An Enzymic Method for the Trace Iodination of Immunoglobulins and other Proteins

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(Received 13 December 1968)

1. A method is described for the trace iodination of immunoglobulins and other serum proteins by a system consisting of lactoperoxidase, hydrogen peroxide and iodide. 2. γ G immunoglobulin that had been labelled to a specific radioactivity of $5\mu\text{Ci}/\mu\text{g}$. by use of carrier-free ^{125}I iodide gave no evidence of denaturation when analysed by electrophoresis and density-gradient ultracentrifugation. 3. Tryptic hydrolysis and peptide 'mapping' of a completely characterized peptide radioiodinated by this method showed that the ^{125}I iodide was bound to tyrosyl residues. 4. Proteins differ in their susceptibility to iodination by this method. Human γ G immunoglobulin, for example, is iodinated more than ten times as readily as is human α_2 -macroglobulin under the same conditions. 5. Lactoperoxidase catalyses the iodination of proteins much more readily than does horseradish peroxidase.

Proteins labelled with radioactive iodine have been extremely useful in biochemical studies. Various applications of the chloramine-T oxidation method of Hunter & Greenwood (1962) have enabled high specific labelling of hormones (Hunter & Greenwood, 1962; Greenwood, Hunter & Glover, 1963), bacterial flagellins (Ada, Nossal & Pye, 1964) and immunoglobulins (McConahey & Dixon, 1966; Brenneman & Singer, 1968; Marchalonis & Nossal, 1968) by using carrier-free ^{125}I iodide. These methods suffer certain drawbacks for precise analysis, because the iodination is carried out in the presence of high concentrations of a powerful oxidizing agent. Protein denaturation is an ever-present danger. This denaturation results from the oxidation of labile residues such as methionine (C. R. Parish & G. L. Ada, personal communication) and can manifest itself as an altered electrophoretic mobility (Landon, Livanou & Greenwood, 1967; Brenneman & Singer, 1968; Marchalonis & Nossal, 1968), the loss of biological activity (Landon *et al.* 1967) and variations in the rate of clearance of injected proteins from the bloodstream (McConahey & Dixon, 1966).

The disadvantages of the chloramine-T oxidation method gave impetus to the search for a more gentle means of radioiodinating proteins to high specific radioactivity. Enzymic iodination has been known for over 20 years. Crude mixtures containing various peroxidases and peroxide or peroxide-

generating systems were shown to be capable of iodinating tyrosine (Keston, 1944), thyroglobulin (De Robertis & Grasso, 1946; Klebanoff, Yip & Kessler, 1962) and bacterial proteins (Klebanoff, 1967). The present paper describes a method that uses catalytic amounts of purified lactoperoxidase to iodinate immunoglobulins and other proteins. This approach, in addition to being a gentle means for the iodination of proteins to high specific radioactivity, may provide a useful model system for the study of the iodination of thyroglobulin *in vivo*.

MATERIALS

Buffers. All buffers were sodium salts. Unless otherwise stated the solvent used to dissolve all reagents and proteins was 0.05M-phosphate buffer, pH 7.3, which was 0.15M with respect to NaCl. This is referred to as diluent buffer.

Peroxidases. A crude preparation of lactoperoxidase was prepared from the whey of fresh skim milk by $(\text{NH}_4)_2\text{SO}_4$ precipitation (Hogg, 1968). Globulins were removed by bringing the whey to 50% saturation with this salt. The enzyme precipitated from the supernatant solution when the salt concentration was brought to 60% saturation. Addition of $(\text{NH}_4)_2\text{SO}_4$ was performed at 25° and the pH was maintained at 8.0 by addition of 10M-NaOH. The enzyme was further purified by chromatography on CM-cellulose by the method of Morrison & Hultquist (1963). The purified lactoperoxidase possessed an E_{412}/E_{280} ratio of 0.65.

Purified horseradish peroxidase was obtained from C.F. Boehringer und Soehne G.m.b.H., Mannheim, Germany.

Peroxidase concentrations were estimated by measuring E_{412} (ϵ_{412} $11.1 \times 10^4 \text{M}^{-1}\text{cm}^{-1}$; Morrison, Hamilton & Stotz,

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1957). Activities of the two enzymes were assayed by the guaiacol oxidation method of Maehly & Chance (1954).

Proteins. (a) Immunoglobulins. Human γ G immunoglobulin (Cohn fraction II) was obtained from Commonwealth Serum Laboratories, Melbourne, Vic., Australia. Rabbit immunoglobulin G was prepared from serum by zone electrophoresis on starch (Kunkel, 1954) followed by gel filtration on Sephadex G-200 (Pharmacia, Uppsala, Sweden). The human γ M myeloma protein Frymac was prepared from serum (gift from Dr S. Whittingham) by application of the same procedures. Other myeloma immunoglobulins were a generous gift from Dr N. L. Warner.

(b) α_2 -Macroglobulin. This protein was prepared from normal human serum by zone electrophoresis and gel filtration as above.

(c) *Salmonella adelaide* flagellar proteins. Purified flagellar protein and its low-molecular-weight sub-unit (mol.wt. approx. 40000) were prepared by procedures described by Ada, Nossal, Pye & Abbot (1964). These proteins were a gift from Dr G. L. Ada.

All these proteins were homogeneous when assayed by zone electrophoresis on cellulose acetate membrane (see below for conditions). Flagellar protein from *Salm. adelaide* did not migrate electrophoretically; it remained at the origin under the conditions used.

[125 I]Iodide. Carrier-free samples were obtained from The Radiochemical Centre, Amersham, Bucks., at a concentration of 90–160 mc/ml.

METHODS

Iodination of human γ G immunoglobulin. The peroxidase-catalysed iodination reaction was carried out at room temperature (about 22°) in polystyrene tubes. The basic protocol was as follows (particular variations are described in the text): 250 μ g. of protein, 1.25 μ g. of lactoperoxidase and 1 μ l. of [125 I]iodide solution. In most experiments this solution consisted of [125 I]iodide diluted to give a specific radioactivity of 4 mc/ml. Sufficient unlabelled KI carrier was added to bring the total iodide concentration to that present in the carrier-free 125 I obtained from The Radiochemical Centre (estimated 0.1 mM). In some cases, 1 mc of carrier-free [125 I]iodide was used. The total reaction volume was 50 μ l. The reaction was initiated by the addition of 1 μ l. of 8.8 mM- H_2O_2 followed by vigorous mixing. When desired, the reaction was stopped by addition of 0.5 ml. of 5 mM-cysteine or 5 mM-2-mercaptoethanol.

Electrophoresis and radioautography of iodinated proteins. In analytical studies, the incorporation of radioactive iodide into proteins was established by taking samples from the reaction mixture, applying them to cellulose acetate membranes (Phoro Slide TM; Millipore Corp., Bedford, Mass., U.S.A.) and subjecting them to electrophoresis (30 mM-sodium veronal buffer, pH 8.6, 40 v/cm., 10 min.). The strips were stained with Amido Black 10B made up in a fixative solution (methanol-acetic acid-water, 5:1:5, by vol.). These were washed extensively in the fixative to remove unbound iodide. Association of iodide label with specific proteins after electrophoresis was demonstrated in two ways: direct counting and radioautography. In the former approach, the strips were cut into 1 mm. sections, which were counted individually in a Packard Autogamma Scintillation Spectrometer. Washing the strips with excess of [127 I]iodide failed to decrease the radioactivity, which

suggested that the radioactive iodide was covalently bound to the protein. In the latter approach, the strips were subjected to radioautography against X-ray film as described by Marchalonis & Nossal (1968). Exposure times varied from 4 to 48 hr. The stained protein on the strips and the exposed X-ray film were scanned with a Chromoscan Recording and Integrating Densitometer (Joyce, Loebel and Co., Gateshead, Co. Durham) as described by Marchalonis & Nossal (1968). A direct correspondence between the position of stained proteins and radioactive label was obtained.

In the kinetic studies, duplicate samples were taken at each time-point and placed on the Phoro Slide membrane. These were fixed, stained and washed as described above. Sections containing protein spots were then cut from the strip and counted in a Packard Autogamma Scintillation Spectrometer. The degree of incorporation of label into protein was calculated by comparison with the radioactivity present in samples of the unfractionated reaction mixture. Variation between duplicate analyses was less than 5%. Values obtained by this method agreed with those obtained by gel filtration on Sephadex G-25.

Determination of residues iodinated. A 21-residue peptide that contained one residue each of tyrosine and histidine (Carnegie, 1969) was obtained from Dr P. R. Carnegie. The peptide was radioiodinated by the peroxidase-catalysed method and subjected to tryptic hydrolysis. The tryptic peptides were separated by electrophoresis at pH 6.5 in pyridine acetate buffer (Offord, 1966) followed by chromatography in butan-1-ol-pyridine-acetic acid-water (15:10:3:12, by vol.). The peptide 'map' was subjected to radioautography against X-ray film.

Immunological analyses. The procedures used for immunoelectrophoresis and immune diffusion in agar gels were as described by Marchalonis & Edelman (1965). Goat antiserum to rabbit γ G immunoglobulin was obtained from Cappel Laboratories, Downington, Pa., U.S.A.

Ultracentrifugation on linear gradients of sucrose. This procedure was performed in accordance with a modification (Marchalonis & Edelman, 1965) of the velocity method of Martin & Ames (1961).

RESULTS

Lactoperoxidase-catalysed incorporation of [125 I]-iodide into human γ G immunoglobulin. Table 1 presents experiments designed to determine the conditions required for lactoperoxidase-catalysed iodination of human γ G immunoglobulin. The iodide used in experiments reported in this section consisted of [125 I]iodide in 0.1 mM-potassium iodide; 250 μ g. of globulin was used. Since the object of this approach was to enable the preparation of radioiodinated proteins for precise biological and biochemical studies, the ratio of enzyme to immunoglobulin had to be kept small. The mass ratio (μ g. of lactoperoxidase/ μ g. of γ G immunoglobulin) in this experiment was 0.005. In addition, the amount of hydrogen peroxide had to be sufficient to activate the lactoperoxidase, but not to oxidize the protein. The fact that the iodide bound was

Table 1. *Conditions for lactoperoxidase-catalysed iodination of human γ G immunoglobulin*Samples (a)–(f) all contained 250 μ g. of γ G immunoglobulin.

Reagents	[125 I]Iodide incorporated (%)	
	At 5 min.	At 30 min.
(a) [125 I]Iodide alone	0.1	1.4
(b) [125 I]Iodide + lactoperoxidase	3.1	3.0
(c) [125 I]Iodide + H ₂ O ₂	0.0	1.4
(d) [125 I]Iodide + H ₂ O ₂ + lactoperoxidase	85.7	94.0
(e) [125 I]Iodide + H ₂ O ₂ + lactoperoxidase + mercaptoethanol	1.8	5.9
(f) [125 I]Iodide + H ₂ O ₂ + lactoperoxidase + cysteine	1.6	7.4
(g) [125 I]Iodide + H ₂ O ₂ + lactoperoxidase (γ G immunoglobulin omitted)	0.7	5.0

actually linked to the γ G immunoglobulin was established by electrophoresis and radioautography.

Optimum iodination occurred when both hydrogen peroxide and lactoperoxidase were present. Little iodination resulted when [125 I]iodide alone or enzyme, but not hydrogen peroxide, was present. The reducing agents mercaptoethanol and cysteine virtually completely inhibited the iodination reaction. This effect occurred at concentrations of thiols that were much lower than those always used to cleave immunoglobulin disulphide bonds or to eliminate antibody activity (Edelman & Marchalonis, 1967). Radioautography gave no evidence of iodinated enzyme in the samples that contained immunoglobulin, although the enzyme itself was iodinated in the absence of other protein. The enzyme possessed an electrophoretic mobility clearly distinct from that of the γ G immunoglobulin and most of the proteins studied here. When enzyme, hydrogen peroxide and immunoglobulin were present (Table 1d), 94% of the iodide was incorporated into protein and the degree of substitution was 0.03 mole of iodide/mole of γ G immunoglobulin.

The effect of varying the concentration of lactoperoxidase when the concentrations of the other reagents are kept constant (250 μ g. of γ G immunoglobulin) is shown in Fig. 1. When the enzyme/ γ G immunoglobulin ratio was 0.005 the reaction went to 90% completion within 10 min. (curve A). A pronounced decrease in the rate of iodination was observed when the enzyme/ γ G immunoglobulin ratio was 5×10^{-5} . In this case (curve C) less than 50% of the iodide was incorporated into immunoglobulin at 60 min.

The effect of variation of γ G immunoglobulin concentration while those of the other reagents were fixed is illustrated in Fig. 2. The amount of enzyme used in each case was 0.05 μ g., which was 4% of the amount used for the experiment described in Table 1. The γ G immunoglobulin concentrations

Fig. 1. Effect of variation of lactoperoxidase concentration on the incorporation of [125 I]iodide into human γ G immunoglobulin. The reaction was carried out under the conditions described in the text. The amount of immunoglobulin used in each experiment was 250 μ g. The amounts of lactoperoxidase were: ●—● (curve A), 1.25 μ g.; ○—○ (curve B), 0.025 μ g.; ○—○ (curve C), 0.013 μ g.

were 250 μ g. (curve A), 25 μ g. (curve B), 12.5 μ g. (curve C) and 5 μ g. (curve D). As would be expected from considerations of mass action, the rate of incorporation of [125 I]iodide into γ G immunoglobulin decreased as the concentration of the protein decreased. Even with 5 μ g. in the 50 μ l.

Fig. 2. Effect of variation of immunoglobulin concentration on the incorporation of $[^{125}\text{I}]$ iodide into human γG immunoglobulin. The reaction was carried out under the conditions described in the text. The amount of lactoperoxidase in each tube was $0.05\text{ }\mu\text{g}$. The amounts of γG immunoglobulin were: $\bullet\text{---}\bullet$ (curve A), $250\text{ }\mu\text{g}$.; $\bullet\text{---}\bullet$ (curve B), $25\text{ }\mu\text{g}$.; $\circ\text{---}\circ$ (curve C), $12.5\text{ }\mu\text{g}$.; $\circ\text{---}\circ$ (curve D), $5\text{ }\mu\text{g}$.

reaction volume, however, 56% of the iodide was incorporated. In this last case, the substitution rate (moles of iodide/mole of γG immunoglobulin) was 0.84. Electrophoresis and radioautography of these samples showed that proteins in the mixtures used to obtain curves A, B and C possessed mobilities identical with those of untreated immunoglobulins. The protein in the mixture used to obtain curve D showed a slightly accelerated mobility.

Preparation of ^{125}I -labelled γG immunoglobulin with high specific radioactivity. Purified rabbit γG immunoglobulin was iodinated as described in the Methods section; 1 mC of $[^{125}\text{I}]$ iodide was used. The reaction was stopped after 15 min. at room temperature (22°) by the addition of 0.5 ml . of 10 mM -mercaptoethanol. Unbound iodide was removed by dialysis against dilution buffer. The specific radioactivity of the iodinated immunoglobulin was $5\text{ }\mu\text{C}/\mu\text{g}$.

A sample of this immunoglobulin was added to normal rabbit serum, which was then subjected to electrophoretic and immunochemical analyses.

Fig. 3. Zone electrophoresis on cellulose acetate of rabbit serum to which ^{125}I -labelled rabbit γG immunoglobulin had been added. These patterns are tracings of densitometer scans. (a) Original strip stained with Amido Black (total protein); (b) X-ray film radioautograph (radioiodinated immunoglobulin); $\downarrow$, origin; (+), anode; (-), cathode.

Fig. 3(a) presents the results obtained by scanning the stained cellulose acetate strip. A typical serum pattern resulted. The strip was subjected to radioautography against X-ray film. A densitometric scan of the exposed X-ray film is given in Fig. 3(b). All of the radioactive iodide was associated with the γG immunoglobulin. This electrophoretic pattern gave no indication of denaturation, which would be manifested by increased electrophoretic mobility or aggregation at the origin.

The fact that the enzymically labelled γG immunoglobulin possessed electrophoretic mobility identical with that of the untreated protein was confirmed by immunoelectrophoresis. A sample of the ^{125}I -labelled immunoglobulin was added to normal rabbit serum. This mixture was then subjected to immunoelectrophoresis, with goat antiserum to rabbit γG immunoglobulin being used to develop the precipitin arcs. The precipitin line of the radioactive γG protein corresponded exactly to that of the untreated protein. Ultracentrifugation of this mixture on a linear density gradient of sucrose showed that the radioactive protein sedimented as a single symmetrical component with a position on the gradient consistent with a sedimentation coefficient of about 7 S , that of the native material.

Effects of denaturation by chloramine-T as shown by electrophoresis. The effects of denaturation are illustrated in Figs. 4(a) and 4(b). In this experiment, two portions of the same sample of human γG -immunoglobulin were iodinated to the same degree of substitution ($0.03\text{ mole of iodide/mole of } \gamma\text{G}$

immunoglobulin) with lactoperoxidase (Fig. 4a) and chloramine-T (Fig. 4b). [^{125}I]Iodide in 0.1 mM-potassium iodide was used. The conditions for chloramine-T oxidation were 250 μg . of γG immunoglobulin and 25 μg . of chloramine-T. Both the enzymic and chemical iodinations were carried out at 22° for 15 min. Pronounced denaturation was observed in the chloramine-T-treated sample, which showed increased mobility and was located about the origin.

Evidence that incorporated [^{125}I]iodide was bound to tyrosyl residues. Since it has been shown that peroxidases can catalyse the iodination of free tyrosine (Serif & Kirkwood, 1958; Alexander, 1959), tyrosyl residues in γG immunoglobulin are probably among the residues iodinated in this study. In addition, work by Lamoureux, Carnegie & McPherson (1967) has shown that histidine, as well as tyrosine, was labelled when an encephalitogenic peptide from ox spinal cord was iodinated by a chloramine-T oxidation method. To test the possibility that the lactoperoxidase method catalyses the iodination of both residues under conditions described here, the completely characterized N-terminal peptide from human encephalitogenic protein (Carnegie, 1969) was studied. This peptide contains 21 amino acids, including one tyrosine at residue 14 and one histidine at residue 10. It was trace-iodinated by the main procedure described in the Methods section. The degree of substitution was 0.01 mole of iodide/mole of peptide. The [^{125}I]

labelled peptide was hydrolysed by trypsin and the tryptic peptides were analysed by peptide 'mapping'. The tyrosyl and histidyl peptides were clearly separated by this method. Two peptides were observed on the radioautogram of the peptide. They were thought to contain monoiodo- and diiodo-tyrosine respectively (Lamoureux *et al.* 1967). The two peptides were present in approximately equal amounts. Under these conditions no radioactivity was found to be associated with the histidyl peptide. A more detailed study is required to examine the possibility that histidine may be labelled under more vigorous conditions in this system and in other proteins and peptides.

Iodination of other proteins by lactoperoxidase. The iodination method developed for γG immunoglobulin was applied to other proteins, as shown in Table 2. The reaction conditions were those described in the Methods section with the use of [^{125}I]iodide in carrier potassium iodide. The enzyme/protein ratio was 0.005.

These results clearly indicated that the proteins varied in their susceptibility to iodination by this procedure. Some, notably normal human γG immunoglobulin and mouse γG myeloma protein HPC-3, incorporated iodine readily. Another group, which included bovine serum albumin, mouse γA myeloma protein HPC-1 and *Salm. adelaide* flagellin monomer, showed moderate capacity for iodination. A third group, which included human α_2 -macroglobulin, a γM myeloma protein (Frymac) and intact *Salm. adelaide* flagella, were iodinated poorly. Electrophoresis and radioautography were carried out on each protein. In all cases the iodine label was associated with the protein, and the protein possessed normal mobility. Intact *Salm.*

Fig. 4. Comparison by electrophoresis and radioautography of γG immunoglobulins radioiodinated by the lactoperoxidase method and by chloramine-T oxidation. (a) γG immunoglobulin iodinated to a degree of substitution of 0.03 mole of I/mole of γG immunoglobulin by lactoperoxidase. (b) γG immunoglobulin iodinated to the same degree of substitution by chloramine-T oxidation. These patterns are tracings of densitometer scans of exposed X-ray film radioautographs. $\downarrow$, origin; (+), anode; (-), cathode.

Table 2. Iodination of various proteins by the lactoperoxidase method

Iodination conditions were as follows: 250 μg . of protein; 1.25 μg . of lactoperoxidase; 1 μl . of [^{125}I]iodide in carrier KI; 1 μl . of 8.8 mM- H_2O_2 .

Sample	[^{125}I]Iodide incorporated (%)	
	At 10 min.	At 60 min.
Human immunoglobulin γG	90.2	97.3
Human γM myeloma protein Frymac	2.6	3.1
Human α_2 -macroglobulin	0.8	2.5
Mouse γM myeloma protein M104	19.3	26.2
Mouse γG myeloma protein RPC-5	67.5	75.9
Mouse γG myeloma protein HPC-3	92.8	104.0
Mouse γA myeloma protein HPC-1	48.2	50.2
Bovine serum albumin	54.4	60.3
<i>Salm. adelaide</i> flagella	8.5	36.5
Flagellin monomer sub-unit	33.0	78.3

Table 3. *Comparison of the abilities of lactoperoxidase and horseradish peroxidase to iodinate proteins*

The results were obtained 30 min. after addition of H_2O_2 to the reaction mixture, which contained 250 μg . of protein. Lactoperoxidase and horseradish peroxidase were added in equal amounts by mass (1.25 μg .) in the first four experiments, and in the last two experiments the lactoperoxidase and horseradish peroxidase were added in amounts possessing equal activities against guaiacol. The horseradish peroxidase showed against this substrate twice the activity of the lactoperoxidase when the enzymes were tested at equal concentration. The horseradish peroxidase was diluted 1:1 in this assay.

Protein	Enzyme	[^{125}I]Iodide incorporated (%)
Human γG immunoglobulin	Lactoperoxidase	85.5
Human γG immunoglobulin	Horseradish peroxidase	10.8
Bovine serum albumin	Lactoperoxidase	54.4
Bovine serum albumin	Horseradish peroxidase	5.0
Human γG immunoglobulin	Lactoperoxidase	95.3
Human γG immunoglobulin	Horseradish peroxidase	6.5

adelaide flagella remained at the origin under these conditions.

Comparison of horseradish peroxidase and lactoperoxidase. Since horseradish peroxidase is readily available commercially it was compared with lactoperoxidase for its capacity to catalyse the iodination of human γG immunoglobulin and bovine serum albumin. The reaction conditions were those used in the preceding section. The enzymes were compared by two criteria. First, they were tested at identical mass concentration. As shown in Table 3, the lactoperoxidase was at least 10 times as effective as the horseradish peroxidase. Secondly, they were normalized to equal activities against guaiacol. Again, the lactoperoxidase was at least an order of magnitude more efficient than was the horseradish peroxidase.

DISCUSSION

The object of this study was to devise a means for the radioiodination of immunoglobulins that would give high specific radioactivities without denaturation caused by the destruction of labile amino acids in the process. It was found that a system consisting of purified lactoperoxidase and hydrogen peroxide would catalyse the binding of iodide to tyrosyl residues on a variety of proteins and peptides. The method was gentle, rapid and required no special conditions. Physical, chemical and immunological analyses gave no evidence of denaturation in proteins iodinated by this method.

Two important characteristics of the system emerged from this study. (1) Proteins vary in susceptibility to iodination. This property probably reflects the accessibility of tyrosyl groups in the molecule. Among immunoglobulins, γG immunoglobulins are more readily iodinated than are the polymerized γM and γA proteins. A possible explanation for this is that the reactive tyrosines

may be obstructed in the polymer. A parallel situation was found to exist between polymerized bacterial flagellin and its monomeric form. Similar phenomena have been observed in studies using chemical methods of iodination (Covelli & Wolff, 1966; McConahey & Dixon, 1966).

(2) All peroxidases do not possess the same capacity to catalyse the iodination of proteins. Horseradish peroxidase was at least an order of magnitude less effective than lactoperoxidase at iodinating proteins. This discrepancy between the properties of the two enzymes has a parallel in their ability to inhibit the growth of streptococci. Jago & Morrison (1962) have shown that growth of *Streptococcus cremoris* strain 972 was inhibited by lactoperoxidase, but not by horseradish peroxidase.

Other peroxidases of animal origin such as myeloperoxidase also exhibit bactericidal properties. Klebanoff (1967) has shown that a system consisting of myeloperoxidase or lactoperoxidase, iodide and hydrogen peroxide iodinated *Escherichia coli*. The possibility exists that animal peroxidases, in addition to facilitating iodination of thyroglobulin in the thyroid gland, may serve as parts of a generalized defence mechanism against microbial infection (Klebanoff, 1967).

Chance (1952) proposed that the reaction of peroxidase with hydrogen peroxide results in the formation of a complex that has the capability to oxidize a variety of substrates. In accordance with previous hypotheses (Klebanoff *et al.* 1962; Yip & Hadley, 1967), peroxidase-catalysed iodination of tyrosine may result from the reaction between the iodine atom and the phenyl radical of tyrosine, both produced by enzymic oxidation. In the present study, the hydrogen peroxide probably functions to form the reactive complex with the enzyme, which then attacks iodide or tyrosyl residues. The difference in ability of lactoperoxidase and horseradish peroxidase to iodinate proteins probably

reflects differences in their capacity to oxidize these substrates.

The lactoperoxidase method for the radioiodination of proteins provides a rapid means by which some proteins, notably immunoglobulins, can be labelled with ^{125}I specific radioactivities greater than $5\mu\text{C}/\mu\text{g}$. The reaction can be carried out at 22° with little danger of denaturation of proteins. However, it must be noted that high substitution of iodide may lead to loss of activity because many proteins such as antibodies possess tyrosine in their combining sites (Singer & Doolittle, 1966). It is hoped that this approach may prove useful in biochemical and immunological studies.

This paper is publication no. 1306 from the Walter and Eliza Hall Institute for Medical Research. I thank Miss Sue Stevens for expert technical assistance. I am grateful to Professor G. L. Ada for helpful advice. I gratefully acknowledge the help of Dr D. McC. Hogg and Dr G. R. Jago in acquainting me with the properties of the lactoperoxidase system and providing samples of the purified enzyme. I thank Dr P. R. Carnegie for valuable advice and assistance with the peptide 'mapping' studies. This work was performed under the tenure of a Postdoctoral Fellowship of the American Cancer Society, and was supported by the National Health and Medical Research Council, Canberra, Australia, the U.S. Public Health Service (AI-0-3958), the U.S. Atomic Energy Commission (AT-30-0-3695) and the International Atomic Energy Agency (470/RB).

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