A Novel Diazonium-Sulfhydryl Reaction in the Inactivation of Yeast Alcohol Dehydrogenase by Diazotized 3-Aminopyridine Adenine Dinucleotide*

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SUMMARY
Diazotized 3-aminopyridine adenine dinucleotide has been found to modify four sulfhydryl groups per molecule of enzyme during the complete inactivation of yeast alcohol dehydrogenase. The reaction of sulfhydryl groups was indicated by titration studies with 5,5'-dithiobis(2-nitrobenzoic acid) as well as isolation and quantitation of the cysteinyl derivative released by acid hydrolysis of the modified enzyme. The cysteinyl derivative was identified as S-(3-pyridyl)cysteine. Authentic S-(3-pyridyl)cysteine was synthesized and structurally characterized for these studies.

Diazonium-sulfhydryl reactions were demonstrated for a number of diazonium derivatives with cysteine, homocysteine, glutathione, and mercaptoethanol at 0-4°C and neutral pH. Second order rate constants were determined in reactions of these sulfhydryl compounds with diazotized 1-methyl-3-aminopyridinium chloride, diazotized 3-aminopyridine adenine dinucleotide, and diazotized 3-aminopyridine adenine dinucleotide phosphate.

EXPERIMENTAL PROCEDURE
Materials
Yeast alcohol dehydrogenase, NAD, NADP, L-cysteine, glutathione (reduced form), D(+)-glucosamine HCl, D(+)-galactosamine HCl, and DTNB were obtained from Sigma Chemical Co.; n-homocysteine was obtained from ICN K & K Laboratories; 3-aminopyridine was obtained from Eastman Kodak Co. and sublimed before use; amino acid calibration mixture type 1 was obtained from Beckman-Spinco.

1-Methyl-3-aminopyridinium chloride was synthesized by the Menschutkin reaction (1), and AAD was prepared by means of the Hofmann hypobromite reaction and extensively diazylzed according to Fisher et al. (1). Diazotized AAD and 3-aminopyridine adenine dinucleotide phosphate was prepared from NADP and 3-aminopyridine through the pig brain nicotinamide adenine dinucleotide nucleosidase-catalyzed pyridine base exchange reaction.2 Bio-Gel P2, 100 to 200 mesh, was obtained from Bio-Rad Laboratories.

In recent studies (1), the chemical conversion of NAD to 3-aminopyridine adenine dinucleotide through the Hofmann hypobromite reaction was demonstrated to proceed with a 68% yield. The chemical, spectrophotometric, and fluorimetric properties of AAD were reported and, as an analog of NAD, this dinucleotide was shown to be a coenzyme-competitive inhibitor of several NAD-requiring enzymes (1). It was further observed that the 3-aminopyridine moiety of AAD could be diazotized by reaction with nitrous acid and the resulting diazonium chloride could be azo-coupled with N-1-naphthylethylendiamine to form an azo dye. The investigation of diazotized AAD as a site-labeling reagent for dehydrogenases revealed a

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‡ The abbreviations used are: AAD, 3-aminopyridine adenine dinucleotide; DTNB, 5,5'-dithiobis(2-nitrobenzoic acid); AADP, 3-aminopyridine adenine dinucleotide phosphate.

1 B. M. Anderson, unpublished results.

2 B. M. Anderson, unpublished results.
Methods

Characterization of Compounds—Thin layer chromatography was performed with the use of Eastman Chromagram cellulose sheets containing fluorescent indicator; the solvents used were 0.1 M acetic acid-95% ethanol (1:1, v/v) and butanol-acetic acid-water (6:2:3, v/v). The spots were first detected by ultraviolet light, then sprayed with ninhydrin spray and observed after 1 hour at room temperature.

Compounds were separated and purified on a Bio-Gel P2 column (6 X 200 cm) with distilled water as the eluting agent.

Ultraviolet spectrophotometric data were obtained with a Beckman ACTA III spectrophotometer. Infrared spectra were obtained with a Beckman 5A spectrophotometer on compounds in KBr pellets. Nuclear magnetic resonance spectra were obtained with a JEOL model JMN-PS-100 nuclear magnetic resonance spectrophotometer at frequency 100 MHz and ambient temperatures with compound dissolved in D2O.

Inactivation of Enzymes—DTNB titrations (8) for free sulfhydryl groups were carried out at pH 8.0 in 0.1 M sodium phosphate solution at room temperature, by measuring the absorbance at 412 nm with a Zeiss PMQ II spectrophotometer. A standard curve was determined for cysteine.

Yeast alcohol dehydrogenase was assayed by measuring the formation of NADH at 340 nm. The assay mixture contained 4.8 mM yeast alcohol dehydrogenase, 350 mM ethanol, 8 mM NAD, 10 mM sodium pyrophosphate, pH 8.8, in a total volume of 3 ml.

Amino acid analysis of enzyme derivatives was performed by sealing samples in 6 M sodium pyrophosphate, pH 8.8, in a total volume of 3 ml. A standard curve was determined for cysteine.

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Enzyme (ymoles)

Time (min)

0

10

20

30

FIG. 1. DTNB titration of yeast alcohol dehydrogenase before and after inactivation by diazotized AAD. Reaction mixtures contained 0, 1, 1.65, and 3.3 X 10^-5 M native or modified enzyme, 6 M guanidine HCl, 10^-4 M DTNB, 0.1 M sodium phosphate buffer, pH 7.6, in a total volume of 1 ml. For the enzyme modification, corresponding amounts of enzyme were inactivated with 7.5 M diazotized AAD at pH 7.0 prior to the addition of guanidine HCl, DTNB, and pH 7.6 buffer. •, native yeast alcohol dehydrogenase; ■, yeast alcohol dehydrogenase inactivated by diazotized AAD.

RESULTS

Inactivation of Yeast Alcohol Dehydrogenase by Diazotized 3-Aminopyridine Adenine Dinucleotide—The incubation of yeast alcohol dehydrogenase with 7.5 mM diazotized AAD at pH 7.0 resulted in complete inactivation of the enzyme in 15 min. Preliminary studies by DTNB titration of sulfhydryl groups indicated a concomitant loss of free sulfhydryl groups of the enzyme during the inactivation process. Experiments were carried out to characterize quantitatively the change in free sulfhydryl groups during complete inactivation of the enzyme. Total free sulfhydryl groups of native yeast alcohol dehydrogenase were determined by DTNB titration at three concentrations of enzyme (Fig. 1). In a second experiment, after complete inactivation by diazotized AAD, the modified enzyme was assayed for free sulfhydryl groups by DTNB titration under identical conditions, and a lower concentration of sulfhydryl groups was indicated (Fig. 1). Comparison of the sulfhydryl contents of the native and the modified enzyme showed a loss of approximately 3.5 to 4 sulfhydryl groups per molecule of yeast alcohol dehydrogenase after the inactivation process.

Diazonium Sulfhydryl Reactions Since the inactivation of yeast alcohol dehydrogenase by diazotized AAD indicated the modification of cysteine sulfhydryl groups of the enzyme, studies were initiated to investigate reactions of diazonium derivatives with simple sulfhydryl-containing compounds. 1-Methyl-3-aminopyridinium chloride was diazotized in 0.1 M nitrous acid at 0-4°. After 10 min, excess nitrous acid was removed by the addition of 0.2 M ammonium sulfamate. After a second 10-min incubation, the pH of the solution was adjusted to 7.0, mixed with sodium phosphate buffer and a cysteine solution. The resulting reaction mixture contained 0.1 M sodium phosphate buffer, pH 7.0, 100 µM cysteine, and diazotized 1-methyl-3-aminopyridinium chloride in a total volume of 6 ml. Aliquots were removed at timed intervals for the DTNB assay of the sulfhydryl concentration. The rates of disappearance of sulfhydryl groups with five different concentrations of diazotized 1-methyl-3-aminopyridinium chloride are shown in Fig. 2. From the linear relationships observed, pseudo-first order rate
The reactions were carried out in 0.1 M sodium phosphate buffer, pH 7.0, at 0-4°, with the use of a fixed sulfhydryl concentration of 0.1 mM, and varying diazonium concentrations 1 to 2.33 mM. Sulfhydryl contents of aliquots were assayed by 1 mM DTNB in 0.1 M sodium phosphate, pH 6.0.

<table>
<thead>
<tr>
<th>Diazonium compounds</th>
<th>Sulfhydryl compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cysteine</td>
</tr>
<tr>
<td>Diazoated AM*</td>
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</tr>
<tr>
<td>Diazoated AAD</td>
<td>14.4</td>
</tr>
<tr>
<td>Diazoated AADP</td>
<td>24</td>
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</table>

* AM, 1-methyl-3-aminopyridinium chloride.

Constants were calculated from the equation $k_{obs} = 0.693/t_{1/2}$. The pseudo-first order rate constants increased linearly with increasing concentration of diazotized 1-methyl-3-aminopyridinium chloride (Fig. 3). From the slope of the line in Fig. 3, a second order rate constant of 30 liters min⁻¹ mole⁻¹ was determined for this reaction. In the same manner, second order rate constants were determined for the reactions of diazotized 1-methyl-3-aminopyridinium chloride, diazotized AAD, and diazotized AADP with cysteine, homocysteine, glutathione, and mercaptoethanol and are listed in Table I. Aromatic diazonium derivatives of aniline, p-aminobenzoic acid, sulfanilic acid, and arsanic acid, as well as diazotized 3-aminopyridine were also shown to react with the above sulfhydryl-containing compounds, the reactions being essentially instantaneous under the reaction conditions employed.

Synthesis and Characterization of S-(3-pyridyl)cysteine—After demonstrating that certain sulfhydryl groups of yeast alcohol dehydrogenase are modified by reaction with diazotized AAD, and that diazonium-sulfhydryl reactions do occur with simple sulfhydryl compounds, studies were initiated to characterize the modified cysteine residue arising during the inactivation of yeast alcohol dehydrogenase by diazotized AAD. Spectrophotometric analysis of the inactivated dehydrogenase indicated covalent attachment of the intact dinucleotide molecule (1). Complete acid hydrolysis of the inactivated dehydrogenase, in addition to releasing free amino acids, would be expected to hydrolyze the pyridine riboside linkage of the attached dinucleotide and produce a cysteine derivative containing the pyridine ring only. Experiments were carried out to synthesize such a compound by reacting diazotized 3-aminopyridine with cysteine.

Sodium nitrite (130 mg) and 3-aminopyridine (188 mg) were dissolved in 10 ml of ice-cold 1 N HCl and allowed to react for 10 min. Ammonium sulfamate (230 mg) was then added, and the solution was stirred vigorously for 10 min. Cysteine (242 mg) was then added and stirring continued for 15 min. All reactions were performed at 0-4°. The resulting solution was chromatographed on a Bio-Gel P2 column (5 x 200 cm) and eluted with distilled water. A major peak with an absorption maximum at 250 nm yielding a single ninhydrin-positive spot on thin layer chromatography was eluted at an elution volume of approximately 3 liters. A minor peak with an absorption maximum at 315 nm was eluted at about 200 ml after the major peak. Thin layer chromatography of this minor component revealed two ninhydrin-positive spots, one of which corresponded to that of the major peak. The fractions from the major peak were collected and lyophilized. Thin layer chromatography of this product showed a single ultraviolet-quenching spot which was also ninhydrin positive, $R_F = 0.75$ for the solvent system, 0.1 M acetic acid-0.5% ethanol (1:1, v/v), and $R_F = 0.65$ for the solvent system, butanol-acetic acid-water (5:2:3, v/v).

Elemental analysis of carbon, hydrogen, and nitrogen supported the formula $C_{1}H_{11}N_{2}O_{8}$.

\[ C_{1}H_{11}N_{2}O_{8} \]

Calculated: C 48.47, H 5.08, N 14.13

Found: C 48.25, H 4.65, N 13.69

The melting point was 183-185° (uncorrected) with decomposition. The ultraviolet spectrum of this product in 0.1 M sodium phosphate buffer, pH 7.0, showed absorption maxima at 210, 250, and 280 nm with molar extinction coefficients of $1.57 \times 10^4$, $1.12 \times 10^4$, and $7.6 \times 10^3$ M⁻¹ cm⁻¹, respectively. The infrared spectrum (KBr pellets) had absorption bands at 703 (s), 800 (m), 1025 (w), 1110 (w), 1200 (w), 1350 (m), 1400 (s), 1440 (m), 1520 (m), 1600 (s), 3020 (s), and 3500 (s) cm⁻¹, of which the 3020 and 1600 cm⁻¹ absorptions suggested a zwitterionic structure. The NMR spectrum of the compound is shown in Fig. 4, with absorption at $\tau = 1.0, \tau = 1.5, \tau = 2.0, \tau = 5.6$, and $\tau = 6.0$ with a proton ratio of 2:1:1:1:2. Analysis of the spectroscopic and chemical data indicated the compound to be the pyridyl thioether, S-(3-pyridyl)cysteine.

When the S-(3-pyridyl)cysteine was analyzed on the amino acid analyzer, with the use of a specially developed amino sugar program, a single peak was obtained at elution time 23.8 min (Fig. 5). The color factor, $K_F$, was determined to be 0.543.0.

Characterization of Modified Yeast Alcohol Dehydrogenase—At 4°, to 0.5 ml of 60 mM AAD was added 0.25 ml of 2.0 N HCl and then 0.5 ml of 1.0 M NaNNO₃. After 10 min, 0.5 ml of 2.0 M ammonium sulfamate was added slowly with stirring in order to destroy the excess nitrous acid. After an additional 10 min, the solution was adjusted to pH 7.0 by adding 0.25 ml of 2.0 N NaOH and 2.0 ml of 0.10 M sodium phosphate buffer, pH 7.0. To the resulting 4.0 ml of 7.5 mM diazotized AAD solution, 0.20 ml of 4.0 M sodium phosphate, pH 7.0, was added and the solution was adjusted to pH 7.0 by adding 0.25 ml of 2.0 M ammonium sulfamate, and incubated for 10 min. Ammonium sulfamate (230 mg) was then added, and the solution was stirred vigorously for 10 min. Cysteine (242 mg) was then added and stirring continued for 15 min. All reactions were performed at 0-4°. The resulting solution was chromatographed on a Bio-Gel P12 column (5 x 200 cm) and eluted with distilled water. A major peak with an absorption maximum at 250 nm yielding a single ninhydrin-positive spot on thin layer chromatography was eluted at an elution volume of approximately 3 liters. A minor peak with an absorption maximum at 315 nm was eluted at about 200 ml after the major peak. Thin layer chromatography of this minor component revealed two ninhydrin-positive spots, one of which corresponded to that of the major peak. The fractions from the major peak were collected and lyophilized. Thin layer chromatography of this product showed a single ultraviolet-quenching spot which was also ninhydrin positive, $R_F = 0.75$ for the solvent system, 0.1 M acetic acid-0.5% ethanol (1:1, v/v), and $R_F = 0.65$ for the solvent system, butanol-acetic acid-water (5:2:3, v/v).

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Characterization of Modified Yeast Alcohol Dehydrogenase—At 4°, to 0.5 ml of 60 mM AAD was added 0.25 ml of 2.0 N HCl and then 0.5 ml of 1.0 M NaNNO₃. After 10 min, 0.5 ml of 2.0 M ammonium sulfamate was added slowly with stirring in order to destroy the excess nitrous acid. After an additional 10 min, the solution was adjusted to pH 7.0 by adding 0.25 ml of 2.0 N NaOH and 2.0 ml of 0.10 M sodium phosphate buffer, pH 7.0. To the resulting 4.0 ml of 7.5 mM diazotized AAD solution, 0.20
TABLE II
Comparison of spectral analysis and amino acid analysis of yeast alcohol dehydrogenase inactivated by diazotized 3-aminopyridine adenine dinucleotide

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Moles of adenyl residues per mole of enzyme</th>
<th>Moles of S-(3-pyridyl)cysteine per mole of enzyme</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>4.4</td>
<td>4.4</td>
</tr>
<tr>
<td>2</td>
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<td>4.0</td>
</tr>
<tr>
<td>4</td>
<td>4.2</td>
<td>4.0</td>
</tr>
</tbody>
</table>

a Calculated from difference spectra of native versus inactivated enzyme.
b Amino acid analysis of acid-hydrolyzed inactivated enzyme.

c the number of moles of AAD per mole of tetrameric enzyme was calculated.

Samples of the dialyzed modified yeast alcohol dehydrogenase were lyophilized and hydrolyzed in constant boiling HCl for 24, 36, and 48 hours at 110°C. The resultant hydrolysates were lyophilized and redissolved in 0.2 M sodium citrate buffer, pH 2.2, and subjected to amino acid analysis by means of the amino sugar program. A peak corresponding to S-(3-pyridyl)cysteine (Fig. 5) was observed. The concentration of S-(3-pyridyl)cysteine was seen to decrease linearly with increasing time of hydrolysis. In order to determine the concentration of the S-(3-pyridyl)cysteine residue present in the modified enzyme, extrapolation to zero time of hydrolysis was employed. Hydrolysates of the unmodified enzyme did not contain any amino acid derivatives eluting after phenylalanine in the amino sugar program.

Synthetic S-(3-pyridyl)cysteine hydrolyzed for 24, 36, and 48 hours under conditions identical with those used for the acid hydrolysis of modified yeast alcohol dehydrogenase showed the same rate of destruction as that observed with S-(3-pyridyl)cysteine released from the modified enzyme. This decay curve is very similar to that of methionine destruction in the acid hydrolysis of proteins.

The number of moles of S-(3-pyridyl)cysteine released from inactivated yeast alcohol dehydrogenase through acid hydrolysis (4 per tetrameric form of enzyme) agreed well with the number of adenyl residues attached to the enzyme during the inactivation process. This is indicated by the comparison of spectral data and amino acid analysis data shown in Table II.

**DISCUSSION**

The complete inactivation of yeast alcohol dehydrogenase by diazotized AAD results from a selective reaction derivatizing four sulfhydryl groups per tetrameric form of the enzyme. The evidence for stoichiometric modification was provided by comparative studies of the modified and native enzymes through DTNB titration, ultraviolet difference spectra, and amino acid analysis. The isolation and identification of the modified cysteine residue of the inactivated enzyme as S-(3-pyridyl)cysteine established the occurrence of a diazonium-sulfhydryl reaction. According to our knowledge, this would constitute the first demonstration of a diazonium-sulfhydryl reaction with protein sulfhydryl groups. Since this reaction occurs under mild conditions of temperature and neutral pH, and appears specific for sulfhydryl groups under these conditions, several important implications can be noted. Diazotized AAD may be used as an active site-directed sulfhydryl reagent for studies of...
other dehydrogenases. The established ultraviolet spectral data as well as amino acid analysis procedures are immediately applicable once modified dehydrogenases are obtained. The AAD residue can also serve as an ultraviolet-absorbing label in peptide analysis studies for the identification of peptides containing the modified sulfhydryl group. Such site-directed studies can be extended to include NADP-requiring dehydrogenases since AADP, recently prepared and characterized,\(^2\) exhibits the same reactivity with sulfhydryl groups after diazotation.

Previous studies of yeast alcohol dehydrogenase have shown that the presence of free sulfhydryl groups is necessary for catalytic activity (10, 11). Several sulfhydryl reagents have been used to inactivate this enzyme. These include iodoacetamide (12), iodoacetamidc (13), N-ethylmaleimide (14), fluorescein mercuric acetate (15), \(p\)-hydroxylmucbenzoate (16, 17) and butyl iodoisocyanate (18). At low concentrations of iodoacetamide, four sulfhydryl groups per molecule are attacked. At higher concentrations and longer reaction time, as many as eight sulfhydryl groups are attacked. N-Ethylmaleimide and the mercurials react with eight sulfhydryl groups during inactivation of the enzyme. Positive chainlength effects in the inactivation of yeast alcohol dehydrogenase by \(N\)-alkylmaleimides indicated the importance of nonpolar interactions in reactions of sulfhydryl groups of this enzyme (14). The fact that NADH protected the enzyme against maleimide inactivation suggested that at least one of the functionally important sulfhydryl groups of the enzyme was located close to the hydrophobic region of the coenzyme-binding site (19-21). More recently, Twu and Wold (18) used butyl iodoisocyanate to study the sensitive sulfhydryl groups of yeast alcohol dehydrogenase. They reported that three sulfhydryl groups per molecule of enzyme were attacked during inactivation. From peptide analysis, the modified sulfhydryl groups were shown to be different from those derivatized by iodoacetamide (22). Twu et al. (22) proposed that there are two distinct "essential" sulfhydryl groups per active site necessary for enzyme activity.

Although the reagents mentioned above can be selective for sulfhydryl groups, they are not necessarily site-directed reagents. However, since diazotized AAD is a structural analog of NAD, it can be preferentially bound at the active site of the enzyme. The parent compound AAD has also been found to be a coenzyme competitive inhibitor of yeast alcohol dehydrogenase (1). Thus, diazotized AAD is both active site directed and sulfhydryl group specific. The fact that four sulfhydryl groups selectively react with diazotized AAD strengthens the argument that one of the functionally important sulfhydryl groups of yeast alcohol dehydrogenase is located nearby the pyridinium ring region of the coenzyme-binding site. Sloan and Mildvan (23), from magnetic resonance studies of the geometry of bound NAD and isobutryramide on spin-labeled yeast alcohol dehydrogenase, have also indicated that the spin label attached to cysteine is close to the dihydropyridine ring of bound NADH. Piopp et al. (24) in studies of the inactivation of yeast alcohol dehydrogenase by 3\((\omega\)-bromacetamidethyl)nicotinamide also suggested the presence of a sulfhydryl group near the pyridinium ring region of the coenzyme-binding site. It will be interesting to identify the amino acid sequence of the peptide containing the diazotized AAD-modified sulfhydryl group and compare with results obtained by Harris (12) and Twu et al. (22). Such experiments are currently in progress.

In view of the general lack of data concerning diazonium-sulfhydryl reactions and the significance of this possibility for protein diazo coupling reactions, reactions with diazonium compounds were demonstrated with cysteine, homocysteine, and glutathione, indicating that sulfhydryl-containing amino acids and small peptides likewise react. The essentially equivalent reactivity of mercaptoethanol confirms that the critical functional group for this reaction is the sulfhydryl group. The rates of reactions involving excess diazotized 1-methyl-3-aminopyridinium chloride, diazotized AAD, and diazotized AADP with simple sulfhydryl compounds were first order with respect to both the diazonium derivatives and the sulfhydryl compounds. This suggests a relatively simple reaction mechanism such as nucleophilic attack on the \(\beta\)-nitrogen of diazonium cation by the sulfhydryl group, or heterolytic diazoniation with the nucleophile (25).

In reactions with cysteine, second order rate constants indicated that 1-methyl-3-aminopyridinium chloride is twice as reactive as diazotized AAD. However, in the inactivation of yeast alcohol dehydrogenase the \(N\)-methyl derivative is only one-tenth as reactive as AAD (1). This latter observation reflects that the specificity of binding of diazotized AAD to the enzyme active site is the important factor in the enzyme inactivation.

To understand the chemistry of diazonium-sulfhydryl reactions, isolation and characterization of products are necessary. The major product from the reaction of diazotized 3-aminopyridine and cysteine under acid conditions was studied in detail. The ninhydrin-positive reaction indicated that the free amino group of cysteine is retained in the product. The ultraviolet spectrum indicated that the compound possesses a chromophore for \(\pi \rightarrow \pi^*\) transitions related to the pyridine nucleus. The infrared data supported the zwitterionic character of the amino and carboxyl functions. The NMR data indicated four pyridyl protons, a methine proton, and two methylene protons. These, together with elemental analysis, confirmed the compound to be the thioether, \(S-(3\text{-pyridyl})\text{cysteine. If this thioether is considered to be derived from the reaction by a one-step mechanism, a heterolytic diazoniation by nucleophile would be involved. It should be noted, however, that a minor ninhydrin-positive product was also obtained.}

The exact nature of the reaction of diazotized AAD with active site sulfhydryl groups of yeast alcohol dehydrogenase is as yet unclear. The concomitant appearance of the 300-nm absorption, together with the 260-nm adenine absorption in the difference spectra of modified versus native enzyme (1), seemed to suggest possible diazomercaptide formation. Whether the initial cysteine derivative formed during the inactivation process is a diazomercaptide or a thioether remains to be established; however, acid hydrolysis of either of these derivatives would release \(S-(3\text{-pyridyl})\text{cysteine. Therefore, the application of diazotized AAD or diazotized AADP in active site sulfhydryl studies should be unaffected by the actual intermediate initially formed.}

This investigation has demonstrated that diazonium-sulfhydryl reactions occur at a significant rate under mild conditions of temperature and pH. This reaction can serve as the basis for developing site-specific reagents for enzymes having catalytically important sulfhydryl groups, for the synthesis of interesting derivatives of sulfhydryl-containing compounds, and is a reaction that must be considered in procedures such as enzyme immobilization.

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A novel diazonium-sulphydryl reaction in the inactivation of yeast alcohol dehydrogenase by diazotized 3-aminopyridine adenine dinucleotide.

J K Chan and B M Anderson


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