Radioiodination of Sulfhydryl-Sensitive Proteins

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Received January 28, 1975; accepted March 17, 1975

A new procedure is described for the radioiodination of proteins with sulfhydryl groups essential for their biological activity. Aniline is iodinated with $^{125}$I-labeled sodium iodide in the presence of chloramine-T, the product separated by solvent extraction, diazotized and coupled to protein.

Radioiodination with $^{125}$I has emerged as the method of choice for labeling protein to high specific radioactivity. A number of procedures have been developed to iodinate proteins under mild conditions and with reproducible results (1–7). Of these techniques, none is suitable for the iodination of proteins having thiol groups. Exposure to the oxidants N-chloro-p-toluene sulfonamide (chloramine-T) (1), hydrogen peroxide (2–4), iodonemonochloride (5), and iodine itself (7) may result in oxidation of sulfhydryl groups with the concomitant disruption of tertiary structure or the chemical modification of a specific amino acid residue in the active site. An alternative method involves the acylation of protein with N-succinimidyl-3-(3-[$^{125}$I]iodo-4-hydroxyphenyl) propionate (6). However, this active ester could react with cysteinyl as well as lysyl residues and thus also disturb the structural integrity of the protein.

The present report describes a simple, rapid, and reproducible technique for the radioiodination of sulfhydryl-containing proteins to high specific activity. Aniline is iodinated with Na[$^{125}$I] in the presence of an oxidant, the product separated by solvent extraction, diazotized, and coupled to the protein. This method which was first suggested by Boyd et al. (8) has been applied successfully to the iodination of three proteins known to require cysteinyl residues for full activity.

METHODS

Protein-iodination-grade Na[$^{125}$I] (~17 Ci/mg) was purchased from New England Nuclear Corp., Boston, MA; N-chloro-p-toluene sulfonamide from Eastman Organic Chemicals, Rochester, NY; and aniline, chloroform, and sodium nitrite from J. T. Baker Chemicals, Phil-

This study was supported by National Institutes of Health Grant No. AI AM 10171 and Phoenix Project Grant.
Radioiodination of *Bandeiraea simplicifolia* lectin by a variety of published procedures resulted in the loss of between 64 and 83% of its type B erythrocyte hemagglutinating activity (Table 1). Since this protein is known to require cysteinyl residues for its carbohydrate-binding activity (9), it was presumed that oxidation to disulfides or cysteic acid residues...
TABLE 1
IODINATION OF B. SIMPLICIFOLIA LECTIN BY PUBLISHED PROCEDURES

<table>
<thead>
<tr>
<th>Reference</th>
<th>Activity remaining (%)</th>
<th>Specific activity (cpm/µg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hunter and Greenwood (1)</td>
<td>35</td>
<td>$8.6 \times 10^5$</td>
</tr>
<tr>
<td>Marchalonis (2)</td>
<td>18</td>
<td>$1.3 \times 10^3$</td>
</tr>
<tr>
<td>Roholt and Pressman (5)</td>
<td>17</td>
<td>0$^a$</td>
</tr>
<tr>
<td>Syvanen et al. (7)</td>
<td>36</td>
<td>0$^a$</td>
</tr>
</tbody>
</table>

$^a$ The reaction was performed with unlabeled NaI.

resulted when the protein was exposed to the oxidants employed. The present technique was developed to circumvent exposure of labile sulfhydryl groups to oxidizing conditions.

As indicated in Table 2, this procedure is suitable for labeling proteins having cysteinyl residues that are required for activity. The hemagglutinating activity of B. simplicifolia lectin is completely inhibited by reaction with Hg$^{2+}$ and dithiobisnitrobenzoic acid (9). This protein was successfully labeled without activity loss by the iodoaniline coupling method. Chromatography of radiolabeled lectin on an affinity column (9) resulted in complete adsorption of both radioactivity and protein. Elution with D-galactose gave a single protein peak with a constant ratio of $A_{280\text{nm}}$ to counts per minute throughout the elution peak, indicating that labeled lectin retains its carbohydrate-binding activity and specificity.

Results of labeling the lima bean lectin III were similar. Gould and Scheinberg have demonstrated the importance of cysteinyl residues for the binding activity of this lectin and noted partial protection of sulfhydryl-sensitive proteins with diazotized iodoaniline

TABLE 2
IODINATION OF SULFHYDRL-SENSITIVE PROTEINS WITH DIAZOTIZED IODOANILINE

<table>
<thead>
<tr>
<th>Protein</th>
<th>Moles of SH/mole of protein</th>
<th>Specific activity, native protein</th>
<th>Specific activity, iodinated protein</th>
<th>Activity remaining (%)</th>
<th>Specific radioactivity (cpm/µg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. simplicifolia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lectin</td>
<td>4.0 (9)</td>
<td>404$^a$</td>
<td>394</td>
<td>98</td>
<td>1085$^b$</td>
</tr>
<tr>
<td>Lima bean lectin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>3.43 (13)</td>
<td>210$^c$</td>
<td>228</td>
<td>108</td>
<td>1115</td>
</tr>
<tr>
<td>Papain</td>
<td>1.0 (14)</td>
<td>72.0$^d$</td>
<td>75.8</td>
<td>105</td>
<td>760</td>
</tr>
</tbody>
</table>

$^a$ Type B erythrocyte hemagglutinating titer/mg of protein/ml.
$^b$ Average of five experiments.
$^c$ Type A erythrocyte hemagglutinating titer/mg of protein/ml.
$^d$ Micromoles of benzoylarginineamide hydrolyzed/min/mg of protein.
TABLE 3
Specific Radioactivity Obtained with Varying $^{125}$I to Protein Ratios$^a$

<table>
<thead>
<tr>
<th>Protein in reaction mixture (mg)</th>
<th>Diazonium coupling reaction volume (ml)</th>
<th>Approximate moles of protein/mole of $^{125}$I</th>
<th>Specific radioactivity (cpm/µg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10</td>
<td>175</td>
<td>1085</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>88</td>
<td>1982</td>
</tr>
<tr>
<td>0.5</td>
<td>0.5</td>
<td>8.8</td>
<td>7109</td>
</tr>
</tbody>
</table>

$^a$ Experiments were performed using *B. simplicifolia* lectin.

dryl groups from thiol reagents in the presence of *N*-acetyl-α-D-galactosamine (13). Labeling with iodoaniline gave a protein with no change in specific hemagglutinating activity.

The sulfhydryl group of papain is at the active site and is required for the enzymatic activity of this enzyme (14). Reaction with diazotized $[^{125}$I]iodoaniline did not detectably alter its catalytic properties.

By varying the ratio of $[^{125}$I]iodoaniline to protein, the resulting specific radioactivity of the protein could be varied (Table 3). It is therefore possible to achieve the approximate degree of radiolabeling desired and adjust the ratio of isotope to protein accordingly.

**DISCUSSION**

A variety of biochemical studies makes use of proteins labeled to high specific activity with $^{125}$I. Metabolic studies, hormone–receptor studies, and radioimmunoassays all depend on the availability of highly radioactive proteins whose biological activity and conformation have not been altered as a result of the labeling process.

The most commonly used radioiodination procedure has been that of Hunter and Greenwood involving direct iodination of tyrosine (and occasionally histidine) residues in the presence of *N*-chloro-p-toluene sulfonamide, a technique that is both rapid and efficient (1). It has been suggested that iodination damage (loss of structural integrity and biological activity) resulting from labeling by this method is caused by impurities in the commercial Na$[^{125}$I] solution (15). Alternatively, loss of activity may result from exposure of the protein to the oxidant. Other methods employing less severe reaction conditions include the lactoperoxidase–hydrogen peroxide technique of Marchalonis (2). Even in this procedure, however, the protein is exposed to an oxidant, the efficiency of labeling is difficult to control, and a protein contaminant (lactoperoxidase) is introduced into the solution being labeled. In a variation of the Hunter–Greenwood procedure, Syvanen *et al.* attempted to obvi-
ate iodination damage by destroying excess oxidant with a change in pH prior to addition of protein (7). They report a 50% reduction in labeling efficiency together with a 15% loss of activity for the enzyme under investigation. The only currently available technique in which neither oxidants nor the commercial Na\(^{125}\)I solution are included in the iodinating reaction mixture is that of Bolton and Hunter (6). These authors successfully iodinated several protein hormones that are denatured under the conditions of the Hunter–Greenwood method (1). They report, however, that the acylating agent is unstable under the iodination conditions. It would also be expected to acylate thiol groups.

We believe the diazotized iodoaniline coupling procedure reported herein offers an important alternative to existing labeling techniques. It does not involve exposure of protein to oxidants or impurities in commercially available Na\(^{125}\)I nor is the labeling reagent unstable under the reaction conditions used. Furthermore, it may be performed in less than 1 hr, gives reproducible results, and is suitable for labeling proteins with reactive thiol groups.

ACKNOWLEDGMENTS

We thank Mr. Allen Eckhardt of this university for preparing papain samples and assaying papain activity. We also acknowledge the generosity of Dr. J. Pollard and Dr. E. Schantz of Calbiochem in making B. simplicifolia seeds available to us. Mr. Tim Ross rendered technical assistance in one phase of this work.

REFERENCES