Modification of Amino Acid Residues in Anti-\(p\)-azobenzenearsonic Acid Antibody during Extensive Iodination*

MARIAN ELLIOTT KOSHLAND, FRIEDA M. ENGLBERGER, MARY JANE ERWIN, AND SUZANNE M. GADDONE

From the Biology Department, Brookhaven National Laboratory, Upton, New York

(Received for publication, October 16, 1962)

The active site of antibody directed against the negatively charged \(p\)-azobenzenearsonic acid group has been shown to contain an iodine-reactive residue. The first evidence was provided by Pressman and Sternberger (1), who demonstrated that iodination destroys the precipitating capacity of this antibody and that the loss can be prevented by the prior addition of the homologous hapten. Subsequently, Koshland, Englberger, and Gaddone (2) showed that the iodination reduces the binding capacity by a direct attack at the active site, and the reaction thus can be used to label with radioactive iodine the specific amino acid involved (3). However, the identity of this iodine-reactive group has not been established.

From studies with other proteins such as pepsin, casein, and lysozyme (4), a variety of side chains is known to be modified during iodination. Iodine may be substituted in the phenolic group of tyrosyl residues (5) and on both the nitrogen and carbon atoms of the imidazole ring of histidyl residues (6). Iodine may also oxidize cysteinyl and cystinyl residues to cysteic acid (7), and tryptophanyl residues to an unidentified compound in which the ring is ruptured (8). Furthermore, on the basis of the reaction of the free amino acid with iodine, the methionyl residues (9) in proteins might also be expected to be oxidized to the corresponding sulfoxide or sulfone, and perhaps the seryl and threonyl residues to carbonyl derivatives (10). In view of these complexities, the first step in the identification of the iodine-reactive group of arsionic antibody was to determine by extensive iodination both the number and kinds of residues that can react. The results of such investigations are presented in this paper.

**EXPERIMENTAL PROCEDURE**

**Antigen Preparations**—The immunizing antigen was prepared by coupling 16.6 pmoles of purified bovine \(\gamma\)-globulin (obtained from Armour and Company) with the diazonium salt of 2 mmoles of \(p\)-aminobenzenearsonic acid. The reaction was carried out at \(0^\circ\) in 0.25 M carbonate buffer, pH 9.5. The azoglobin was dialyzed exhaustively to remove excess diazonium salt and was then precipitated with alum by the method of Karush and Marks (11).

In the precipitating antigen, human fibrinogen, purified by a modification of Laki’s method (12), was substituted as the protein carrier. Aliquots containing 4 pmoles were coupled to 2 mmoles of the diazonium salt under the conditions described above. After dialysis, the azofibrinogen was precipitated three times with 3 M NaCl to remove any remaining adsorbed dye.

**Preparation of Purified Arsonic Antibody**—Antisera were obtained from New Zealand white rabbits which were killed after a single course of immunization. The immunization consisted of four injections per week in increasing dosage over a period of 4 weeks, so that each animal was administered a total of 80 mg of alum-precipitated antigen. The sera from 12 to 24 animals which contained more than 100 \(\mu\)g of antibody N per ml were pooled and stored in the deep freeze.

The method developed for the isolation of antibody directed only against the azobenzenearsonic acid moiety has been described in detail elsewhere (2). Briefly, it consists of the removal of antiprotein carrier antibody and complement by the addition of 20 \(\mu\)g of bovine \(\gamma\)-globulin N per ml of antisera pool and the subsequent precipitation of the antihapten antibody by the addition of the azofibrinogen antigen in amounts that provide a slight antibody excess. The antihapten antibody is then eluted from the washed immune precipitate with sufficient \(p\)-nitrobenzenearsonic acid in 0.02 M phosphate buffer, pH 7.2, so that the final concentration of hapten is 0.025 M. The supernatant solution is applied to a DEAE-cellulose column, 1.2 x 20 cm, equilibrated with the same phosphate buffer. The antibody appears in the eluate as a single peak at column volume.

**Iodination of Antibody**—The method of iodination used in these experiments was adapted from the procedure of McFarlane (13), in which iodine monochloride is the iodinating agent. This method has the advantages that (a) the reagent is stable, (b) the theoretical yield is 100\%, so that less reagent need be added and side reactions are minimized, and (c) the iodine uptakes obtained are more reproducible.

A stock solution was made by triturating 100 g of ICl (obtained from K and K Laboratories, Inc.) with NaCl and dilute HCl to a volume of 2 liters. The final concentrations of the salt and acid were 1 M and 0.01 N, respectively. The mixture was warmed to 37\(^\circ\) for several hours and then allowed to stand for 1 week at room temperature before the remaining undissolved ICl was filtered off. The solution was titrated against a standardized preparation of \(\text{As}^\text{+}\) by the method of Singh, Kashyap, and Sahota (14) and was found to be 0.267 M. No significant change in concentration was observed after storage in the dark for 2 years. Just before use, the stock ICl solution was diluted 1:12 by the addition of 5 parts of cold 2 \(\times\) glycine buffer, pH 9.0, and
6 parts of cold distilled H$_2$O containing the appropriate amount of carrier-free radioactive iodine. This solution was colorless and remained stable for at least several hours as long as it was maintained at less than 5°.

For the substitution of small amounts of iodine, the appropriate quantity of diluted I$_2$ was added directly to a cold solution of arsenic antibody buffered with 0.2 M glycine, pH 8.5, and 0.025 M in NaCl. Since the direct addition of large amounts of reagent resulted in considerable precipitation of the antibody protein and corresponding irreproducible yields, a continuous dialysis apparatus similar to that described by Craig, King, and Stracher (15) was used for extensive iodination. Five to 10 ml of cold, buffered arsenic antibody at a concentration of 4 X 10$^{-3}$ M were introduced into the bulb at the top of the apparatus, an amount of I$_2$ not exceeding 50 atoms of I per molecule of protein was added, and the solution was quickly forced down into the dialysis bag. The solution was allowed to dialyze for 1 hour against 1 liter of glycine buffer kept at 3-5° by a surrounding ice bath before it was drawn back up into the bulb for analysis and successive iodination. By the repeated addition of small amounts of I$_2$ and the rapid dilution of the by-products of the reaction after each addition, less than 5% of the antibody was precipitated during iodination to saturation.

Analyzed of Iodinated Antibody—1. Specific activity: To determine specific activities, 1.2 to 2.0-mg samples of iodinated antibody were precipitated in the cold in the presence of 0.13% NaHSO$_3$ and 7% CCl$_3$COOH (trichloroacetic acid). The precipitates were washed three times with cold 7% trichloroacetic acid to remove nonprotein-bound iodine and glycine buffer and then were dissolved in 0.05 ml of 2 N NaOH. After appropriate dilution, triplicate aliquots were taken for counting and nitrogen analysis by the micro-Kjeldahl method. The control in each case consisted of an equivalent dilution in NaOH of a volume of the last wash equal to that of the trichloroacetic acid precipitate. The measurements of radioactivity were made in a deep well scintillation counter. Corrections were applied for background, decay, and sample volume.

2. Amino acid residues: Amino acid analyses were carried out on 4-mg samples which were either precipitated with trichloroacetic acid as described above or dialyzed exhaustively against water or dilute phosphate buffer, pH 7.2. Acid hydrolyses were performed by the standard procedure in which 0.4 ml of constant boiling HCl is added per mg of dried sample, the tube is evacuated, boiled, and sealed, and the contents are heated at 110° for 21 hours. Basic hydrolyses were performed by the Ba(OH)$_2$ procedure originally developed by Dröge for tryptophan determination and subsequently modified first by Moore and then by Ray and Koshland (18). After 16 hours of hydrolysis at 105°, the barium was eliminated by precipitation with ammonium bicarbonate and the remaining salt was largely removed by sublimation under vacuum. A detailed description of the method used has been given by Ray and Koshland (18). The dried basic or acidic hydrolysates were dissolved in 5 ml of the standard pH 2.2 buffer, and 2-ml quantities were applied to both the long and the short columns of the automatic amino acid analyzer. The values obtained for the amino acids in each chromatogram were normalized to the leucine peak and expressed as residues per mole of antibody by multiplying by the factor of 89.

Leucine was chosen as the standard because of its stability to acid hydrolysis and its lack of reactivity with iodine. The value of 89 represents the average number of leucine residues per mole of antibody as calculated from the leucine yields after acid hydrolysis, the nitrogen analyses of the antibody protein before hydrolysis, and the assumptions of a nitrogen content of 16% and a molecular weight of 160,000.

The correction factor was also applied to the data from the basic hydrolysates, although the number of leucine residues recovered per mole of antibody was 10% less than the value obtained after acid hydrolysis, and the other amino acids showed greater or less recoveries. To demonstrate that these differences were caused by the basic hydrolysis, samples of a mixture of amino acids approximating the composition of arsenic antibody as determined by acid hydrolysis were treated with Ba(OH)$_2$ under the conditions described above. Amino acid analyses gave results similar to those found with the basic hydrolysates of the antibody protein. Reproducible recoveries of 90% were obtained for proline, valine, isoleucine (sum of isoleucine and alloisoleucine), leucine, and tyrosine; values of 87, 83, and 80% were obtained for phenylalanine, methionine, and tryptophan, respectively. The yield of aspartic acid ranged from 40 to 56%, and glutamic acid from 69 to 76%, whereas serine, threonine, histidine, and arginine were completely or almost completely destroyed. Variable quantities of the degradation products accumulated under the glycine and alanine peaks, and ornithine, the product of arginine breakdown, appeared under the lysine peak. Only the values of those amino acids that gave reproducible recoveries were used.

Because of unreliable recoveries from both acid and base hydrolysates, the half-cystine content was determined by measurements of the carboxymethylated derivative. The -S-S- bonds were reduced and alkylated by the method of Edelman and Poulak (17). The precipitated antibody fragments were then hydrolyzed in acid by the procedure described above. Extreme care was taken to remove all oxygen from the HCl solution of the fragments before hydrolysis. Under these conditions, the recovery of carboxymethylcysteine has been shown to be better than 98% (18).

Control experiments were performed with 3,5-diiodotyrosine and 2,5-diiodohistidine to determine their stability in acid and basic hydrolysis, the extent of regeneration by deiodination, and the possibility of transfer of iodine or oxidation of other residues in the antibody protein. The L-3,5-diiodotyrosine was purchased from the California Corporation for Biochemical Research. The diiodohistidine was prepared by a modification of the method of Brunings (19), in which iodine dissolved in heptane was added dropwise to a solution of L-histidine·HCl·2H$_2$O (purchased from the California Corporation for Biochemical Research) in 0.5 N NaOH. The excess iodine and iodide were extracted into heptane after the aqueous phase was acidified with HCl, and the appropriate quantity of KIO$_3$ was added. After flash evaporation to reduce the volume, the diiodohistidine was precipitated by adjusting the pH to 2.72 with concentrated NH$_4$OH, and then purified by repeated precipitation. The final product decomposed at 220° and showed a single peak 30 to 32 ml before lysine when chromatographed on the short column (23 cm in length) of the amino acid analyzer.

RESULTS

When the expected products of iodination were examined for stability to hydrolysis, only cysteic acid was found to give quanti-
The recoveries after treatment with 6 N HCl as previously shown by Schram, Moore, and Bigwood (20). Of the others tested, diiodohistidine and oxidized tryptophan were completely destroyed by hydrolysis with either HCl or Ba(OH)₂. Diiodotyrosine was deiodinated in acid to regenerate tyrosine, and the recoveries after basic hydrolysis were not quantitative or reproducible. Methionine sulfoxide gave results similar to those obtained for diiodotyrosine (16). These data established that the iodine-reactive residues in arsenic antibody, other than cysteinyl or cystinyl groups, could be identified only by difference between the amino acid recoveries of iodinated and untreated samples. Furthermore, the comparative analyses of both acid and basic hydrolysates were required because of the known destruction of histidine in base, the observed regeneration of tyrosine and methionine, and the destruction of tryptophan in acid solution.

The validity of the difference method depended on the demonstration that (a) the preparations of arsenic antibody gave reproducible amino acid recoveries after basic and acidic hydrolysis and (b) the presence of oxidized and iodinated derivatives did not significantly alter the recoveries of the residues not modified by iodination. The data from the first of these control experiments, the analyses of untreated arsenic antibody, are presented in Table I. The accompanying standard errors of an individual determination showed that the recoveries in each case were very reproducible since the individual variations fell within the experimental error of the analysis. The results from the acid hydrolysates represented at least 10 separate preparations of arsenic antibody, each isolated from the pooled sera of 8 to 30 animals and each shown to be 95% pure by equilibrium dialysis measurements (2). Thus the reproducibility of the analyses also indicated that any differences in amino acid composition caused by animal variation or impurities were too small to be detected.

To determine any losses or incomplete recoveries during acid hydrolysis, samples of arsenic antibody were treated with 6 N HCl for periods of 20, 24, 30, 48, 72, and 96 hours. The greatest losses were observed in the serine and threonine yields, as shown in Fig. 1, where the logarithm of their recoveries is plotted as a function of the time of hydrolysis. The shapes of the curves indicated that the serine and threonine residues were not destroyed in a simple first order reaction, but exhibited different labilities to acid hydrolysis. In addition, the tyrosine recoveries decreased slightly on longer hydrolysis, but even after extrapolation to zero hydrolysis time the average yield was 5% lower than that obtained from basic hydrolysates. Although Smith et al. (22) reported incomplete release of valine and isoleucine after 20 hours of hydrolysis of rabbit antipneumococcal antibodies, no significant increase in the recoveries of these residues was observed upon prolonged hydrolysis of arsenic antibody. Furthermore, the chromatograms of 20-hour hydrolysates did not exhibit any extraneous peaks of ninhydrin-positive material, which would indicate the presence of incompletely hydrolyzed peptides. However, it should be noted that the valine determinations were less reliable because of the early application of the second buffer required for the subsequent separation of glucosamine from tyrosine and phenylalanine. The experimental error in the valine values was, therefore, sufficiently large that an increased yield of several residues (3%) would not be detected.

### Table I

**Amino acid composition of rabbit antibody expressed as residues per 160,000 g**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Ascorbic acid</th>
<th>Antipneumococcal antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Data from this paper</td>
<td>Data from Smith et al. (22)</td>
</tr>
<tr>
<td></td>
<td>Acid hydrolysates⁶</td>
<td>Basic hydrolysates⁶</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>71.4 ± 1.66</td>
<td>18.3 ± 0.24</td>
</tr>
<tr>
<td>Lysine</td>
<td>16.4 ± 0.35</td>
<td>65</td>
</tr>
<tr>
<td>Histidine</td>
<td>46± 1.19</td>
<td>44</td>
</tr>
<tr>
<td>Arginine</td>
<td>105 ± 0.72</td>
<td>108</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>174± 1.40</td>
<td>161</td>
</tr>
<tr>
<td>Threonine</td>
<td>116 ± 1.57</td>
<td>117 ± 4.49</td>
</tr>
<tr>
<td>Serine</td>
<td>110 ± 1.41</td>
<td>110</td>
</tr>
<tr>
<td>Glycine</td>
<td>79.2 ± 1.02</td>
<td>104</td>
</tr>
<tr>
<td>Half-cystine</td>
<td>43.7 ± 0.50</td>
<td>31</td>
</tr>
<tr>
<td>Valine</td>
<td>127 ± 2.93</td>
<td>132</td>
</tr>
<tr>
<td>Methionine</td>
<td>14.0 ± 0.40</td>
<td>12.8 ± 0.22</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>48.7 ± 0.67</td>
<td>49.8 ± 0.16</td>
</tr>
<tr>
<td>Leucine</td>
<td>89</td>
<td>89</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>55.9 ± 1.54</td>
<td>58.3 ± 0.59</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>44.2 ± 0.56</td>
<td>42.7 ± 0.88</td>
</tr>
</tbody>
</table>

| Molecular weight calculated from residues | 156,700 | 152,900 | 165,200 |

⁶ Average of 12 determinations on 20-hour hydrolysates.
⁵ Average of 5 determinations on 16-hour hydrolysates.
⁷ The values following the ± sign represent the standard error of an individual determination.
⁸ Corrected to zero hydrolysis time.
⁹ Determined as carboxymethylcysteine.
¹⁰ Sum of isoleucine and alloisoleucine.

---

**Fig. 1. Effect of the time of hydrolysis in 6 N HCl on the recoveries of threonine and serine from arsenic antibody.**

- ○, Threonine recoveries; ○, serine recoveries.
When the amino acid composition of arsenic antibody was corrected for the serine and threonine losses and the tyrosine and tryptophan values from the basic hydrolysates were substituted, the total residues recovered gave a molecular weight of 156,700. Since the approximately 3% contribution of the carbohydrate moiety known to be associated with antibody globulin was not included, this value was in excellent agreement with the average molecular weight of 160,000 determined for rabbit antibodies by various physical measurements (17, 23-25). Furthermore, these results supported the normalization to a leucine value of 89 residues and the assumption of a nitrogen content of 16% used in the calculation of the number of residues per mole.

The amino acid composition of rabbit arsenic antibody determined in these studies is compared in Table I with the results of two previous investigations, one also carried out on arsenic antibody (21) and the other representing an average of several anti-pneumococcal antibodies (22). The data have been recalculated as residues per mole on the assumption of a molecular weight of 160,000. Although the compositions are generally similar, significant differences occur, for example, in the alanine, aspartic acid, leucine, glycine, and phenylalanine values, which are reflected in the molecular weights calculated from the sum of the residues so obtained (last line of Table I). However, the differences in composition between the antibodies of the same specificities are as large as those between the antibodies of different specificities. Since in the previous two studies the purity of the antibody preparations was not established by criteria as stringent as binding capacity and since the amino acid assays were not carried out by use of the more accurate automatic analyzer, some of the observed differences may have resulted from small impurities in the starting material and experimental errors in analysis. On this basis, no immunological significance can be attached to the comparative data given in Table I.

The data from the second of the control experiments, the effect of iodinated derivatives on the amino acid recoveries of untreated arsenic antibody, are shown in Table II. It may be seen that the addition of 1 μmole of diiodotyrosine per 0.01 μmole of antibody did not alter the yields of any amino acid under the conditions of hydrolysis used. Thus, the iodine liberated from diiodotyrosine during acid hydrolysis was not transferred to histidine, and the destruction of diiodotyrosine in base did not result in the regeneration of tyrosine or the oxidation of methionine and tryptophan.

The data in the last column of Table II show that the presence of both diiodotyrosine and diiodohistidine did not cause modifications of amino acid recoveries other than those observed with the separate addition of the iodinated derivatives. These results do not support a previous report (6) that the deiodination of diiodotyrosine during HCl hydrolysis is increased in the presence of decomposing diiodotyrosine.

On the basis of these control experiments and the demonstration that methionine sulfoxide (16) and oxidized tryptophan were not reduced during basic hydrolysis, the differences observed between the amino acid recoveries of iodinated and untreated arsenic antibody were considered a valid measure of iodine-modified residues. These differences are summarized in Table III. The data for the iodinated antibody were obtained from five preparations iodinated to saturation by the successive addition of ICl equilibrated with trace amounts of P and containing 40 to 50 iodine atoms per molecule of protein. The extent of the reaction was measured by the protein-bound iodine, since no rapid quantitative procedure was available for the assay of oxidized residues. The value for maximal incorporation was found to be very reproducible; the average from the five experiments, each carried out under slightly varying experimental conditions, was 100.3 atoms per molecule with a standard error of 2.04. None of these preparations showed any detectable immunological activity as assayed by equilibrium dialysis techniques.

A comparison of the amino acid analyses of iodinated and untreated antibody showed that at saturation iodine was substituted in 44 of the 59 tyrosyl residues and 7 of the 16 histidyl residues. The observed loss of six histidyl groups was corrected for deiodination during acid hydrolysis as discussed previously. If 2 atoms of iodine were assumed to be bound to each residue of reacted tyrosine and histidine, the total iodine incorporated was calculated to be 102 atoms per molecule. This value corresponded very well with the average maximal incorporation determined from measurements of the specific activity of the iodinated antibody. The possibility that the value of 2 represented an average of some monosubstituted tyrosyl and trisubstituted histidyl groups was eliminated by the demonstration that the observed specific activities of the iodinated antibody were not altered by prior treatment with bisulfite. Under these conditions any iodine bound to the imino N of the imidazole ring would be expected to be released. Thus, the formation of a N—I bond.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Lysine</th>
<th>Histidine</th>
<th>Arginine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>71.4</td>
<td>16.4</td>
<td>45.3</td>
</tr>
<tr>
<td>Acceptor</td>
<td>4.5</td>
<td>4.4</td>
<td>4.4</td>
</tr>
</tbody>
</table>

* Hydrolysis time was 20 hours.
* Data are corrected to lysine = 71 residues per 160,000 g of antibody.
* Hydrolysis time was 16 hours.
* Sum of isoleucine and alloisoleucine.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Residues per 150,000 g of antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antibody control</td>
</tr>
<tr>
<td>Acid hydrolysates</td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>71.4</td>
</tr>
<tr>
<td>Histidine</td>
<td>16.4</td>
</tr>
<tr>
<td>Arginine</td>
<td>45.3</td>
</tr>
<tr>
<td>Basic hydrolysates</td>
<td></td>
</tr>
<tr>
<td>Tryptophan</td>
<td>18.3</td>
</tr>
<tr>
<td>Proline</td>
<td>117</td>
</tr>
<tr>
<td>Methionine</td>
<td>12.8</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>49.8</td>
</tr>
<tr>
<td>Leucine</td>
<td>89</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>58.8</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>42.7</td>
</tr>
</tbody>
</table>

### Table II

**Effect of added diiodotyrosine and diiodohistidine on amino acid recoveries of arsenic antibody**

* Hydrolysis time was 20 hours.
* Data are corrected to lysine = 71 residues per 160,000 g of antibody.
* Hydrolysis time was 16 hours.
* Sum of isoleucine and alloisoleucine.
Threonine
Histidine
Lysine
Tryptophan
Tyrosine
Isoleucine
Valine
Half-cystine
Alanine
Glycine
Proline
Glutamic acid
Serine
Phenylalanine

The application of these procedures to arsenic antibody established that iodine was substituted in tyrosyl and histidyl groups and that it oxidized methionyl, tryptophanyl, and cysteinyl residues. Phenylalanyl, seryl, threonyl, and cystinyl residues did not react under the conditions of iodination employed. In view of the number of residues and the size of arsenic antibody, it would be reasonable to expect a similar pattern of reactivity in other proteins. Hence this study not only reveals which residues can be identified by the reagent, but also, because of the easily detected iodine radioactivity, provides an immediate identification of a tyrosyl or histidyl group in those proteins in which loss of biological activity can be correlated with iodine uptake.

## DISCUSSION

Before these studies, the iodination reaction has not been used extensively as an amino acid reagent because methods were not available (a) to obtain reproducible yields and (b) to measure quantitatively the residues that were modified. These limitations have been largely overcome by the procedures presented in this paper. By the combined use of a stable reagent, iodine monochloride, and a method of rapid dialysis, the amounts of iodine incorporated could be reproduced in successive experiments within 3 to 5%. Second, although most of the iodinated derivatives were partially unstable to hydrolysis, the differences obtained between the amino acid recoveries of iodinated and untreated samples after both basic acid hydrolysis were shown to provide an accurate measure of the iodine-modified residues.

The application of these procedures to arsenic antibody established that iodine was substituted in tyrosyl and histidyl groups and that it oxidized methionyl, tryptophanyl, and cysteinyl residues. Phenylalanyl, seryl, threonyl, and cystinyl residues did not react under the conditions of iodination employed. In view of the number of residues and the size of arsenic antibody, it would be reasonable to expect a similar pattern of reactivity in other proteins. Hence this study not only reveals which residues can be identified by the reagent, but also, because of the easily detected iodine radioactivity, provides an immediate identification of a tyrosyl or histidyl group in those proteins in which loss of biological activity can be correlated with iodine uptake.

In the case of arsenic antibody, the identification of the group at the active site among the 5 iodine-reactive residues could be narrowed by evidence previously presented (2) that a direct relationship exists between iodine incorporation and the destruction of binding capacity. Moreover, it was observed in protection experiments (3) that approximately one site per molecule was destroyed by the uptake of 4.6 of the 4.8 iodine atoms, and that the remaining unbound iodine, which represented reagent available for oxidative reactions, was insufficient to account for the observed destruction. This result was consistent with the elimination of cysteine, since the complete loss of immunological activity in the extensively iodinated preparations required the recovery of at least 2 modified cysteinyl residues per mole and only 0.8 residue per mole was obtained. Thus the correlation of immunological studies with the amino acid data indicated that loss of antibody activity cannot be explained by the oxidation of methionyl, tryptophanyl, or cysteinyl groups but can be by the modification of a histidyl or tyrosyl group at the active site.

## SUMMARY

Procedures have been developed for the extensive iodination of proteins without producing significant amounts of insoluble product and for the quantitative determination of the iodine-modified residues. These procedures have been used to identify the available reactive residues in anti-p-azobenzenearsonic acid antibody, which is known to contain an iodine-reactive group at its active site. Iodine was found to be substituted in 7 of the 16 histidyl and 44 of the 59 tyrosyl residues. In addition, 10 of the 13 methionyl and 6 of the 18 tryptophanyl residues were oxidized, and traces of cysteic acid suggested that sulfhydryl groups were also labile. These results narrowed the identification of the iodine-reactive group to 4 residues and, in conjunction with previous immunological studies, implicate a tyrosyl or a histidyl residue.

## REFERENCES

Modification of Amino Acid Residues in Anti-\(p\)-azobenzenearsionic Acid Antibody during Extensive Iodination
Marian Elliott Koshland, Frieda M. Englberger, Mary Jane Erwin and Suzanne M. Gaddone


Access the most updated version of this article at
http://www.jbc.org/content/238/4/1343.citation

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/238/4/1343.citation.full.html#ref-list-1