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Biosynthesis, transport and processing of myeloperoxidase in the human leukaemic promyelocytic cell line HL-60 and normal marrow cells

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The processing and intracellular transport of myeloperoxidase were studied in the human promyelocytic leukaemia cell line HL-60 and in normal marrow cells labelled with [^{35}S]methionine or [^{14}C]leucine. Myeloperoxidase was precipitated with anti-myeloperoxidase serum; the immunoprecipitates were subjected to sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and radiolabelled myeloperoxidase visualized by fluorography. During a 1 h pulse, myeloperoxidase was labelled in a chain of apparent M_r 90000. With a subsequent chase, the M_r 90000 polypeptide disappeared and was replaced by chains of M_r 62000 and 12400 corresponding roughly to the size of neutrophil myeloperoxidase subunits. The identification of the radioactive polypeptides as different forms of myeloperoxidase was established also by the similarity in patterns generated by partial proteolysis with V8 proteinase from *Staphylococcus aureus*. Processing of myeloperoxidase in HL-60 was slow; mature polypeptides were significantly increased only after 6 h. Another myeloperoxidase chain of apparent M_r 82000 was an intermediate precursor or degradation form. Pulse–chase experiments in combination with sucrose-density-gradient separations of homogenates showed that the M_r 90000 precursor was located in light density organelles only and not in granule fractions, whereas the M_r 82000 precursor was located only in intermediate density organelles, suggesting that the latter is a product of the former. Processed mature myeloperoxidase was concentrated in the granule fraction, but some occurred in lower density organelles, which may indicate processing during intracellular transport. Only the M_r 90000 polypeptide was secreted into the culture medium; this was also the only form found in the cytosol fraction.

During maturation of neutrophil precursor cells specific proteins are synthesized, transported to the Golgi apparatus for sorting and packaging into membrane-bound granules. Two distinct classes of granules are formed at subsequent stages of maturation (Bainton & Farquar, 1970; Bainton *et al.*, 1971). Azurophil (primary) lysosomal-like granules are formed in promyelocytes, whereas specific (secondary) granules are produced in myelocytes. Myeloperoxidase is a unique constituent of azurophil granules (Agner, 1941; Schultz & Kaminker, 1962; Bretz & Baggiolini, 1974; Spitznagel *et al.*, 1974), which also contain a number of cationic proteinases (Olsson & Venge, 1980). The mechanisms by which neutrophil granule enzymes are synthesized, processed and stored are largely unknown. Some lysosomal enzymes are synthesized as precursors and modified during or subsequent to storage in lysosomes (Hasilik & Neufeld, 1980*a*; Erickson *et al.*, 1981; Skudlarek & Swank, 1981).

To elucidate the subcellular progression and processing of newly synthesized neutrophil granule enzymes, we undertook to radiolabel them biosynthetically, to isolate them by immunoprecipitation followed by polyacrylamide-gel electrophoresis and fluorography. We have chosen to study a major constituent of azurophil granules, myeloperoxidase, to which rabbit antibodies have been generated (Olsson *et al.*, 1972). For biosynthetic labelling the human promyelocytic leukaemia cell line HL-60 (Gallagher *et al.*, 1979) was employed as well as marrow cells from healthy individuals. Our results demonstrate that myeloperoxidase is synthesized as larger precursors and shortened to the size of granule-bound myeloper-

Abbreviation used: SDS, sodium dodecyl sulphate.

oxidase prior to or subsequent to storage in azurophil granules.

Experimental

Chemicals

L-[^{35}S]Methionine (93 Ci/mmol) was from The Radiochemical Centre, Amersham. Sodium [^{35}S]-sulphate (0.5 Ci/mmol). [^{3}H]uridine (7.1 Ci/mmol), [^{3}H]arginine (15 Ci/mmol), L-[^{14}C]leucine (342 m Ci/mmol) and En^{3}Hance were from New England Nuclear. Protein A–Sepharose CL-4B was from Pharmacia, Uppsala, Sweden. Endo-*N*-acetyl-glucosaminidase H (endoglycosidase H) was from Miles Laboratories Inc., Rehovot, Israel. Phenyl-methanesulphonyl fluoride, monensin, and V8 proteinase from *Staphylococcus aureus* were from Sigma Chemical Co. Acrylamide/bisacrylamide (29:1) was from Bio-Rad.

Cell culture

HL-60 cells (passages 26–40) were maintained in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated foetal bovine serum. Cells were incubated at 37°C in a humidified atmosphere of air/CO_2 (19:1). All studies were made with exponentially growing cells with 95–100% viability judging from Trypan Blue exclusion.

Isolation of marrow cells

Bone marrow cells from healthy individuals were collected in heparinized McCoy's medium and separated on Isopaque/Ficoll (1.077 g/ml). The cells from the interphase were washed and used for biosynthetic labelling.

Labelling of cells

Biosynthetic labelling of myeloperoxidase. HL-60 or marrow cells were resuspended in methionine-free minimum essential medium (Eagle) with 10% foetal bovine serum and incubated at 37°C for 60 min to allow the depletion of the intracellular methionine pool. The labelling medium was the same deficient medium; the addition of 10% foetal bovine serum resulted in optimal biosynthetic protein labelling judging from overall incorporation of ^{35}S into trichloroacetic acid precipitable macromolecules. The cells, $(2–3) \times 10^6$/ml, were incubated with 50 μCi of [^{35}S]methionine/ml. In chase experiments, cells pulsed with [^{35}S]-methionine for 1–4 h were washed with and resuspended in RPMI 1640 medium with 10% foetal bovine serum at a density of 10^6 cells/ml, and incubated for 3–18 h.

Identification of subcellular sites for newly synthesized protein. Cells (10 ml, 3×10^6/ml) were incubated at 37°C for 10 min with 100 μCi of [^{35}S]-methionine/ml in methionine-free minimum essential medium (Eagle) containing 10% foetal-bovine serum, followed by the addition of cycloheximide, 200 μg/ml, which was also added to the homogenization medium. For identification of the intracellular site of sulphation, presumed to be localized to the Golgi apparatus (Young, 1973), cells (10 ml, 3×10^6/ml) were incubated at 37°C for 3 min with 50 μCi of [^{35}S]sulphate/ml in Dulbecco's medium. Substrates for sulphation in the form of glycosaminoglycans are produced in immature myeloid cells (Olsson, 1969). Ribosomal RNA was identified by incubation at 37°C for 3 h of 8 ml of cells (10^6/ml) with 100 μCi of [^{3}H]uridine/ml of RPMI 1640 medium with 10% foetal-bovine serum. Homogenates of the labelled cells were used for preparation of subcellular fractions by differential centrifugation or were analysed by sucrose-density-gradient centrifugation as described below. The distribution of macromolecular incorporation of radioisotope-labelled precursor was determined by trichloroacetic acid precipitation of aliquots from subcellular fractions followed by liquid-scintillation counting. β-Hexosaminidase and α-mannosidase were determined as described by Hultberg *et al.* (1976).

Subcellular fractionation

Before homogenization of labelled cells, 5×10^7 unlabelled carrier cells were added. Homogenization was at a concentration of 10^8 cells/ml in 0.34 M-sucrose/5 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] (pH 7.3)/0.5 mM-EDTA with 40 strokes of a Dounce glass homogenizer. The homogenate was diluted with the same solution and unbroken cells and nuclei were recovered by centrifugation at 700***g*** for 10 min, after which the granule fraction was collected at 8000***g*** for 15 min. The microsomal fraction was obtained by centrifugation of the post-granule supernatant at 200000***g*** for 2 h. The resulting supernatant was the cytosol. The granule and microsomal fractions are crude subcellular fractions which are to some degree cross-contaminated (see Table 1).

Subcellular organelles were also separated in a sucrose-density gradient. The 700***g*** supernatant was layered on 10 ml continuous sucrose gradients (density range 1.10–1.30 g/ml). Centrifugation was performed at 65000***g*** for 16 h in rotor model SW 25.3 (Beckman Instruments, Bromma, Sweden). Fractions (1 ml) were collected with a peristaltic pump. Density was read in a refractometer. The distribution in the gradient of Golgi elements and protein synthesis sites was determined by isotope labelling of cells as described above. Immunochemical determination of the total myeloperoxidase content of the fractions by single radial

immunodiffusion (Odeberg *et al.*, 1976) was used for localization of the azurophil granules.

Extraction of cells and subcellular fractions

Extraction was with a radioimmunoprecipitation assay (RIPA) buffer consisting of 0.15M-NaCl/30mM-Hepes (pH7.3)/1% (v/v) Triton X-100/1% (w/v) sodium deoxycholate/0.1% (w/v) SDS. Approx. 1ml of RIPA buffer containing 1mM-phenylmethanesulphonyl fluoride was used/10^7 cells. The extracts were kept on ice for 1h and centrifuged at 4°C at 32000*g* for 2h. The clear supernatant was stored frozen until used for immunoprecipitation. Sucrose-density-gradient fractions were supplemented with 1/10 the volume of a 10-fold concentrated RIPA buffer and dialyzed against RIPA buffer to remove sucrose. The fractions were then clarified by centrifugation at 4°C at 32000*g* for 2h and the supernatants were used for immunoprecipitation.

Immunoprecipitation of radioactive myeloperoxidase

Anti-myeloperoxidase serum was produced by immunization of rabbits (Olsson *et al.*, 1972). Adsorbed anti-myeloperoxidase was obtained by adding myeloperoxidase to the antiserum followed by centrifugation to remove the precipitate.

RIPA buffer extracts, 100–300μl, of whole cells and subcellular fractions or the RIPA buffer extract of sucrose-density-gradient fractions (1.0–1.5ml) were mixed with 15μl of anti-myeloperoxidase. Up to 3ml of culture medium or cytosol was used for immunoprecipitation with the addition of 50μl of anti-myeloperoxidase. After being left on ice for at least 60min, 40μl of a Protein A–Sepharose (200mg/ml) solution in RIPA buffer was added for collection of the immunoprecipitate by rotation at 4°C for 30–60min. The Protein A–Sepharose was washed five times with RIPA buffer and the supernatant was carefully removed. The pellet was resuspended in 50μl of water plus 15μl of electrophoresis sample buffer [0.4M-Tris/HCl (pH6.8)/50% (v/v) glycerol/10% (w/v) SDS/5% (w/v) mercaptoethanol], boiled for 5min and used for electrophoresis. In some experiments the Protein A–Sepharose was extracted with 0.5ml of 0.5M-NaOH and counted for radioactivity after addition of 10ml of Aquasol for determination of the total ^{35}S activity of immunoprecipitated myeloperoxidase.

SDS/polyacrylamide-gel electrophoresis and fluorography

SDS/polyacrylamide-gel electrophoresis was performed on slab gels 18cm long, 1.5mm thick, and 16cm wide, with ten wells, in an LKB 2001 vertical electrophoresis unit (LKB Products, Bromma, Sweden). The gels and the electrode buffers were made according to Laemmli (1970). Samples were loaded on a linear gradient of 5–20% polyacrylamide gel with a 3% stacking gel. The electrophoresis was run at 25mA/gel without cooling. After electrophoresis, gels were fixed in 10% (w/v) trichloroacetic acid/10% acetic acid/30% (v/v) methanol for 1h and then treated with En^{3}Hance for 1h and water for 1h. Gels were dried on filter paper, and exposed to X-ray film (Kodak X-Omat S) at −80°C for 2–3 days.

Apparent M_r values were determined by use of the following [^{14}C]methylated standards (New England Nuclear): cytochrome *c*, (M_r 12300), carbonic anhydrase (M_r 30000), ovalbumin (M_r 46000), bovine serum albumin (M_r 69000) and phosphorylase *b*, (M_r 97400).

To quantify radioactivity, individual bands, localized on the dried gel by fluorography, were excised and treated overnight at 37°C in 1ml of a solution containing 30% H_2O_2/30% NH_3 (19:1, v/v); 15ml of scintillation liquid was added for counting.

Peptide mapping

For peptide mapping HL-60 cells were labelled with [^{14}C]leucine. Cells were starved in leucine-free minimum essential medium (Eagle) with 1% foetal bovine serum for 60min at 37°C. Incubation was for 16h in the same deficient medium with 5% foetal bovine serum with 3×10^6 cells/ml and 10μCi of leucine/ml. Cell extraction, immunoprecipitation, and electrophoresis was as described above. Regions of the gel corresponding to myeloperoxidase species were cut out, equilibrated for 30min in buffer [0.125-Tris/HCl (pH6.8)/10mM-dithiothreitol/1% SDS) and packed in sample wells containing the same buffer (Myerowitz & Neufeld, 1981). Digestion with V8 proteinase from *S. aureus* in gel slices was as described by Cleveland *et al.* (1977) using a linear gradient of 5–20% polyacrylamide gel with 3% stacking gel (3cm). Buffer (20μl) containing 10% (v/v) glycerol with 5μg of V8 proteinase was layered over the slices in each well. Electrophoresis was performed at room temperature at 15mA/gel and was interrupted for 30min when the dye marker was close to the bottom of the stacking gel. Electrophoresis was continued at 25mA/gel without cooling followed by fluorography.

Results

Our results (Figs. 1–4) show that myeloperoxidase is synthesized as a precursor polypeptide with an apparent M_r of 89700 ± 1200 (S.D.). An intermediate form with an M_r of 81500 ± 900 (S.D.) was also found. The mature myeloperoxidase stored in the azurophil granules consisted of two polypep-

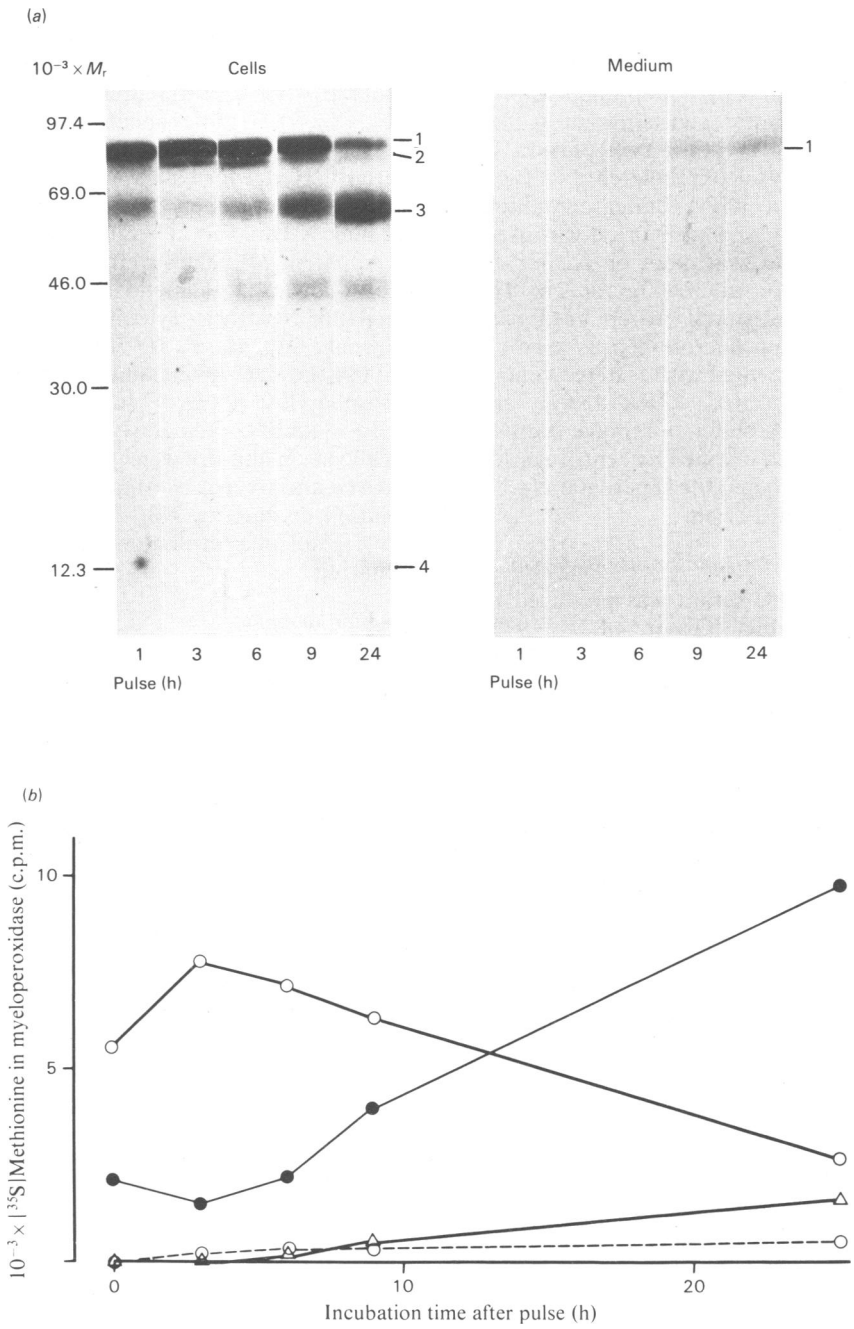

Fig. 1. *Pulse–chase labelling with [^{35}S]methionine of myeloperoxidase in HL-60 cells*

(*a*) HL-60 cells were pulse-labelled with [^{35}S]methionine for 60 min and the label was chased for 3, 6, 9 and 24 h. Extraction, immunoprecipitation with anti-myeloperoxidase serum, SDS/polyacrylamide-gel electrophophoresis and fluorography were performed as described in the text. The positions of the two myeloperoxidase precursor polypeptides (1 and 2) and the two mature myeloperoxidase polypeptides (3 and 4) are indicated to the right; M_r markers ($\times 10^{-3}$) are shown to the left. (*b*) The individual bands corresponding to the M_r 90000 (1) and 82000 (2) precursor polypeptides (○), the M_r 62000 (3) (●) and M_r 12400 (4) (△) mature polypeptides were localized on the dried gel using fluorography, excised and counted by liquid-scintillation. Data are also given for the M_r 90000 polypeptide of the medium (○----○), which was the only species of myeloperoxidase detected extracellularly.

tides with M_r values of 61670±2400 (S.D.) and 12375±100 (S.D.). In addition, bands were usually seen on the gels corresponding to M_r values of approx. 40000–45000. The latter components are regarded as degradation products of myeloperoxidase.

Biosynthesis of myeloperoxidase in HL-60

A pulse–chase experiment with [^{35}S]methionine is shown in Fig. 1. After 1h pulse, the major biosynthetically labelled myeloperoxidase consists of an M_r 90000 polypeptide (component 1); label is also associated with polypeptides with M_r values of 82000 (component 2), 62000 (component 3) and 12400 (component 4). During a subsequent 24h chase, radioactivity of components 1 and 2 decreases steadily (after an initial increase) with a concomitant increase of components 3 and 4. Significant increases in components 3 and 4 do not, however, occur until after 6h of chase. These observations indicate that myeloperoxidase is synthesized as precursor polypeptides with M_r values of 90000 and 82000. Furthermore it is shown in Fig. 1 that the only form of myeloperoxidase which is released to the culture medium is the M_r 90000 precursor polypeptide (component 1). Release to the culture medium occurs at a slow rate during the whole chase period, but only small amounts are released.

Preincubation of HL-60 cells for 30min with cycloheximide (10μg/ml) followed by [^{35}S]-methionine labelling for 4h abolished all myeloperoxidase biosynthesis (results not shown). Specificity controls showed that myeloperoxidase-absorbed anti-myeloperoxidase serum did not precipitate any labelled myeloperoxidase (results not shown).

Subcellular processing of newly synthesized myeloperoxidase in HL-60 cells

HL-60 cells were pulsed with [^{35}S]methionine for 1h and the label was chased for 4, 8 and 18h. Microsomal, granule and cytosol fractions were prepared, extracted and immunoprecipitated with anti-myeloperoxidase serum. Fluorograms obtained after SDS/polyacrylamide-gel electrophoresis are shown in Fig. 2. ^{35}S-labelled M_r 90000 polypeptide (component 1) is formed in pulse-labelled cells present both in crude microsomal, granule and cytosol fractions. Approximately the same pattern is seen after a 4h chase. However, significant changes occur during an 8h chase, with occurrence of the M_r 62000 and M_r 12400 polypeptides in both microsome and granule fractions. In addition the M_r 82000 polypeptide is visible at 8h. Its presence in the granule fraction is interesting because component 1 is faint in that fraction but predominant in the microsomal fraction.

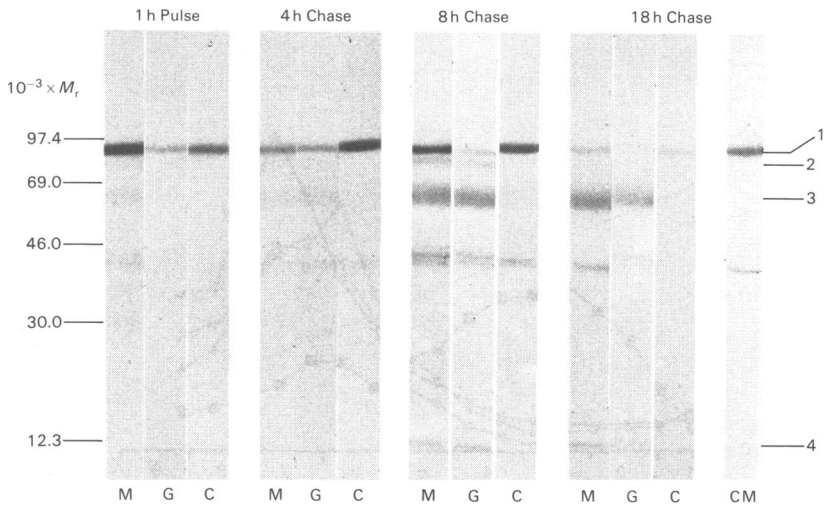

Fig. 2. *Pulse–chase labelling with [^{35}S]methionine of myeloperoxidase in HL-60 cells and distribution of labelled myeloperoxidase in subcellular fractions*

HL-60 cells were pulse-labelled with [^{35}S]methionine for 60min and the label was chased for 4, 6 and 18h. Crude subcellular microsome (M), granule (G) and cytosol (C) fractions were prepared. The analysis result for 18h chase culture medium (CM) is also given. Extraction, immunoprecipitation with anti-myeloperoxidase serum, SDS/polyacrylamide-gel electrophoresis and fluorography were performed as described in the text. The positions of the two myeloperoxidase precursor polypeptides, M_r 90000 (1) and M_r 82000 (2), as well as the two mature myeloperoxidase polypeptides, M_r 62000 (3) and M_r 12400 (4), are indicated to the right; M_r markers ($\times 10^{-3}$) are shown to the left.

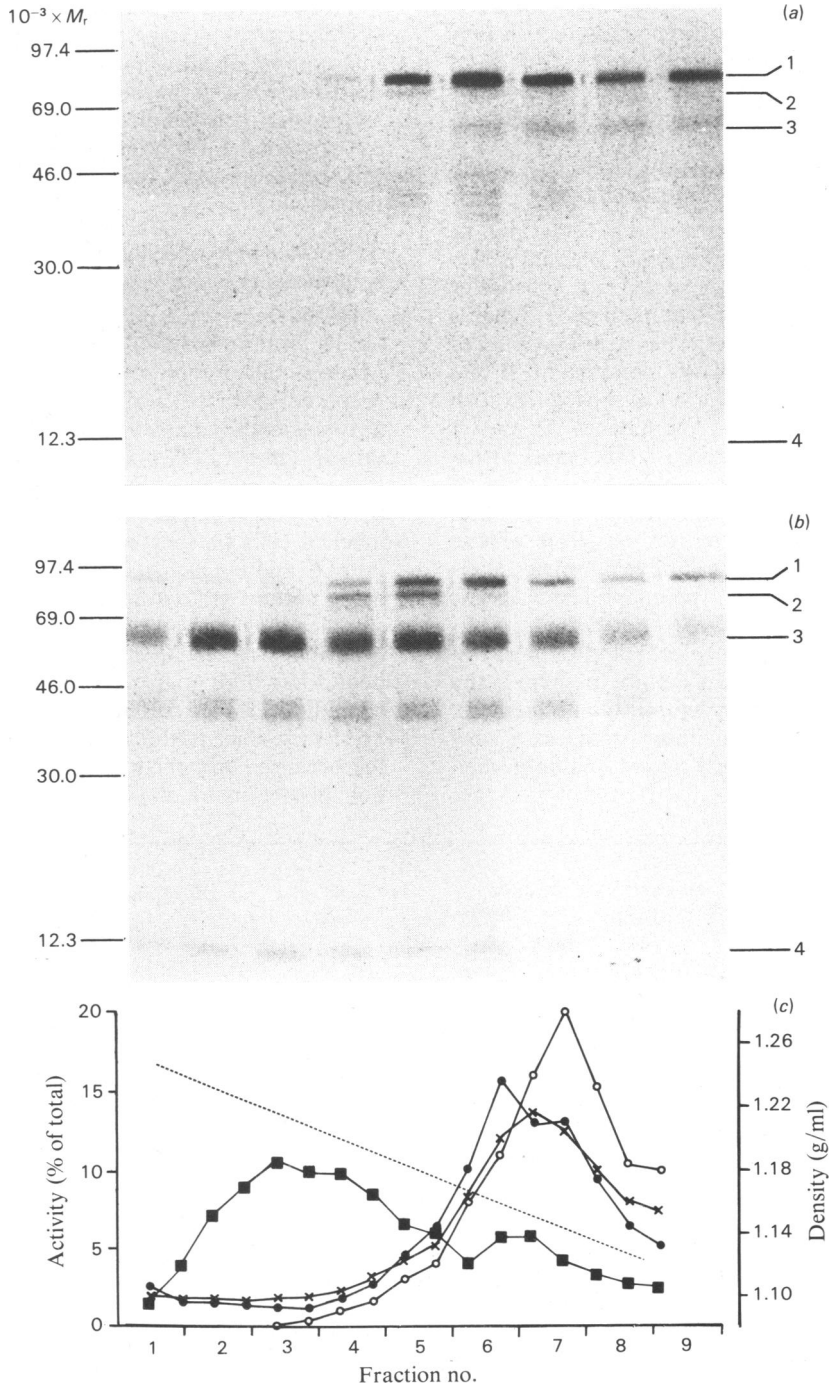

Fig. 3. *Pulse–chase labelling with [^{35}S]methionine of myeloperoxidase in HL-60 cells and distribution of labelled myeloperoxidase in subcellular fractions*

HL-60 cells were pulse-labelled with [^{35}S]methionine for 4h and the label was chased for 18h. The post-nuclear supernatants obtained after pulse (*a*) and 18h chase (*b*) were centrifuged in a sucrose density gradient for separation of subcellular organelles. Extraction, immunoprecipitation with anti-myeloperoxidase serum, SDS/polyacrylamide-gel electrophoresis and fluorography were performed as described in the text. The positions of the two myeloperoxidase precursor polypeptides, M_r 90000 (1) and M_r 82000 (2), as well as the two mature myeloperoxidase polypeptides, M_r 62000 (3) and M_r 12400 (4), are indicated to the right; M_r markers are shown to the left. Also shown (*c*) are the

During an 18h chase most of the radioactivity of the M_r 90000 and 82000 components disappeared with a concomitant increase of M_r 62000 and 12400 components. The results of Fig. 2 also show that only the M_r 90000 precursor form is released to the cytosol. Furthermore, this was the only form of myeloperoxidase which was released to the culture medium.

In a separate experiment, repeated twice, HL-60 cells were pulse-labelled with [^{35}S]methionine for 90min and the label was chased for 4, 8 and 18h. The total ^{35}S activity of immunoprecipitated myeloperoxidase was determined in culture medium, microsomal, granule and cytosol fractions. Compared with the ^{35}S-labelled myeloperoxidase of the culture medium present after the pulse an increase by 63% was seen during the chase period, after which labelled myeloperoxidase amounted to 2.1% of the total labelled protein of the medium. Similarly, microsomal myeloperoxidase decreased by 29% during the chase and granule myeloperoxidase increased by 59%, while cytosol myeloperoxidase decreased by 38%. Microsomal, granule and cytosol [^{35}S]myeloperoxidase amounted to 0.31%, 0.63% and 0.13%, respectively, of total labelled protein after 18h chase. These data are mean values from two separate experiments.

Interpretation of the data of Fig. 2 is complicated by the fact that cross-contamination exists between the granule and microsomal fractions. This is obvious from Table 1, showing results from lysosomal enzyme assays as well as distribution of protein synthesis sites and Golgi elements. Lysosomal enzymes are, however, concentrated in the granule fraction, whereas protein synthesis sites and Golgi elements are concentrated in the microsomal fraction. The cross-contamination between the granule and microsomal fractions may explain the presence of M_r 90000 precursor myeloperoxidase also in the granule fraction after 1h pulse labelling (Fig. 2). Therefore attempts were made to separate these organelles by using sucrose-density-gradient centrifugation of post-nuclear supernatant (Fig. 3). Newly synthesized protein, identified by a brief pulse-labelling with [^{35}S]-methionine, banded at a density of 1.14–1.17g/ml; [^{3}H]uridine incorporation sites into RNA banded at an identical density; Golgi-derived elements, identified by a 3min pulse with [^{35}S]sulphate, banded at a density of 1.14g/ml. Thus any separation between endoplasmic reticulum and Golgi elements was not accomplished. However, a complete separation was achieved between these organelles and granules identified by the distribution in the gradient of total myeloperoxidase (density 1.19–1.23g/ml), which is a specific marker for azurophil granules.

HL-60 cells were pulse-labelled for 4h with [^{35}S]methionine and the label was chased for 18h. Post-nuclear supernatants were analysed by sucrose-density-gradient centrifugation (Fig. 3). The density gradient fractions were extracted and immunoprecipitated with anti-myeloperoxidase serum. The fluorograms obtained upon electrophoresis are shown in Fig. 3. In pulse-labelled cells, the M_r 90000 precursor polypeptide was located in subcellular fractions with a density of 1.14–1.17g/ml and in the cytosol, represented by the top

Table 1. *Distribution of α-mannosidase and β-hexosaminidase in subcellular fractions obtained by differential centrifugation of an HL-60 cell homogenate*

Enzyme activity is expressed as units (1 μmol of substrate split/min) per 10^9 cell equivalents. Data are also given for the percentage distribution of ^{35}S-labelled macromolecules after a 3min pulse with [^{35}S]sulphate (identifying Golgi elements) and the distribution of [^{35}S]methionine-labelled macromolecules after a 10min pulse (identifying sites for newly synthesized protein; endoplasmic reticulum).

	pH	Whole homogenate	Nuclear fraction	Granule fraction	Microsomal fraction	Cytosol
α-Mannosidase	4.5	0.72	0.11	0.25	0.19	0.12
α-Mannosidase	5.5	0.56	0.11	0.16	0.08	0.18
β-Hexosaminidase	4.5	1.34	0.10	0.52	0.20	0.55
[^{35}S]Sulphate (Golgi)		78%	5.5%	15.0%	57.5%	22.5%
[^{35}S]Methionine (endoplasmic reticulum)		98%	5.2%	23.9%	46.3%	24.6%

density gradient (----), the distribution of ^{35}S-labelled macromolecules after a 3min pulse with [^{35}S]sulphate (○) identifying Golgi elements, the distribution of [^{35}S]methionine-labelled macromolecules after 10min pulse labelling (×) identifying sites for newly synthetized protein and the distribution of [^{3}H]uridine incorporation into RNA after 3h labelling (●). Immunochemical determination of total myeloperoxidase content (marker for azurophil granules) of the fractions is also shown (■).

fractions of the gradient; virtually no M_r 90000 polypeptide was associated with the dense granule-containing fractions. During an 18h chase, significant changes occurred; the M_r 90000 polypeptide is still visible in fractions with a density of 1.15–1.18g/ml and in the cytosol, the M_r 82000 polypeptide is present with a peak density of 1.18g/ml, but most of the radioactivity of the M_r 90000 polypeptide has now disappeared, resulting in an increase of the mature myeloperoxidase polypeptides with M_r values of 62000 and 12400. The latter were concentrated in the fractions containing azurophil granules, but some was also present in organelles with a lower density. By excising the gel and counting the ^{35}S activity associated with precursor and mature myeloperoxidase forms, about 70% transfer of radiolabel to the mature form was demonstrated to take place during the chase period.

Synthesis of myeloperoxidase by normal marrow cells

Experiments with normal marrow cells from healthy individuals gave similar results as for HL-60. Marrow cells were labelled with [^{35}S]-methionine for 18h and crude subcellular fractions were prepared, extracted and analysed by SDS/polyacrylamide-gel electrophoresis (Fig. 4). The microsomal fraction contained the myeloperoxidase precursor polypeptides as well as the mature polypeptides. The granule fraction lacked the M_r 90000 polypeptide but contained the other myeloperoxidase forms. As for HL-60 only the M_r 90000 polypeptide was detected in the cytosol. The culture medium from incubations with marrow cells was difficult to analyse by immunoprecipitation, because unspecific precipitates occurred also with non-immune serum, but the results indicated secretion exclusively of the M_r 90000 polypeptide (results not shown).

Peptide mapping

Partial proteolysis of the [^{14}C]leucine-labelled M_r 90000, 82000 and 62000 myeloperoxidase peptide forms, as well as the presumed degradation forms with M_r values of 40000–45000, is shown in Fig. 5. When the major precursor (M_r 90000) and mature form (M_r 62000) were compared the only difference was in the relative intensity of some peptide bands in the M_r range 30000–40000. Thus these two forms have profound structural similarities. Partial proteolysis of the M_r 12400 subunit gave very small fragments and the data are therefore not included in Fig. 5. Regarding the M_r 82000 component, the track in Fig. 5 is faint because of lack of labelled material; the pattern seems, however, to be identical with that of the M_r 90000 polypeptide. Also the track in Fig. 5 for the presumed degradation forms is faint but the pattern is clearly related to that for the myeloperoxidase forms.

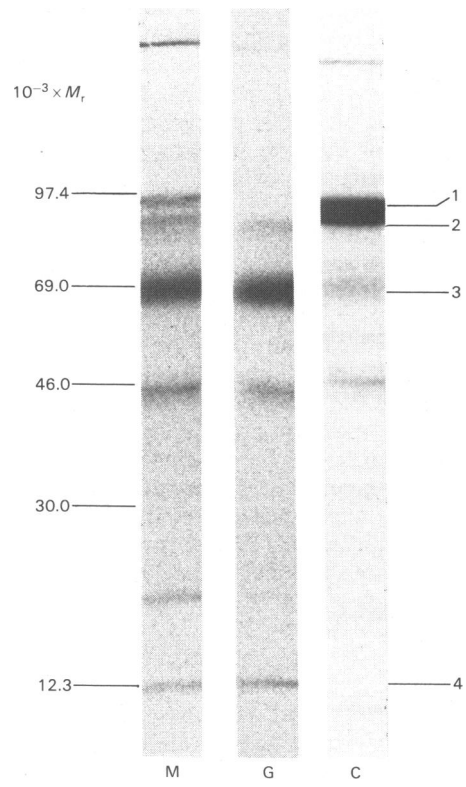

Fig. 4. [^{35}S]*Methionine labelling of myeloperoxidase in human marrow cells and distribution of labelled myeloperoxidase in subcellular fractions*

Marrow cells were labelled with [^{35}S]methionine for 18h. Crude subcellular microsome (M), granule (G) and cytosol (C) fractions were prepared. Extraction, immunoprecipitation with anti-myeloperoxidase serum, SDS/polyacrylamide-gel electrophoresis and fluorography were performed as described in the text. The positions of the two myeloperoxidase precursor polypeptides, M_r 90000 (1) and M_r 82000 (2) as well as the two mature myeloperoxidase polypeptides, M_r 62000 (3) and M_r 12000 (4) are indicated.

Discussion

Studies of biosynthesis and processing of neutrophil granule proteins require cells with active granulogenesis. The promyelocytic HL-60 cell line (Gallagher *et al.*, 1979) is useful because it produces azurophil granules with a high myeloperoxidase content. The present results indicate that myeloperoxidase is synthesized as M_r 90000 and M_r 82000 precursor polypeptides, which are trimmed to M_r 62000 and 12400 polypeptides,

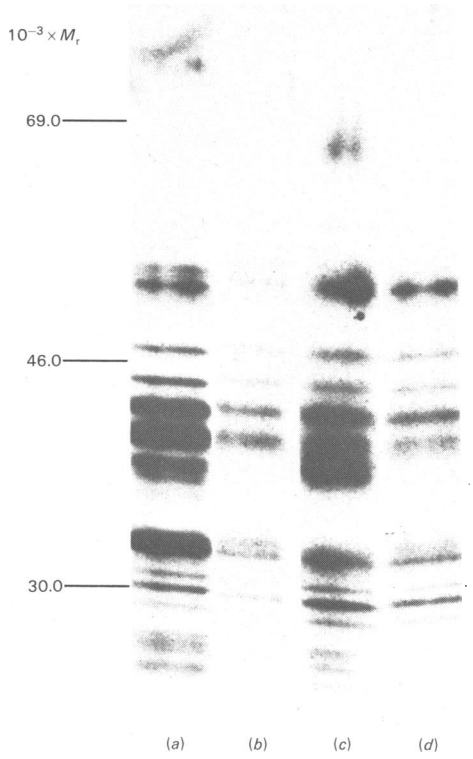

Fig. 5. *Peptide maps of* [^{14}C]*leucine-labelled myeloperoxidase*

HL-60 cells were labelled with [^{14}C]leucine for 16h, extracted and immunoprecipitated with anti-myeloperoxidase followed by SDS/polyacrylamide-gel electrophoresis. Regions of the gel corresponding to the M_r 90000 (*a*); 82000 (*b*), 62000 (*c*) and 40000–45000 (*d*) myeloperoxidase species were localized and cut out. Electrophoresis was carried out as described in the text.

corresponding to the size of chains found in mature neutrophils (Andrews & Krinsky, 1981). The data confirm some results from a recent report on myeloperoxidase synthesis in HL-60 cells (Yamada, 1982).

The polypeptide chains of higher M_r were identified as precursors to those of lower M_r because they were precipitated by antibodies raised against neutrophil myeloperoxidase and because the large chains decreased during pulse–chase experiments with the appearance of mature myeloperoxidase polypeptide chains. Thus the different forms are not synthesized independently. The peptide patterns generated by partial proteolysis with V8 proteinase also supported a precursor–product relationship. Native myeloperoxidase contains two heavy–light protomers, which are joined by a single disulphide bond between the heavy subunits (Andrews & Krinsky, 1981). The M_r reported for the two subunits is 57500 and 14000 (Andrews & Krinsky, 1981) roughly corresponding to the apparent M_r values of 62000 and 12400 that we found for fully processed myeloperoxidase localized in the granule fractions (see Fig. 3). The species seen with M_r values of 40000–45000 were regarded as degradation products because they do not correspond to any known form of myeloperoxidase; they may represent intracellular degradation of newly synthesized myeloperoxidase. Since the apparent M_r of the major precursor form was 90000, the conversion to M_r 62000 and 12400 subunits most likely occurs by proteolytic cleavage. It is unlikely that this conversion is a result of modification of carbohydrate side chains only. The relationship between the M_r 90000 and 82000 precursor polypeptides of myeloperoxidase is not clear. Our data from subcellular fractionation of HL-60 cells suggest that the M_r 82000 form is an intermediate or degradation product; it is localized in a subcellular organelle which is distinct from that holding most of the M_r 90000 precursor or mature myeloperoxidase. The subcellular site for the M_r 82000 peptide may be a pregranule structure.

Subcellular fractionation showed that completely processed myeloperoxidase is concentrated in the granule fraction, which is the storage site for myeloperoxidase (Bainton & Farquar, 1970; Bainton *et al.*, 1971; Bretz & Baggiolini, 1974; Spitznagel *et al.*, 1974). The finding of some processed myeloperoxidase also in fractions with lower density than granules either indicates contamination with granule material, release of some granular myeloperoxidase during sedimentation in the gradient or processing during intracellular progression of newly synthesized material. The sucrose gradient system used did not resolve clearly endoplasmic reticulum and Golgi elements, judged from brief pulse-labelling experiments to localize subcellular sites for protein synthesis and sulphation, respectively. Processing of myeloperoxidase in HL-60 cells is relatively slow; mature myeloperoxidase polypeptides were significantly produced only after 4–8h of chase with [^{35}S]methionine.

The finding of the myeloperoxidase precursors mainly associated with microsomal elements and the mature polypeptides with granules is inconsistent with results from a recent report, which showed that myeloperoxidase precursors were present exclusively in the soluble fraction of the cells, but the matured polypeptide chains were in both the soluble and 'granule' fractions (Yamada, 1982). However, in the latter report the homogenate was treated with a detergent, which could have solubilized precursor myeloperoxidase and some granule myeloperoxidase.

The M_r 90000 precursor was the only myeloperoxidase form detectable in cytosol and extracellular medium. It cannot be ruled out that cytosol myeloperoxidase originated from solubilization during the homogenization procedure of, e.g., vesicles involved in transport of the precursor polypeptide. Further work is needed to clarify this possibility. Despite the fact that considerable leakage occurred of both lysosomal and microsomal contents to the cytosol (Table 1), the latter was totally devoid of labelled mature myeloperoxidase. Thus mature myeloperoxidase is tightly bound to subcellular sites. The exclusive secretion of the M_r 90000 precursor form of myeloperoxidase is of interest because myeloperoxidase is detectable in plasma (Olofsson *et al.*, 1977; Olsson *et al.*, 1979). It remains to be determined if it is circulating in precursor form and has peroxidase activity.

Biosynthesis of lysosomal enzymes differs from that of secretory proteins because high-mannose oligosaccharide side chains of lysosomal enzymes are modified by phosphorylation (Hasilik & Neufeld, 1980*b*; Neufeld, 1981). A receptor for mannose 6-phosphate may therefore play an important role in directing acid hydrolases to lysosomes (Kaplan *et al.*, 1977; Sahagian *et al.*, 1981). Both the precursor polypeptides of myeloperoxidase, as well as the mature M_r 62000 polypeptide, were susceptible to digestion with endoglycosidase H indicating that they have high-mannose oligosaccharide side chains (I. Olsson, A.-M. Persson & K. Strömberg, unpublished work). It remains, however, to be determined if myeloperoxidase and other products of the lysosomal-like azurophil granules of neutrophils such as neutral proteinases (Olsson & Venge, 1980) contain phosphomannosyl residues to establish that the mannose 6-phosphate receptor is part of a general pathway for lysosomal protein segregation.

Several lysosomal enzymes have now been shown to have precursor forms (Hasilik & Neufeld, 1980*a*; Erickson *et al.*, 1981; Skudlarek & Swank, 1981), which may be a prerequisite for transport to the lysosomes or protection against destructive enzymes. Lysosomal enzymes of fibroblasts are present in the extracellular medium in precursor form (Neufeld, 1981) and may be recaptured by the cell through a receptor for mannose-6-phosphate (Kaplan *et al.*, 1977) and inserted into lysosomes. Further studies on the processing and intracellular transport, storage and secretion of neutrophil granule proteins are now feasible using the systems and techniques employed in the present work.

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