 10 ml. The gels were scanned at 620 nm (protein) or 520 nm (zymograms) using a Beckman Acta gel scanning accessory.

Electrophoresis in the presence of detergents: Electrophoresis in the presence of urea and SDS was performed according to the method of MacGuillivray et al. [8] Electrophoresis in the presence of cetyltrimethyl ammonium

bromide was performed according to Fairbanks and Avruch [9]. The gels were stained by the Coomassie Blue G-250-$HClO_4$ method [10] and scanned at 615 nm.

Gel filtration: The molecular weight of quinate (shikimate) dehydrogenase was determined by gel filtration on A Sephadex G-100 column (2.5 X 90 cm) according to Andrews [11]. The Stokes radius was measured using a Sephadex G-200 column (1.2 X 85 cm) equilibrated with Buffer A plus 0.1 M NaCl [12].

Sucrose density gradient centrifugation: Sucrose density gradient centrifugations were performed according to the method of Martin and Ames [13] at 55000 rev./min and 4°C for 19 h in a SW65K rotor. The gradients were prepared in Buffer A plus 50 mM KCl. Horseradish peroxidase ($s_{20,w}$ = 3.48 S) was included as an internal standard. The crude extracts were precipitated at 50% $(NH_4)_2SO_4$ saturation and extensively dialyzed against Buffer A prior to being loaded on the gradient.

Analytical ultracentrifugation: Sedimentation velocity analysis was performed in a Beckman model E ultracentrifuge as described by Schachman [14].

Isoelectric focusing: Isoelectric focusing of quinate (shikimate) dehydrogenase was performed using a 100 ml column containing a linear 0—47% sucrose density gradient with an ampholyte concentration of 1%.

## Results

*Affinity chromatography of Quinate (shikimate) dehydrogenase*

The enzyme showed affinity for Cibacron Blue F3G-A Sepharose, especially

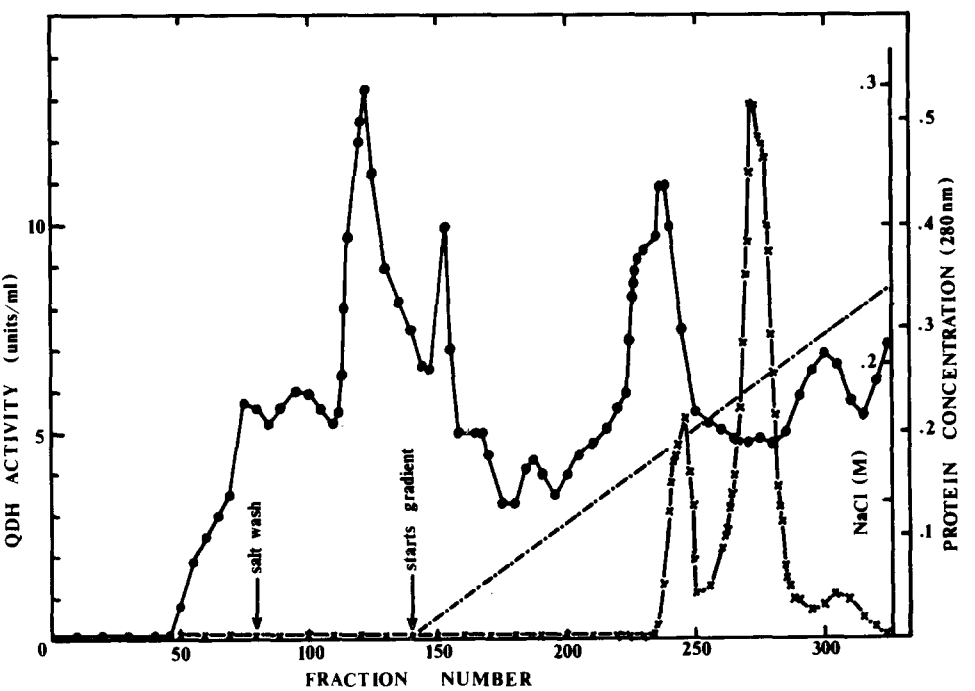


Fig. 1. DEAE-cellulose chromatography of quinate (shikimate) dehydrogenase. The enzyme was chromatographed in DEAE-cellulose as described in Materials and Methods with a flow rate of 100 ml/h collecting 300-drop fractions. ●, protein; X, quinate dehydrogenase (QDH) activity.

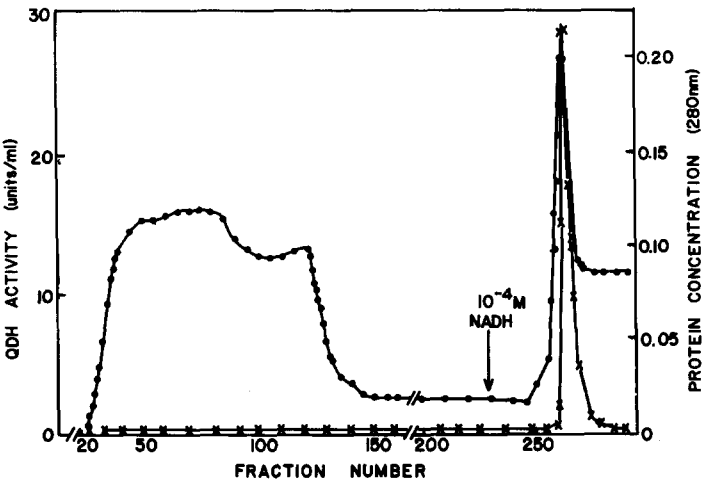


Fig. 2. Affinity chromatography of quinate (shikimate) dehydrogenase (QDH) in Cibacron Blue-Sepharose. The enzyme was chromatographed as described in Materials and Methods with a flow rate of 20 ml/h collecting 80-drop fractions. ●, protein; X, quinate dehydrogenase.

after the enzyme had been partially purified via DEAE-cellulose chromatography, and Buffer B was used to equilibrate the gel. A 0—1 M NaCl gradient in the same buffer eluted the quinate dehydrogenase, but a more specific elution method was required. Neither 20 mM quinic, 20 mM dehydroquinic, 20 mM shikimic acids, nor 50 mM $NAD^+$ could release the enzyme from the gel. Fig. 2 shows, however, that 0.1 mM NADH eluted the quinate dehydrogenase without appreciable loss. A concentration of 0.01 mM NADH did not release the enzyme.

*Purification of quinate dehydrogenase*

Quinate dehydrogenase has been purified 450-fold to a specific activity of 87 units/mg protein with a 10% recovery (Table I). Since the quinate dehydrogenase bound to DEAE-cellulose at 0.1 M NaCl, a significant number of the contaminating proteins were removed by extensive washing with 0.1 M NaCl (Fig. 1). The activity was then eluted in three peaks by a shallow linear salt gradient (Fig. 1). The enzyme bound efficiently to a Cibacron Blue-Sepharose

TABLE I

PURIFICATION OF QUINATE (SHIKIMATE) DEHYDROGENASE OF *N. CRASSA*

| Purification (step) | Total activity * (units) | Total protein (mg) | Specific activity (units/mg) | Purification (-fold) | Recovery (%) |
|---|---|---|---|---|---|
| Crude extract | 5157 | 26 650 | 0.19 | 1 | 100 |
| $(NH_4)_2SO_4$ (40—50%) | 2179 | 2 975 | 0.73 | 3.8 | 42 |
| DEAE-cellulose | 1485 | 372 | 3.99 | 20.6 | 29 |
| Cibacron Blue | 864 | 10.4 | 82.82 | 428 | 17 |
| Sephadex G-100 | 520 | 6 | 86.87 | 448 | 10 |

* Quinate dehydrogenase.

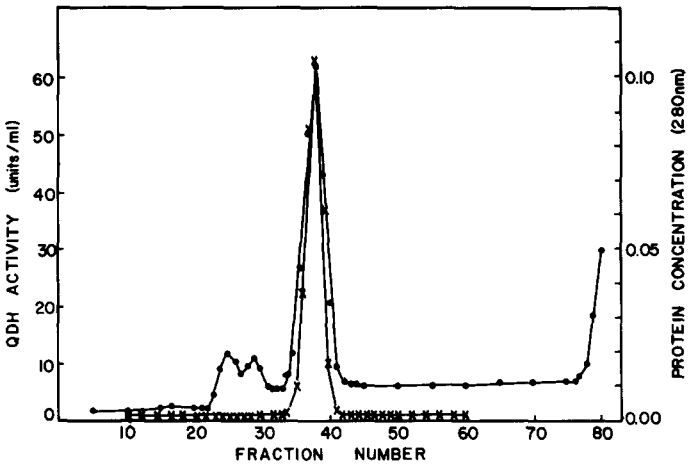


Fig. 3. Gel filtration of quinate (shikimate) dehydrogenase (QDH) through Sephadex G-100. The enzyme was subjected to gel filtration as described in Materials and Methods with a flow rate of 18 ml/h collecting 80-drop fractions. ●, protein; X, quinate dehydrogenase activity.

affinity support and was specifically eluted by 0.1 mM NADH (Fig. 2). Gel filtration on Sephadex G-100 yielded a single narrow peak of activity which matched exactly the main protein peak (Fig. 3). At this stage, the protein was apparently homogeneous as shown by a single moving boundary in sedimentation velocity experiments. Throughout the purification, shikimate dehydrogenase activity copurified with quinate dehydrogenase activity. Polyacrylamide gel electrophoresis of the pure enzyme demonstrated the presence of three protein species (Fig. 4) although the central band was clearly predominant. All the bands had quinate and shikimate dehydrogenase activities (Fig. 4). Zymograms of crude extracts showed the same three bands, although the pattern changed somewhat during purification.

*Molecular size*

Sucrose density gradient centrifugation of the purified enzyme or the crude extract yielded a single peak of quinate dehydrogenase activity with a sedimentation coefficient of 2.8 S (Fig. 5). Gel filtration of quinate dehydrogenase on a calibrated column of Sephadex G-100 produced a single peak corresponding to 41700 daltons for both purified and crude preparations.

Gel electrophoresis in urea-SDS showed a single protein band with a molecular weight of 39000. Electrophoresis in the presence of ceyltrimethyl ammonium bromide also gave a single protein band with a molecular weight of 41000.

The Stokes radius of quinate (shikimate) dehydrogenase was determined to be 28.8 Å. Sedimentation velocity experiments indicated a value of 2.9 S for the sedimentation coefficient. From these data, a molecular weight of 43000 and a frictional ratio of 1.22 were calculated.

*Kinetic properties*

The purified enzyme is $NAD^+$ specific since its activity is 10 times higher

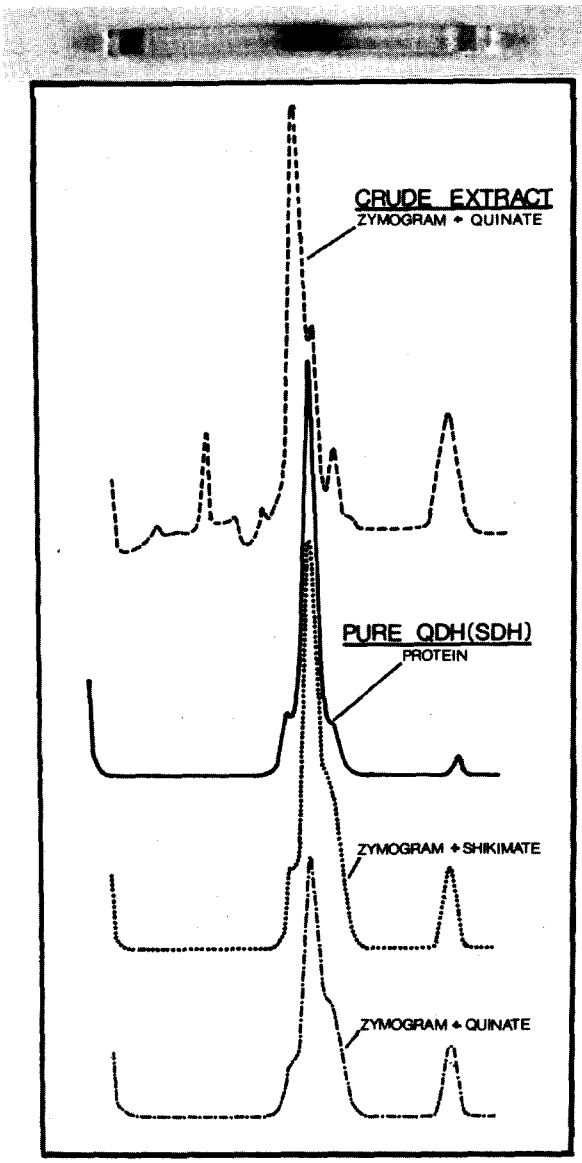


Fig. 4. Electrophoretic patterns of quinate (shikimate) dehydrogenase (QDH/SDH). Samples of 100 $\mu$l containing either 0.2 unit of activity (crude extract) or 50 $\mu$g of protein (pure enzyme), were subjected to disc gel electrophoresis as described in Materials and Methods. After staining for protein, or for quinate or shikimate dehydrogenase activity, the gels were scanned at 620 nm (protein), or 520 nm (zymograms).

with $NAD^+$ than with $NADP^+$. The enzyme shows about 3-fold higher affinity for quinate ($K_m = 3.7 \cdot 10^{-4}$ M) than for shikimate ($K_m = 11.7 \cdot 10^{-4}$ M); however, the $V$ is approx. three times higher with shikimate as substrate (Figs. 6A and 6C). At saturating concentrations of both shikimate and quinate, the activity observed was only 41% of the sum of the separate activities. Dehydroquinate, a product of the quinate dehydrogenase reaction, produced an identi-

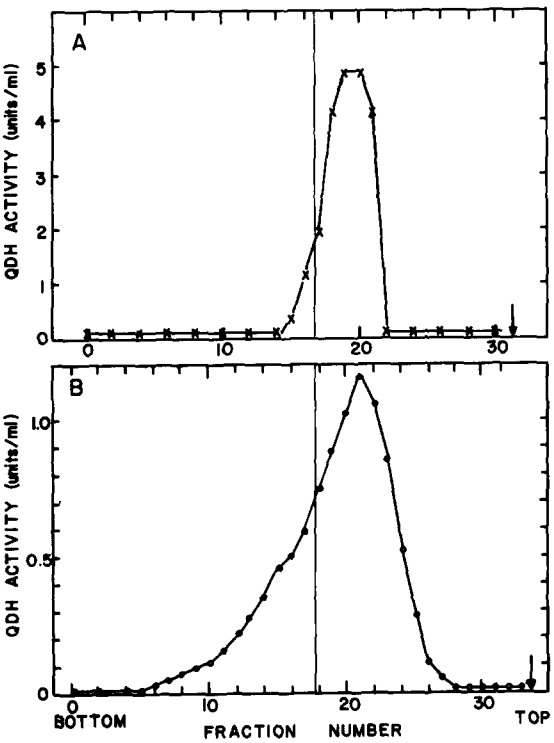


Fig. 5. Sucrose density gradient centrifugation of quinate (shikimate) dehydrogenase (QDH). 100 $\mu$g of pure enzyme (A), or 3 units of enzyme from crude extracts (B), plus 5 $\mu$g horseradish peroxidase, were centrifuged as described in Materials and Methods. 10-drop fractions were collected and quinate dehydrogenase and peroxidase activities determined. The vertical line indicates the position of horseradish peroxidase.

cal inhibition of both the quinate and shikimate dehydrogenases (Figs. 6B and 6D), as was also the case for NADH.

The effect of several sulphydryl reagents on the quinate (shikimate) dehydrogenase activity is shown in Table II. Thiols, especially dithiothreitol, enhanced the activity. *p*-Chloromercuribanzoate, even at a concentration as low as 50 $\mu$M, totally inhibited the enzyme, but the activity partially recovered after treatment with $\beta$-mercaptoethanol. None of the substrates, products, or cofactors (quinate, dehydroquinate, shikimate, $NAD^+$, NADH, $NADP^+$ or NADPH) could protect the enzyme against *p*-chloromercuribenzoate inhibition.

*Thermal stability*

The relative instability of quinate dehydrogenase, especially during final stages of purification, led us to investigate possible ways to stabilize the protein. Preliminary experiments showed that high ionic strength, such as 0.1 M $(NH_4)_2SO_4$, NaCl or phosphate buffer, or the presence of 0.1 M quinate or shikimate, protected the enzyme markedly against thermal inactivation. Fig. 7 shows the effect of different concentrations of quinate, shikimate and NaCl in preventing thermal inactivation of quinate dehydrogenase at 40°C. Both substrates can protect the enzyme completely at concentrations at least one order

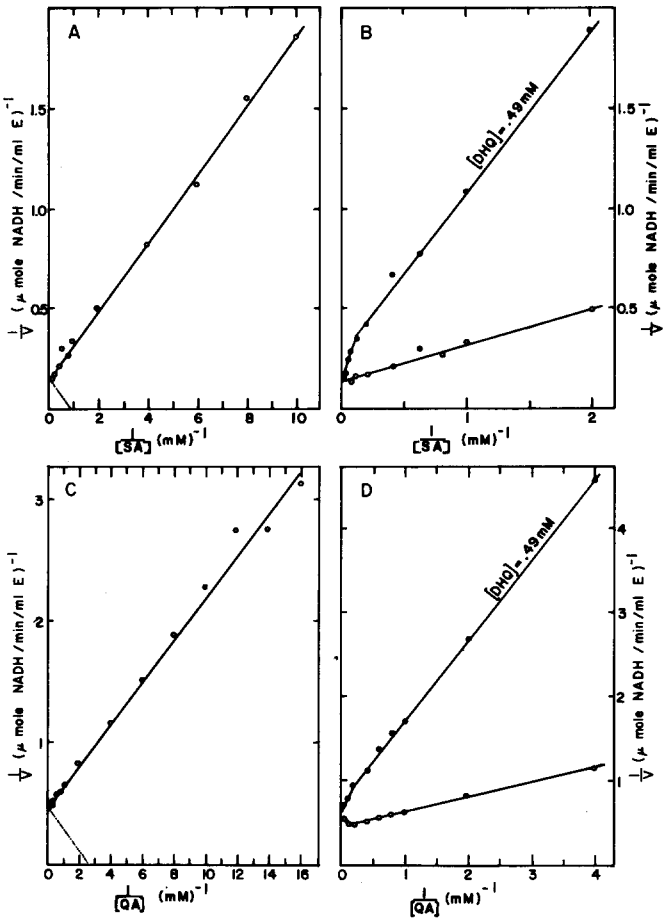


Fig. 6. Double reciprocal plots of quinate (QA) and shikimate dehydrogenase activities versus substrate concentration. 1 $\mu$g of pure enzyme was assayed in the presence of different concentrations of shikimate (SA) (A and B), or quinate (C and D), with or without added dehydroquinate.

TABLE II

EFFECT OF SEVERAL SULPHYDRYL REAGENTS ON THE ACTIVITY OF QUINATE (SHIKIMATE) DEHYDROGENASE FROM *N. CRASSA*

50 $\mu$g of pure enzyme were preincubated for 2 h at room temperature in Buffer A plus 0.1 M NaCl in the presence of the substances indicated at the given concentrations. Shikimate dehydrogenase activity was then measured in 2-$\mu$l samples. The activity in the control was 139 units/ml. Reactivation with $\beta$-mercaptoethanol was tested in the mixture with *P*-chloromercuribenzoate after 2 h incubation.

| System | Concentration (mM) | Activity (%) |
|---|---|---|
| Control | — | 100 |
| *P*-chloromercuribenzoate | 0.05 | 0 |
| $\alpha$-Thioglycerol | 1 | 121 |
| Thioglycolic acid | 1 | 102 |
| $\beta$-Mercaptoethanol | 1 | 119 |
| Dithiothreitol | 1 | 152 |
| *P*-Chloromercuribenzoate, $\beta$-mercaptoethanol | 0.05, 0.2 | 74 |

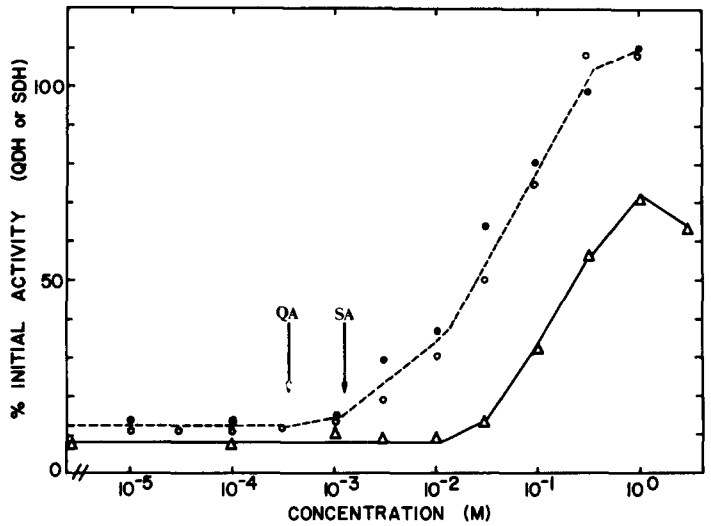


Fig. 7. Protection by quinate, shikimate, or NaCl, against thermal denaturation. Crude extracts were subjected to heat treatment, as described in Materials and Methods, in the presence of quinate (○), shikimate (●), or NaCl (△), at the indicated concentrations. Then the activities quinate dehydrogenase (○, △) and shikimate dehydrogenase (●) were determined. 100% activities were 3 and 8.5 units/ml, respectively. $K_m$ values for quinate (QA), and shikimate (SA) are indicated by the arrows.

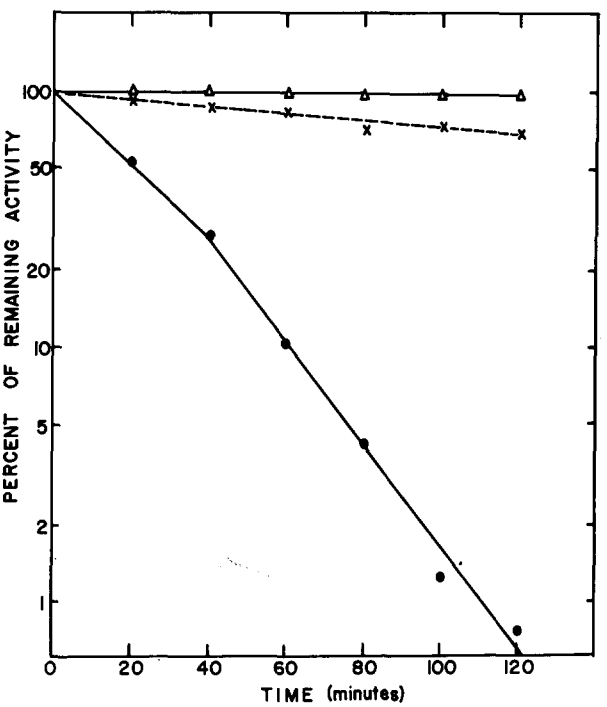


Fig. 8. Time-course of heat inactivation. Crude extracts were subjected to heat inactivation, as described in Materials and Methods, in the absence (●), or in the presence of quinate, shikimate or NADH (△), or NaCl (X). Quinate dehydrogenase or shikimate dehydrogenase (in the mixture with shikimate) were determined at the indicated times. 100% activity was 3.5 and 10.5 units/ml, respectively.

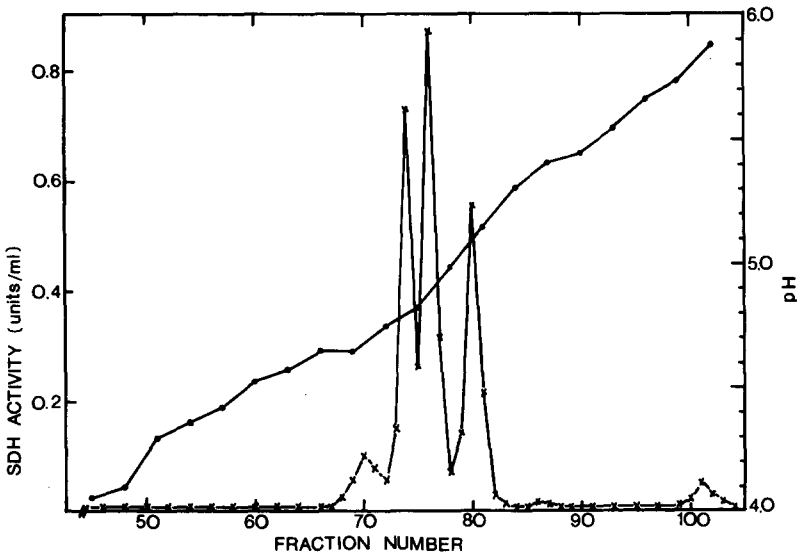


Fig. 9. Isoelectric focusing of quinate (shikimate) dehydrogenases. 1 mg of pure protein was submitted to isoelectric focusing for 3 days as described in Materials and Methods. Then 30-drop fractions were collected and shikimate dehydrogenase activity (X) and pH (•) were determined.

of magnitude lower than the required NaCl concentration. Fig. 8 presents the time course of thermal inactivation of the enzyme at 40°C. The unprotected enzyme decays with a half-life of about 20 min. However, in the presence of NaCl the half-life increased to about 180 min, and when quinate, shikimate or NADH are present, the enzyme is completely stable. However, $NAD^+$ did not protect to any extent. In the presence of 0.1 M NaCl the pure enzyme can be stored at −20°C without appreciable loss of activity (half-life approx. 40 days).

*Separation of electrophoretic forms of quinate dehydrogenase*

As seen in Fig. 1, gradient elution of the DEAE-cellulose column yielded three peaks of activity, which upon electrophoresis, showed a partial separation of the three species. The first peak was enriched in the least mobile band, the second in the central band, and the third in the most mobile band. The results of isoelectric focusing of the pure protein are shown in Fig. 9. Three main peaks of activity with pI values of 4.79, 4.88, and 5.09 can be distinguished. However, isoelectric focusing was not used for purification purposes, since the protein was significantly inactivated by this procedure.

## Discussion

In 1954 Davis et al. [15] found in *Aerobacter aerogenes* a $NAD^+$-dependent quinate dehydrogenase unable to oxidize shikimic acid. They considered this enzyme to be involved in the biosynthesis of aromatic amino acids. Several other bacteria and fungi [3,4,16—18] possess a soluble, $NAD^+$-dependent dehydrogenase which can oxidize both quinate and shikimate. Other bacteria have a particulate, pyridine nucleotide-independent dehydrogenase which also acts on both substrates [19—22]. Higher plants, on the other hand, possess a $NADP^+$-

dependent shikimate dehydrogenase which functions in the reverse direction in the aromatic amino acid biosynthetic pathway [23—31]. The present study involves the purification and characterization of the $NAD^+$-dependent quinate dehydrogenase of *N. crassa*.

Cibacron Blue has been reported to bind different proteins, including several dehydrogenases, having in common a "dinucleotide fold" [32]. Quinate dehydrogenase bound to the immobilized ligand when a low ionic strength buffer was used. NADH, a reaction product, was found to release the enzyme efficiently from the column even at a concentration of 0.1 nM which is one or two orders of magnitude lower than that required for elution of other dehydrogenases [32]. The enzyme eluted in a narrow peak with a high specific activity and the minor contaminant proteins were eliminated by gel filtration on Sephadex G-100.

Earlier genetic and biochemical studies [1,3,4] had suggested that a single enzyme was responsible for both the quinate and shikimate dehydrogenase activities. The current study confirmed this conclusion. First, both activities copurified at a constant ratio (shikimate dehydrogenase:quinate dehydrogenase approx. 3) which is identical to the activity ratio of the pure enzyme. In addition, both activities, as assayed by specific zymograms, showed the same pattern on gel electrophoresis. The enzyme shows slightly more affinity for quinate, but its activity is higher with shikimate. Consequently, we suggest it be designated as quinate (shikimate) dehydrogenase.

The activity in the presence of both substrates, quinate and shikimate, and the similar type of inhibition of both activities, possibly of a competitive type, suggest the existence of a single binding site for which both substrates and the reaction product compete (Fig. 6). The lack of preotection against *p*-chloromercuribenzoate inhibition suggests that the essential sulphydryl groups are not localized in the binding site (Table II).

The thermal stability of the enzyme was greatly enhanced by the presence of either high ionic strength or lower concentrations of substrates and cofactors: quinate, shikimate, or NADH (but not $NAD^+$). Increasing concentrations of quinate or shikimate protected both activities to the same extent, another indication of a single enzyme. However, the concentrations required for half-protection are at least one order of magnitude above their respective $K_m$ values indicating that the protection is not exclusively due to interaction with the binding site. The inactivation of the unprotected enzyme showed, in different experiments, two steps. A similar biphasic inactivation has been described for dehydroshikimate reductase of potato root [26].

The pure enzyme has an activity 10-fold higher with $NAD^+$ as cofactor, behaving in this respect as does the enzyme from several bacteria [15,17] and fungi [18]. The NAD independence of other catabolic quinate (shikimate) dehydrogenases [19—22] may be related to their particulate nature. In any case, the specificity of the catabolic enzyme is different from that of the biosynthetic dehydroshikimate reductase, which is $NADP^+$ dependent. The lack of any spectral features in quinate (shikimate) dehydrogenase from *N. crassa* (Barea, J.L., unpublished) indicates that this soluble enzyme is also very different from the particulate enzyme of *Acetomonas oxidans* [19] for which a tightly bound flavoprotein and cytochrome *c*-555 have been postulated.

Several dehydrogenases have been shown to have a multimeric structure [33]. With quinate (shikimate) dehydrogenase, the presence, in both the pure material and crude extracts, of three differently charged species might be indicative of a similar situation (Fig. 4). However, the existence of a single peak of activity upon Sephadex G-100 filtration and sucrose density gradient centrifugation as well as the single moving boundary in sedimentation velocity experiments, pointed to a monomeric structure. Gel electrophoresis under denaturing conditions has confirmed the existence of a single polypeptide chain of 41000 ± 1600 daltons. This molecular weight is in good agreement with that calculated from a sedimentation coefficient of 2.94 S and a Stokes radius of 28.8 Å (43000). The frictional coefficient of 1.22 suggests a non-globular shape for the molecule.

It might be argued that the three electrophoretic species are an artifact. However, the partial separation of the quinate (shikimate) dehydrogenase into three different species by DEAE-cellulose chromatography and resolution of the pure enzyme into three distinct peaks by isoelectric focusing confirms their reality. Several other monomeric proteins have been shown to have species with different electrical charges [34—36]. Since quinate (shikimate) dehydrogenase is encoded in a single gene [4], and the enzyme is a monomer, only a post-translational modification could account for the charge differences. The presence during purification of protease inhibitors and thiol reagents should exclude either proteolytic cleavage or sulphydryl oxidation as the mechanism of modification. The protein does not contain polysaccharides (Reinert, W., unpublished) nor does it appear to be a phosphoprotein (Barea, J.L., unpublished). Deamidation of asparagine and glutamine residues could thus be an explanation of the molecular heterogeneity [37].

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