conjugated ecdysteroids cannot be eliminated as precursors of 20-hydroxyecdysone in the hemolymph[13-15]. Recent in vitro studies on prothoracic glands revealed 3-DHE to be the major product in several insects, e.g. *Manduca, Papilio, Precis*. The 3-DHE secreted by the prothoracic glands was demonstrated to be converted into ecdysone by hemolymph 3-oxoecdysteroid 3β-reductase (3-DHE reductase), and successively into the active molting hormone, 20-hydroxyecdysone[6,7,16]. Metabolic experiments were therefore attempted with ecdysone and 3-DHE. After incubation of the Y-organs with [^{3}H]ecdysone, most of the radioactivity was confined to ecdysone (fig. 6). Similar radiograms were also obtained for Y-organ homogenates. When Y-organs were incubated with hemolymph, 3-DHE was scarcely converted into ecdysone (not shown). Thus, in the present experiments, 3-DHE was shown to be neither the descendant nor the precursor of ecdysone; this result is in agreement with that for *Cancer antennarius*[4]. Further studies are necessary to clarify the physiological role of 3-DHE in crustaceans.

Acknowledgments. The authors wish to express their gratitude to Dr Sho Sakurai of Kanazawa University (Japan) for the gifts of antiserum S-3, and Dr Lawrence I. Gilbert of the University of North Carolina (USA) for H-22. Thanks are also due to Dr Isamu Nakatani of Yamagata University (Japan) for his technical advice on the dissection of the crayfish.

1 Chang, E. S., and O'Connor, J. D., Proc. natl Acad. Sci. USA *74* (1977) 615.
2 Keller, R., and Schmid, E., J. comp. Physiol. B *130* (1979) 347.
3 Lachaise, F., Meister, M. F., Hetru, C., and Lafont, R., Molec. cell. Endocr. *45* (1986) 253.
4 Spaziani, E., Rees, H. H., Wang, W. L., and Watson, R. D., Molec. cell. Endocr. *66* (1989) 17.
5 Naya, Y., and Sonobe, H., Heredity *44* (1990) 62.
6 Warren, J. T., Sakurai, S., Rountree, D., Gilbert, L. I., Lee, S., and Nakanishi, K., Proc. natl Acad. Sci. USA *85* (1988) 985.
7 Kiriishi, S., Rountree, D. B., Sakurai, S., and Gilbert, L., Experientia *46* (1990) 716.
8 Carrow, G. W., Calabrese, R. L., and Williams, C. M., Proc. natl Acad. Sci. USA *78* (1981) 5866.
9 Dinan, L., and Rees, H. H., Steroids *32* (1978) 2368.
10 Webster, S. G., and Keller, R., in: Ecdysone; From Chemistry to Mode of Action, p. 211. Ed. J. Koolman. George Thieme Verlag, Stuttgart, New York 1989.
11 Naya, Y., Ohnishi, M., Ikeda, M., Miki, W., and Nakanishi, K., Proc. natl Acad. Sci. USA *86* (1989) 6826.
12 Warren, J. T., and Gilbert, L. I., in: Immunologic Techniques in Insect Biology, p. 181. Eds L. I. Gilbert and T. A. Miller. Springer-Verlag, Heidelberg 1988.
13 Charmantier-Daures, M., and Vernet, G., C.r. Acad. Sci. Paris *278* (1974) 3367.
14 Connat, J. L., and Diehl, P. A., Insect Biochem. *16* (1986) 91.
15 Snyder, J. M., and Chang, E. S., Gen. comp. Endocr. *81* (1991) 133.
16 Sakurai, S., Warren, J. T., and Gilbert, L. I., Archs Insect Biochem. Physiol. *10* (1989) 179.

0014-4754/91/090948-05$1.50 + 0.20/0

Self-labeling of human polymorphonuclear leucocyte myeloperoxidase with ^{125}iodine

G. Deby-Dupont[a,b], J. Pincemail[c], A. Thirion[d], C. Deby[c], M. Lamy[b] and P. Franchimont[a]

[a] Laboratoire de Radioimmunologie, B23, Université de Liège, Sart-Tilman, B-4000 Liège 1, [b] Service d'Anesthésiologie, CHU, B35, Université de Liège, Sart-Tilman, B-4000 Liège 1, [c] Laboratoire de Biochimie et de Radiobiologie, Institut de Chimie, B6, Université de Liège, Sart-Tilman, B-4000 Liège 1, and [d] Centre de Transfusion de Liège, rue Dos Fanchon, B-4000 Liège (Belgique)
Received 3 January 1991; accepted 18 February 1991

Abstract. In order to obtain a radioimmunoassay (RIA) technique for the measurement of human plasma myeloperoxidase (MPO), we purified the enzyme from polymorphonuclear granulocytes (neutrophils), and compared three methods of labeling it with ^{125}Iodine: chloramine T, lactoperoxidase, and an original technique of 'self labeling' based on the ability of the enzyme to oxidize and bind ^{125}I in the presence of H_2O_2. The chloramine T technique produced a degraded protein, as well shown by a high non-specific binding of tracer to antibody. The lactoperoxidase technique did not succeed in labeling MPO with an adequate specific activity. In contrast, the self-labeling method gave a stable tracer with a specific activity of 23 µCi/µg MPO (85 MBq), a satisfactory level of immunoreactivity, and a low-specific binding ($\leq 3\%$). After labeling, purification of tracer was achieved by gel filtration chromatography in phosphate buffer (0.05 M; pH7) to which 0.1% poly-L-lysine was added. The labeled molecule remained stable for 40 days and could be used for RIA with a polyclonal antibody raised in rabbits.
Key words. Human; leucocytes; myeloperoxidase-iodination-radioimmunoassay.

During phagocytosis, or in the presence of particular stimuli (endotoxins, complement fraction C_{5a}, etc.), polymorphonuclear leucocytes (PMNLs) are activated and release active oxygen species[1,2], proteases[3] and myeloperoxidase[4] into both the intra- and extracellular medium. In the presence of a halide and hydrogen peroxide (H_2O_2), MPO yields chlorinated species: the result is the formation of a highly toxic system which has antimi-

crobial, antiparasitic and cytotoxic properties[2], but also plays an important role in tissue damage at sites of inflammation. As MPO is essentially located in the azurophilic granules of PMNLs, the presence of high concentrations of MPO in plasma can thus provide specific evidence of the activation and degranulation of PMNLs, which happens during many inflammatory states and almost certainly during the development of the adult respiratory distress syndrome (ARDS)[7,8].

Direct enzymatic measurements of MPO in plasma or serum are problematic because of the presence of interfering material, so that we decided to develop a sensitive and specific immunological assay of MPO to study the release of this enzyme into the plasma of patients who were at risk of developing an ARDS. In 1972, MPO was measured in serum of leukemic patients by a radioimmunodiffusion technique, but with a low sensitivity[9]. Several specific immunological methods were then published[10–14], most of them using ^{125}I-labeled anti-MPO immunoglobulins[11,12] or enzyme-linked anti-MPO immunoglobulins[15], but avoiding the labeling of MPO. Indeed, the tracer obtained after labeling of MPO using classical methods of iodination (free lactoperoxidase[16], or lactoperoxidase fixed on polyacrylamide[17], or chloramine T in the presence of H_2O_2[18]) always had low specific activity and low immunoreactivity[10] for 'unexplained reasons'. In order to obtain a reliable ^{125}I-MPO tracer for our radioimmunoassay (RIA) of MPO, we tried an original method of 'self-labeling' based on the capacity of MPO to oxidize and bind halide in the presence of H_2O_2, and determined the best procedure for purification of this ^{125}I-MPO. Its immunoreactivity and time-dependent degradation were compared with those of tracers obtained by conventional labeling techniques (chloramine T and lactoperoxidase).

Material and methods

Purification of MPO. Human MPO was extracted from isolated normal human leucocytes[19]. The enzyme purification procedure involved detergent solubilization (cetyltrimethylammonium bromide Sigma M-7635), ammonium sulphate precipitation, cation exchange chromatography on SP-50 (Pharmacia) and gel filtration chromatography on Aca 34 (IBF)[20]. The absorbance of each fraction from the last chromatography step was determined at two wavelengths, 430 and 280, and the ratio A_{430}/A_{280} (Reinheitszahl: RZ) was taken as an index of purity[21]. The fractions with a RZ greater than 0.65 were pooled and kept at −20 °C. Our final enzyme preparation was tested for contamination with leucocytic elastase by a spectrophotometric enzymatic test using Suc-Ala-Ala-Ala-p-nitranilide as specific substrate (Bachem L-1385)[22]. Electrophoresis was carried out either on SDS-PAGE 7–15% with 0.1 M tris-glycine buffer pH 8.3, 0.1% SDS and 5% 2-mercaptoethanol, or on 9% polyacrylamide gel at pH 4.6 according to the procedure of Brewer and Ashworth[23]. Gels were stained with Coomassie blue for protein and by the periodic acid-Schiff method for carbohydrate[24]. The enzymatic activity of MPO on polyacrylamide gel at pH 4.6 was evidenced by immersion of the gel for 10 min in 1 mM o-dianisidine followed by 1 mM H_2O_2[20]. The specific enzymatic activity of the pure MPO was measured with o-dianisidine as hydrogen donor in presence of H_2O_2[25]. One unit of enzymatic activity was defined as a change in absorbance of 1.0 optical density unit per minute at 460 nm.

Iodination procedure. The iodination was always performed at room temperature in an 11 × 55 mm polystyrene tube, under continuous agitation. Three methods were tried:

a) *Lactoperoxidase assay.* 5 µg of pure MPO dissolved in 20 µl Na acetate buffer 0.5 M, pH 5.6 were incubated with the different reagents added in the following order: 1 mCi (10 µl) of Na^{125}I (NEN; specific activity ±17 Ci/mg), 4 µg (0.4 U) of lactoperoxidase (Sigma) in 10 µl Na acetate buffer and 0.33 µg of H_2O_2 (20 µl of an appropriate dilution of perhydrol Merck). Ten minutes later, a second addition of 0.33 µg of H_2O_2 was performed and, after a further reaction period of 10 min, 400 µl Na acetate buffer was added to stop the reaction by dilution.

b) *Chloramine T assay.* To 10 µl phosphate buffer 0.5 M, pH 7.4, containing 5 µg of pure MPO, were added 1 mCi (10 µl) of Na^{125}I and 20 µg of chloramine T (Merck, analytical grade) dissolved in 10 µl phosphate buffer 0.05 M, pH 7.4. After 60 s, the reaction was stopped by addition of 25 µg (10 µl) of Na metabisulphite (Merck analytical grade) in phosphate buffer 0.05 M, and the reaction mixture was diluted by addition of 400 µl of phosphate buffer.

c) *'Self-labeling' assay.* 5 µg of pure MPO were dissolved in 10 µl Na acetate buffer 0.5 M, pH 5.5, and 1 mCi (10 µl) of Na^{125}I and 10 µl of $1.9.10^{-4}$ M H_2O_2 were successively added to the enzyme solution. After 10 min, 300 µl of Na acetate buffer was added to stop the reaction by dilution.

Purification of radioiodinated MPO. After labeling, ^{125}I-MPO was separated from denatured MPO and unfixed ^{125}I by gel filtration chromatography (column length: 70 cm; ∅ 0.8 cm) on Aca 23 (IBF), a gel filtration medium on which proteins with molecular weights (MW) in the range from 350,000 to 20,000 can be fractionated. Elution was performed with phosphate buffer 0.05 M, pH 7.5 containing 0.5% bovine serum albumin (Boehringer) and 0.05% sodium azide (Merck analytical grade). In some purification assays, 0.1% cetyltrimethylammonium bromide (Sigma) or 0.1% poly-L-lysine hydrobromide (Sigma) were added to the phosphate elution buffer.

Assay of immunoreactivity. After chromatography, the elution fractions corresponding to ^{125}I MPO were tested for their immunoreactivity with a rabbit antiserum against human MPO. 100 µl portions of a convenient dilution (±20,000 cpm) of each fraction were incubated

for 48 h at room temperature in the presence of 100 µl of buffer and 100 µl of the antiserum at an initial dilution of 1/40,000. The buffer used was 0.05 M phosphate, pH 7.5 with 0.5% bovine serum albumin and 0.05% sodium azide. After the incubation, the tracer bound to antibody was separated from the free labeled antigen by a second antibody precipitation (normal rabbit serum at a 1/100 dilution and sheep anti-rabbit gammaglobulin serum at a 1/200 dilution) in the presence of 6% polyethylene glycol 6000, 0.5% Tween 20 and 0.2% microcrystalline cellulose[26]. After centrifugation and discarding of the supernatant, the precipitate was counted in a gamma-spectrometer (LKB). The non-specific binding of tracer was tested by incubation in the absence of antiserum.

Results

Purification of MPO. The purified enzyme was free of elastase activity and presented an RZ value >0.72. Its specific activity was 375 U/mg protein. The SDS-polyacrylamide (7–15%) gel electrophoresis pattern showed three bands of proteins (fig. 1, lane 2): an intense one of MW ± 58,000 and two minor bands of MW ±90,000 and 15,000. Carbohydrate staining was negative on these two last bands (lane 3).

Iodination with lactoperoxidase. The elution of the labeling mixture on an Aca 34 column with phosphate buffer separated three peaks (fig. 2). The first one (A) and the second one (B) with a low radioactivity were found in fractions 13–16 (MW ≥ 150,000) and 27–30 (MW ± 20,000). The last peak (C), which was highly radioactive, was eluted in fractions 35–39 and corresponded to non-reacted iodide. As a considerable amount of radioactivity remained on the column, 0.1% cetyltrimethylammoniumbromide was added to the buffer, but no other radioactive peak could be eluted.

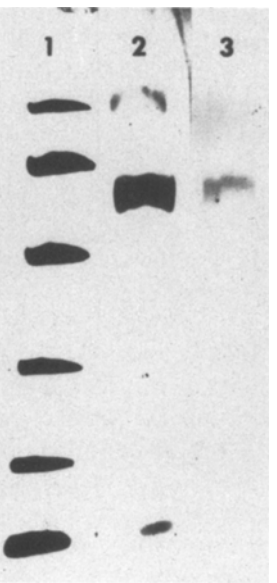

Figure 1. SDS-Polyacrylamide gel electrophoresis of pure human leucocyte myeloperoxidase. Lane 1: standard proteins: (a) phosphorylase b, MW 94,000; (b) bovine serum albumin, MW 67,000; (c) ovalbumin, MW 43,000; (d) carbonic anhydrase, MW 30,000; (e) trypsin inhibitor, MW 20,100; (f) α-lactalbumin, MW 14,400. Lane 2: MPO was stained for proteins. Lane 3: MPO was stained for carbohydrates.

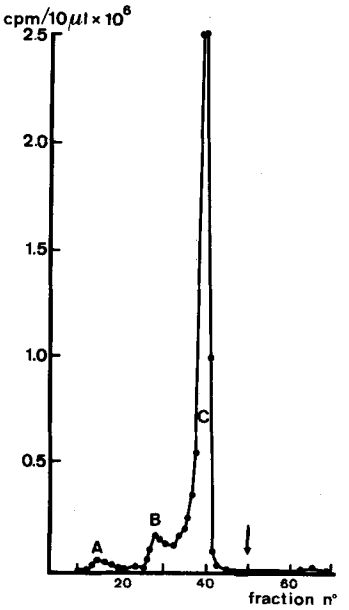

Figure 2. Purification of ^{125}I-MPO by gel filtration chromatography on Aca 34 (radioiodination of MPO by lactoperoxidase). cpm: counts per minute measured on a 10-µl aliquot for each eluted fraction: ↓: buffer added with 0.1% cetyltrimethylammonium bromide. A, B, C: see text.

Table 1. Identification and test of immunoreactivity for ^{125}I-material obtained after iodination of MPO by the lactoperoxidase (MPO) technique. B/T: % of tracer bound to antibodies (B) versus total radioactivity added to the incubation medium (T). NSB: binding of tracer in the absence of antibodies.

	B/T	NSB	Identification
Peak A	46%	29%	Degraded or polymerized ^{125}I-MPO
Peak B	6%	4%	^{125}I-MPO fragments or ^{125}I-lactoperoxidase
Peak C	2.2%	2%	Non-reacted iodide

The three peaks were tested for their immunoreactivity with an anti-MPO serum (table 1): peak A was immunoreactive, but it contained degraded materials, as shown by the high non-specific binding (29%), and was not suitable for RIA. Peak B was not immunoreactive and contained either radioactive MPO fragments or ^{125}I-lactoperoxidase. The last peak, C, containing the non-reacted iodide, had no immunoreactivity.

Iodination with chloramine T. The fractionation of the labeling mixture on Aca 34 with phosphate buffer produced two peaks (fig. 3). The first one (A), with a low radioactivity, corresponded to a protein with a molecular weight about 150,000, and the second one (B) contained the non-reacted iodide. Another labeled protein remained on the column and could only be eluted when 0.1% cetyltrimethylammonium bromide was added to the elution buffer (peak C).

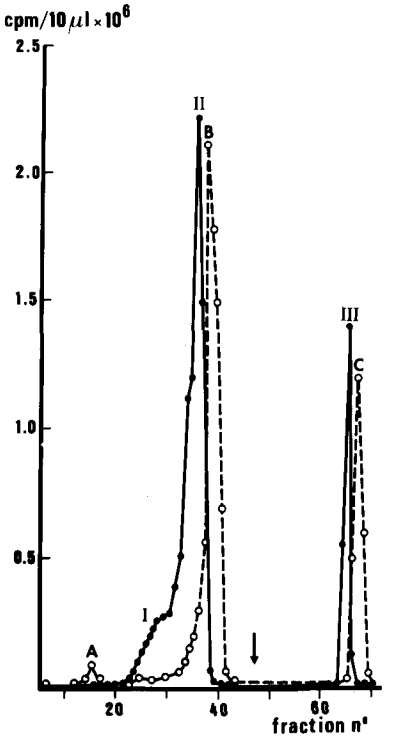

Figure 3. Purification of ^{125}I-MPO by gel filtration chromatography on Aca 34 (radioiodination with chloramine T [o---o] or by self-labeling [●—●]). cpm: counts per minute measured on a 10-µl aliquot for each eluted fraction. A, B, C, I, II, III: see text.

Table 2. Identification and test of immunoreactivity for ^{125}I material obtained after iodination by chloramine T. (B/T, NSB: see legend of table 1).

	B/T	NSB	Identification
Peak A	46%	35%	Polymerized or degraded ^{125}I-MPO
Peak B	2.2%	2%	Non-reacted iodide
Peak C	68%	27%	Degraded ^{125}I-MPO

The first peak (A) contained immunoreactive material but also degraded material (non-specific binding: 35%), and the peak B was without immunoreactivity (table 2). The last peak (C) contained immunoreactive material (B/T: 68%), but this was largely degraded (NSB: 27%), and thus unsuitable for RIA. Moreover, the degradation process continued, even at −20 °C, and was complete after 10 days (total loss of immunoreactivity).

Iodination by 'self-labeling' assay. After gel filtration on Aca 34 in phosphate buffer (fig. 3) a large radioactive peak (II) without immunoreactivity (table 3) was observed in fractions 31–35 and identified as non-reacted iodide. It was preceded by a weakly radioactive shoulder peak (I) containing immunoreactive ^{125}I-MPO (B/T: 72%) but with a high non-specific binding (32%). A last peak (III) could be eluted afterwards, but only when 0.1% cetyltrimethylammonium bromide was added to the phosphate buffer. It showed a significant immunoreactivity (B/T: 65%) with a low non-specific binding (2.5%), and was identified as ^{125}I-MPO.

Table 3. Identification and test of immunoreactivity for ^{125}I-material obtained after self-labeling of MPO. (B/T, NSB: see legend of table 1).

	B/T	NSB	Identification
Peak I	72%	32%	Degraded MPO
Peak II	1.1%	1%	Non-reacted iodide
Peak III	65%	2.5%	^{125}I-MPO

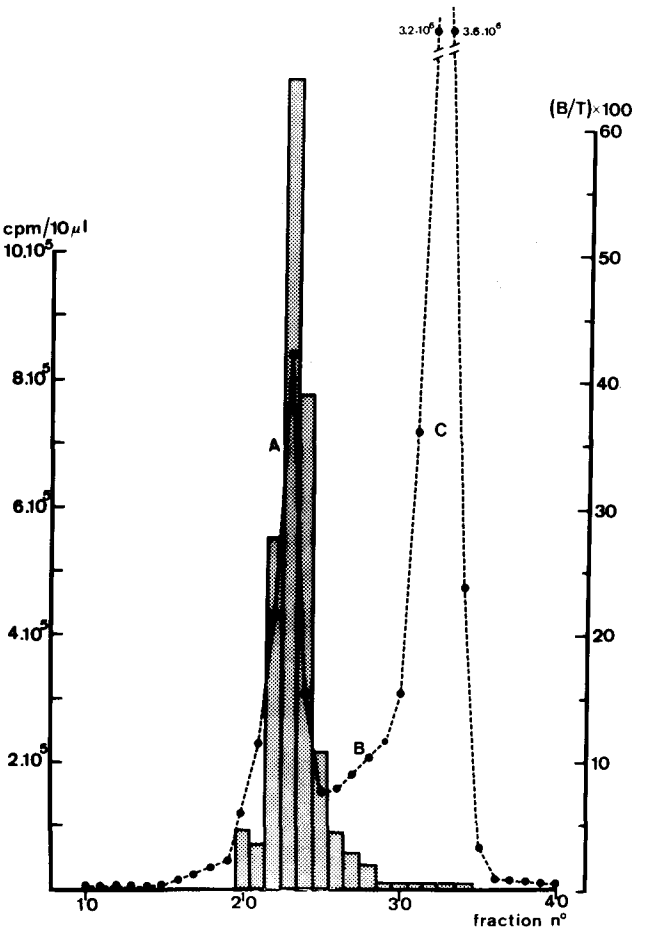

Figure 4. Self-labeling of MPO: purification of ^{125}I-MPO an Aca 34 with phosphate buffer added with 0.1% poly-L-lysine. Left ordinate: radioactivity [●---●] in counts per minute (cpm) for a 10-µl aliquot of each eluted fraction. Right ordinate: immunoreactivity (shaded column) expressed as the ratio of tracer bound to antibody (B) on total activity (T). A, B, C: see text.

Unfortunately, this tracer lost its immunoreactivity within 15 to 20 days, by interaction with the detergent (cetyltrimethylammonium) used for elution. Therefore, we eluted with phosphate buffer containing 0.1% poly-L-lysine, which could reduce the binding of ^{125}I MPO on Aca 34 without having a detergent effect. With this new elution buffer, three peaks of radioactivity were observed (fig. 4). Peaks B (degraded ^{125}I-MPO) and C (non-reacted iodide) were similar to peaks I and II of figure 3. The

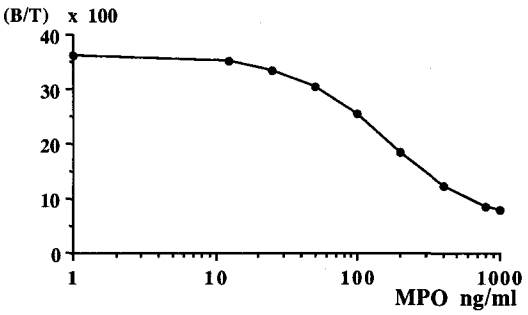

Figure 5. Standard curve for RIA of human MPO. Ordinate: percent of tracer bound to antibodies in the presence of increasing concentrations of unlabeled MPO (B) versus total radioactivity in the incubation medium (T). Abscissae: concentration (logarithmic scale) of unlabeled human MPO (ng/ml).

first peak (A) appeared in fractions 22–24, where proteins with MW ± 60,000 are eluted. Its immunoreactivity was excellent (B/T: 30%) with a low non-specific binding (≤3%). This tracer remained stable until day 40 to 45 after labeling without significant loss of immunoreactivity. Its specific activity was 23 μCi/μg or 85 MBq (around 1.5 atom of iodine per molecule of MPO).

Ria of MPO. The tracer obtained after self-labeling, purified on Aca 34 in the presence of poly-L-lysine, was used to establish a standard RIA curve with unlabeled MPO concentrations ranging from 10 to 1000 ng/ml (fig. 5). This RIA technique allowed us to measure MPO in human plasma (anticoagulated with EDTA), where we observed a mean normal value of 340 ± 100 ng/ml for 152 healthy volunteers [27].

Discussion

Myeloperoxidase, one of the major components of polymorphonuclear cells (5% of total dry weight) [2], plays a key role in the host defence of the organism by its ability to produce toxic and bactericidal agents [2]. However, myeloperoxidase-dependent reactions resulting from hyperactivation of PMNLs are implicated in inflammatory diseases [28, 29]. Therefore, the development of an effective measurement technique for MPO (as an index of granulocyte activation) could be of prime importance in clinical investigation. We developed a radioimmunoassay procedure which needed a highly purified enzyme, a specific antiserum and a stable tracer.

The enzyme, purified according to Bakkenist et al. [20] with minor modifications, exhibits an RZ (ratio A_{430}/A_{280}) of 0.72, which is considered as a good level of purity [18]. No elastase (a protein present in the same granules as MPO) was found in our final preparation. Gel electrophoresis confirmed the purity of the MPO preparation. Only one band with peroxidase activity was evidenced after electrophoresis on 9% polyacrylamide gel, pH 4.6 (data not shown). SDS-PAGE (7–15%) in presence of 2-mercaptoethanol separated three proteins of MW 90,000, 58,000 and 15,000. Although the molecular weights of the subunits of MPO are still in dispute [4, 14, 20, 30–36], and although different myeloperoxidases do exist [37], it is generally agreed that human myeloperoxidase is composed of two heavy subunits (MW 60,000) containing the heme group, and two light subunits (MW 15,000) [31]. The presence of a supplementary weak band with MW 90,000 has been already mentioned; it was identified either as a minor form of MPO [35] or as a myeloperoxidase precursor already containing the heme [38, 39]. In agreement with previous work [31, 35], periodic acid-Schiff staining showed that only the heavy chains contain carbohydrates.

Labeling of MPO with ^{125}I was a critical step in the development of the radioimmunoassay. Two classical labeling techniques (chloramine T and lactoperoxidase) gave us unsatisfactory results. The chloramine T assay produced two iodinated fragments of MPO (peak A and C of fig. 3) with significant immunoreactivity, but with a high non-specific binding, which is an index of degradation. Similar results were observed with the lactoperoxidase assay. Moreover, it seems that lactoperoxidase itself was labeled instead of MPO, as indicated by peak B, which showed radioactivity, but no immunoreactivity (fig. 2, and table 1).

In 1971, Thorell et al. [16] published the first procedure for labeling MPO with $Na^{125}I$, using lactoperoxidase immobilized on acrylamide. Although this method gave excellent results with several proteins, no satisfactory results were obtained with MPO despite a long period of dialysis to eliminate non-reacted iodide: ^{125}I incorporation into MPO was weak, only 32% compared to 50–70% for other proteins. Using chloramine T, the same authors also found that MPO was largely denatured during the iodination procedure, and Hansen et al. have also indicated that MPO remained difficult to label for 'unknown reasons' [10]. In 1977, Olofsson et al. [9] described iodination of MPO by chloramine T and H_2O_2: their method was apparently successful, but they gave no data about the immunoreactivity or the non-specific binding of their tracer, nor did they explain why they had used H_2O_2 together with chloramine T.

Our technique of self-labeling in the presence of H_2O_2 and $Na^{125}I$ gave us indisputably better results. It is well known that MPO catalyses the oxidation of halides (Cl^-, Br^-, F^-) in the presence of hydrogen peroxide, to yield hypohalous acids (HOCl, HOBr, HOF) [40]. By its strong oxidant activity, HOCl can transfer Cl to several molecules. A good example is the chlorination of monochlorodimedon to produce dichlorodimedon, which is a commonly-used test for the enzymatic activity of MPO [41]. Another enzymatic test is the radioiodination of serum proteins on tyrosine residues by $MPO/H_2O_2/Na^{125}I$, well documented by Klebanoff et al. [42, 43]. Since the human MPO contains 24–27 tyrosine residues per mol [20, 30], a binding of ^{125}I on these residues is likely, resulting in a 'self-labeling' of MPO. However, the purification of the tracer by gel filtration on Aca 34 remains a

critical step since ^{125}I-MPO often remains absorbed on the gel filtration medium. We partially resolved this problem by adding 0.1% cetyltrimethylammoniumbromide detergent to the buffer, and were able to obtain a tracer with high immunoreactivity and low non-specific binding. However, the elution of the tracer remained time-consuming (resulting in potential risks of degradation) and was not always reproducible. Moreover, during storage, the presence of the detergent led to denaturation of the ^{125}I MPO. The replacement of the detergent by 0.1% poly-L-lysine allowed us to obtain a tracer well separated from non-reacted iodide, with a good immunoreactivity and a low non-specific binding (maximum 3%), after only 2–3 h of elution. In contrast to tracers obtained with conventional techniques, our tracer has the major advantage of being stable for more than one month without loss of immunoreactivity.

However, under our experimental conditions, MPO totally loses its enzymatic activity, since H_2O_2 is used at a final concentration of $1.9\ 10^{-4}$ M, so that MPO is inactivated by compound II formation[44]. This suggests that the enzymatic site of the enzyme is quite different from the immunological site. The enzymatic activity was not conserved with the conventional labeling techniques either.

Using ^{125}I-MPO obtained by the 'self-labeling' method, we have developed a radioimmunoassay technique for measurement of this enzyme in biological samples, with a sensitivity of 20 ng/ml with an antiserum at an initial dilution of 1/40,000[27]. A normal mean value of 340 ± 100 ng/ml was found in EDTA-anticoagulated plasma of healthy volunteers. The coefficient of variation of this RIA (within or between assays) never exceeded 7% for MPO values ranging from 100 to 800 ng/ml. With this method, we have already produced evidence of neutrophil activation in patients with acute inflammatory reactions[45], in patients undergoing cardiopulmonary bypass[46] and in volunteers submitted to physical exercise[47].

Acknowledgments. The authors wish to thank Mrs Salemme-Casertano for typing the manuscript and Miss A. Dethier for preparing the diagrams. This work was supported by the Fonds National de la Recherche Scientifique (FNRS) grant n° 3.4574.89.

1 Babior, B. M., N. Engl. J. Med. *298* (1978) 659 and 721.
2 Klebanoff, S., J. Bact. *95* (1968) 2131.
3 Janoff, A., White, R., Carp, H., Harel, S., Dearing, R., and Lee, D., Am. J. Path. *97* (1979) 111.
4 Bentwood, B. J., and Henson, P. M., Immun. *124* (1980) 855.
5 Agner, K., Acta physiol. scand. *2* (1941) 1.
6 Schultz, J., and Kaminker, K., Archs Biochem. *96* (1962) 465.
7 Powe, J. E., Short, A., Sibbald, W. J., and Driedger, A. A., Crit. Care Med. *10* (1982) 712.
8 Simon, R. H., and Ward, P. A., in: Inflammation Basic Principles and Clinical Correlates, p. 815. Eds J. I. Gallin, I. M. Golstein and R. Snyderman. Raven Press, New York 1988.
9 Malmquist, J., Scand. J. Haemat. *9* (1972) 311.
10 Hansen, N. E., Malmquist, J., and Thorell, J. I., Acta med. scand. *198* (1975) 437.
11 Olofsson, T., Olsson, I., and Venge, P., Scand. J. Haemat. *18* (1977) 73.
12 Venge, P., Hällgren, R., Stalenheim, G., and Olsson, I., Scand. J. Haemat. *22* (1979) 317.
13 Oberg, G., Dahl, R., Ellegaard, J., Sundström, C., Vaeth, M., and Venge, P., Eur. J. Haemat. *38* (1987) 148.
14 Olsen, R. L., Steigen, T. K., Holm, T., and Little, C., Biochem. J. *237* (1986) 559.
15 Neumann, S., Gunzer, G., Lang, H., Jochum, M., and Fritz, H., Fresenius Z. analyt. Chem. *324* (1986) 365.
16 Thorell, J. I., and Larsson, I., Biochim. biophys. Acta *251* (1971) 363.
17 Thorell, J. I., and Johansson, B. G., Immunochemistry *11* (1974) 203.
18 Greenwood, F. C., Hunter, W., and Glover, J., Biochem. J. *89* (1963) 114.
19 Borgeat, P., and Samuelsson, B., Proc. natl Acad. Sci. USA *76* (1979) 2148.
20 Bakkenist, A. R. J., Wever, R., Vulsma, T., Plat, H., and Van Gelder, B. F., Biochim. biophys. Acta *524* (1978) 45.
21 Agner, K., Acta chem. scand. *12* (1958) 89.
22 Baught, R. J., and Travis, J., Biochemistry *15* (1976) 836.
23 Brewer, J. M., and Ashworth, R. B., J. chem. Educ. *46* (1969) 41.
24 Segrest, J. P., and Jackson, R. L., Meth. Enzym. *28* (1972) 54.
25 Hurst, J. K., Albrich, J. M., Green, T. R., Rosen, H. J., and Klebanoff, S. J., J. biol. Chem. *259* (1984) 4812.
26 Wood, W. G., Stella, G., Müller, O. A., and Seriba, P. C., J. Clin. Chem. clin. Biochem. *17* (1979) 111.
27 Pincemail, J., Deby-Dupont, G., Deby, C., Thirion, A., Torpier, G., Faymonville, M. E., Damas, P., Tomassini, M., Lamy, M., and Franchimont, P., J. Immun. Meth. *137* (1991) 181.
28 Matheson, N. R., Biochem. biophys. Res. Commun. *108* (1982) 259.
29 Wong, P. S., and Travis, J., Biochem. biophys. Res. Commun. *96* (1980) 1449.
30 Matheson, N. R., Wong, P. S., and Travis, J., Biochemistry *20* (1981) 325.
31 Andrews, P. C., and Krinsky, N. I., J. biol. Chem. *256* (1981) 4211.
32 Harrison, J. E., Pablan, S., and Schultz, J., Biochim. biophys. Acta *493* (1977) 247.
33 Andersen, M. R., Atkin, C. L., and Eyre, H. J., Archs Biochem. Biophys. *214* (1982) 273.
34 Nauseef, W., Root, R. K., and Malech, H. L., J. Clin. Invest. *71* (1983) 1297.
35 Olsen, R. L., and Little, C., Biochem. J. *222* (1984) 701.
36 Olsson, I., Olofsson, T., and Odeberg, H., Scand. J. Haemat. *9* (1972) 483.
37 Svensson, B. E., Domeij, K., Lindvall, S., and Rydell, G., Biochem. J. *242* (1987) 673.
38 Olsson, I., Eur. J. clin. Invest. *17* (1978) Poster 322 (21st Annual Meeting of the Eur. Soc. for Clin. Invest., March 21–24, Copenhagen Denmark).
39 Arnljots, K., and Olsson, I., J. biol. Chem. *262* (1987) 10430.
40 Zgliezynski, M., Stelmzynska, T., Domanski, J., and Ostrowski, W., Biochim. biophys. Acta *235* (1971) 419.
41 Hager, L. P., Morris, D. R., Brown, F. S., and Eberwein, H., J. biol. Chem. *241* (1966) 1769.
42 Klebanoff, S. J., J. exp. Med. *126* (1967) 1063.
43 Klebanoff, S. J., and Clark, R. A., J. Labo. clin. Med. *89* (1977) 675.
44 Odijama, T., Yamazaki, I., Biochem. biophys. Act. *206* (1970) 71.
45 Pincemail, J., Faymonville, M. E., and Lamy, M., in: Update in Intensive Care and Emergency Medicine, vol. 8, pp. 24–32. Ed. J. L. Vincent. Springer-Verlag, Berlin 1989.
46 Faymonville, M. E., Pincemail, J., Duchateau, J., Paulus, J. M., Adam, A., Deby, C., Albert, A., Larbuisson, R., Limet, R., and Lamy, M., J. Thorac. cardiovasc. Surg. (1991) in press.
47 Pincemail, J., Camus, G., Roesgen, A., Dreezen, E., Bertrand, Y., Lismonde, M., Deby-Dupont, G., and Deby, C., Eur. J. appl. Physiol. *61* (1990) 319.

0014-4754/91/090952-06$1.50 + 0.20/0