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Purification of hemopoietin 1: A multilineage hemopoietic growth factor

(growth factors/hemopoiesis/colony-stimulating factor/cell proliferation/differentiation)

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ABSTRACT **Hemopoietin 1 (H-1) and the mononuclear phagocyte specific growth factor CSF-1 act synergistically on developmentally early bone marrow cells to generate primitive CSF-1 receptor-bearing cells. The H-1 activity of the serum-free medium conditioned by the human urinary bladder carcinoma cell line 5637 was shown to result from the sum of the activities of two charged species (pI ≈4.8, ≈85%; pI ≈5.3, ≈15%) of similar size. No qualititative difference in the biological activity of these two species was detected. A four-step procedure, involving batch DEAE-cellulose chromatography, chromatofocusing, gel filtration, and hydrophobic chromatography has been developed for the major (pI ≈4.8) species. H-1 was purified approximately 65,000-fold and recovered as 32% of the total activity of the starting material. The lowest concentration yielding maximal biological activity was ≈0.25 ng/ml. The ^{125}I-labeled purified H-1, in either native or reduced form, behaved as a homogeneous single band that coelectrophoresed with the biological activity of purified H-1 on sodium dodecyl sulfate gel electrophoresis ($NaDodSO_4$/PAGE). The molecular mass of the purified reduced H-1, determined by $NaDodSO_4$/PAGE was ≈17 kDa. Recent studies indicate that the purified H-1 is a multilineage hemopoietic growth factor.**

Growth factors play an important role in regulating the production of the different mature blood cell types from the totipotent hemopoietic stem cell. Those involved in early steps in this pathway stimulate proliferation and differentiation of cells capable of forming more than one blood cell type (multilineage growth factors). Those controlling the later stages act on cells determined to form one particular blood cell type (lineage-specific growth factors) (reviewed in ref. 1). Studies of Iscove *et al.* (2, 3) and Bradley and co-workers (4–7) have indicated that synergism between lineage specific and multilineage growth factors is an important aspect of the regulation of hemopoiesis.

The colony-stimulating factor CSF-1 is a monouclear phagocyte lineage-specific growth factor (8–11). The ability of mononuclear phagocytic precursors to bind ^{125}I-labeled CSF-1 has been used to assay two multilineage growth factors, hemopoietin 1 (H-1) and hemopoietin 2 (H-2). These factors have been detected by their ability to stimulate proliferation of developmentally early CSF-1 receptor bearing cells (12, 13). In the presence of either H-1 or H-2, these cells eventually develop the capacity for increased expression of the CSF-1 receptor, an event that is soon followed by their differentiation into adherent mononuclear phagocytes.

H-2 (12) shares properties with, and is possibly identical to, interleukin 3 (14), the factor with erythroid burst-promoting activity (2, 3), P-cell stimulating factor (15), and hemopoietic-cell growth factor (16). H-1 differs from H-2 in several respects. The physical properties of the factor are different, its target cells are more primitive hemopoietic cells, and its activity in generating CSF-1 receptor-bearing cells requires CSF-1 (13). In this paper, we describe the purification and some properties of human H-1.

MATERIALS AND METHODS

H-1 Bioassay. Samples to be tested for hemopoietin activity were diluted in 10% α medium (see below), dialyzed against phosphate-buffered saline (P_i/NaCl, three changes, 24 hr, 4°C), sterilized by membrane filtration, and bioassayed in triplicate. The method used to bioassay H-1 was based on the ability of H-1 to synergize with CSF-1 to generate nonadherent CSF-1 binding cells from developmentally early bone marrow cells and is described in detail elsewhere (13). Briefly, nonadherent bone marrow cells from C3H/HeJ mice (The Jackson Laboratory) that had received an injection of 5-fluorouracil (150 mg/kg, i.v.) 1 day previously, were cultured in 35-mm tissue culture dishes (Falcon 3001, 2.0-ml cultures, 2×10^5 cells per ml) for 3 days in α medium containing 20% (vol/vol) fetal calf serum (Flow Laboratories), glutamine at 0.292 mg/ml, asparagine at 0.02 mg/ml, 50 μM 2-mercaptoethanol, penicillin at 0.2 g/liter, and streptomycin at 0.2 g/liter (20% FCS-α medium). All culture dishes except for controls (no addition, H-1 alone) contained 800 units of purified murine CSF-1 and 0.2 ml of the preparation to be tested. Following incubation, the nonadherent cells of each culture were resuspended in 3.0 ml of 20% FCS-α medium and incubated for a further 9 hr to up-regulate CSF-1 receptors. The capacity of the cells to specifically bind ^{125}I-labeled CSF-1 at saturating concentrations was then determined at 0°C (2 hr). The bioassay was standardized using a stable partially purified (stage 2) reference preparation defined (arbitrarily) as containing 20 units of H-1 per ml. Stage 2 H-1, in contrast to the starting material and stage 1 H-1, is devoid of inhibitory activity. Dilutions of the reference preparation were included in each bioassay to provide a standard curve from which the bioactivities of test samples could be determined.

Preparation of Serum-Free 5637 Cell-Conditioned Medium (5637 CM). The urinary bladder carcinoma cell line 5637 (17) obtained from Jorgen Fogh, Sloan-Kettering Institute, was maintained by subculture in 20% FCS-α medium in plastic roller bottles (3027, Falcon Labware). For preparation of serum-free 5637 cell conditioned medium (5637-CM), cells were trypsinized from the roller bottle by a 30-min incubation at 37° with 50 ml of 0.25% trypsin (Difco) in P_i/NaCl. Twenty milliliters of horse serum was added and the cells were

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Abbreviations: CSF-1, colony-stimulating factor; H-1, hemopoietin 1; H-2, hemopoietin 2; FCS, fetal calf serum; P_i/NaCl, phosphate-buffered saline; CM, conditioned medium.

centifuged (800 × g, 10 min, 4°C) and washed three times by centrifugation in α medium. The washed cells were seeded into round-bottomed flasks (4422 Pyrex; Corning; 4.3×10^7 cells per flask) each containing 1 liter of high-bicarbonate/glucose α medium (α medium diluted 1.26-fold with water and containing bicarbonate at 3.54 g/liter, D-glucose at 4.30 g/liter, penicillin at 0.2 g/liter, and streptomycin at 0.2 g/liter). Culture flasks were gassed with 10% CO_2 in air, sealed and incubated at 37°C until the monolayer began to disintegrate (≈14 days). After incubation, the medium was harvested from cultures of viable, attached cells and filtered through filter paper (Whatman 2V). The resulting 5637 CM was brought to 0.02% with respect to NaN_3 and stored at −20°C until used.

Purification of H-1 from 5637 CM. 5637 CM (≈7 liters) was diluted with 5 volumes of cold distilled water and DEAE-cellulose (DE52, Whatman), equilibrated and deaerated in 100 mM Tris·HCl was added (19 g dry weight/liter of diluted 5637 CM). The mixture was stirred, the pH was adjusted to 8.2 with 0.5 M NaOH, and the DEAE-cellulose was allowed to settle. The supernatant fluid was siphoned off and discarded and the DEAE-cellulose was collected on a sintered glass funnel (Pyrex, ASTM 40-60 C). The DEAE-cellulose was then washed six times each with a volume of deaerated, ice-cold 10 mM Tris·HCl, pH 8.2, equal to the packed volume of DEAE-cellulose, and the washings were discarded. Elution of H-1 was effected with six to eight washes, each with 1.5 volumes of deaerated, ice-cold, 50 mM NaCl in 100 mM Tris·HCl, pH 8.2. To ensure complete elution of H-1 the DEAE-cellulose was similarly washed with 150 mM NaCl in 100 mM Tris·HCl, pH 8.2. If this eluate was found to contain significant H-1 activity it was also included in the stage 1 pool. The eluate(s) was immediately concentrated to 1/10th volume by vacuum rotary evaporation at 40°C, then dialyzed (3500 M_r cut-off dialysis membrane; Spectrum Medical Industries, Los Angeles) against three changes of 100 volumes of polyethylene glycol 1000 at 0.02 g/liter (PEG 1000; Sigma) for 24 hr, and the precipitate was discarded. It was further concentrated by vacuum rotary evaporation to ≈3.0 ml and equilibrated in 25 mM histidine·HCl buffer, pH 6.2 (stage 1). Stage 1 material was applied to a chromatofocusing column (dimensions 27.0 × 0.7 cm) of PBE-94 (Pharmacia) that had been previously equilibrated with 25 mM histidine·HCl buffer, pH 6.2. Elution was carried out with 180 ml of 11% (vol/vol) Polybuffer 74 (Pharmacia), pH 3.9. Fractions (3.7 ml) were collected at a flow rate of 15 ml/hr. The pool of active fractions was brought to 80% saturation with respect to $(NH_4)_2SO_4$ by addition of powdered $(NH_4)_2SO_4$ and allowed to stand for 2 hr prior to centrifugation (2000 × g, 40 min, 4°C). The precipitate was dissolved in ≈3.0 ml of 50 mM NaCl/50 mM Tris·HCl, pH 7.4 (stage 2) and applied to a Sephadex G-50 (medium grade; Pharmacia) column (dimensions 150 × 2 cm) equilibrated in the same buffer. Fractions (3.7 ml) were collected at a flow rate of 20 ml/hr. The pool of active fractions was concentrated to ≈4 ml by vacuum rotary evaporation and dialyzed against 10 mM phosphate, pH 6.5 (stage 3). It was adjusted to 30% saturation with respect to $(NH_4)_2SO_4$ and applied to a phenyl-Sepharose CL-4B (Pharmacia) column (dimensions 3.5 × 0.7 cm) equilibrated in 27% saturated $(NH_4)_2SO_4$ and 10 mM phosphate, pH 6.4. Elution was carried out at a flow rate of 3.8 ml/hr using a linear 100-ml gradient from 27% saturated $(NH_4)_2SO_4$ to 50% ethylene glycol in 4% saturated $(NH_4)_2SO_4$ and collecting 3.0-ml fractions. Pooled active fractions were dialyzed against PEG 1000 at 0.01 g/liter and concentrated to ≈1 ml by vacuum dialysis (stage 4).

All operations were carried out at 4°C. All buffers used in the purification (except the Polybuffer 74 at stage 2) contained 3 mM NaN_3 to prevent bacterial contamination and PEG 1000 at 0.01 g/liter to prevent loss of biological activity in dilute solution (18). For stages 3 and 4, fraction collection tubes and other glassware were siliconized to prevent further losses due to adsorption. Triton X-100 (final concentration, 0.001%) was also added to some stage 4 preparations to further reduce the possibility of adsorption (19). Each stage of the purification could be stored at −20°C without loss of activity. Samples of each stage were saved for determination of biological activity and of protein (20). Small samples (≈1/100th) of stages 2 and 3 were iodinated as described below and used to trace-label stage 2 and stage 3 material for further purification, thus providing a sensitive means of following protein during fractionation (21). Lyophilization of stage 4 H-1 resulted in a loss of activity that could be prevented by addition of acetonitrile (10% vol/vol, HPLC grade; Fisher) immediately prior to shell-freezing.

Iodination. H-1 (stages 2–4) was iodinated using a modification (8) of the method of Greenwood *et al.* (22). For iodination of stage 4 H-1, incubation mixtures (31 μl) contained stage 4 H-1 (12 ng of protein), 0.56 nmol of carrier-free $Na^{125}I$ [13–17 mCi (1 Ci = 37 GBq)/μg of I; Amersham], 0.31 μg of PEG 1000, 52 μmol of $(CH_3)_2SO$, and 2.5 nmol of chloramine-T (Eastman) in 50 mM Na phosphate, pH 6.5. Incubation (30 min, 0°C) was stopped by addition of 3.0 nmol of $K_2S_2O_3$ (7 μl). KI (4 μl, 0.1 M) was added and the protein-bound and free ^{125}I was determined following their separation on a column (5 × 0.5 cm) of Sephadex G-50 (medium grade; Pharmacia) equilibrated in 0.3 mM Tris·HCl, pH 7.4, containing PEG 1000 at 0.01 g/liter. Fractions in the protein-bound peak were pooled and stored at −20°C. Iodination efficiencies and specific radioactivities were calculated as described elsewhere for CSF-1 (8). Iodination efficiencies for stage 4 H-1 were ≈2% (≈10^6 cpm/ng of protein).

Other Techniques. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis ($NaDodSO_4$/PAGE) was carried out in gel slabs according to the method of Laemmli (23). Reduction of stage 4 H-1 was accomplished by heating in 0.1 M 2-mercaptoethanol in sample buffer for the indicated times. Those lanes to be assayed for H-1 bioactivity were cut out and fractionated using a Mickle gel slicer (Mickle Laboratory Engineering, Comshall, Surrey, England), and the H-1 was eluted from each segment by incubation in 0.8 ml of 10% FCS (16 hr, 4°C) (24). Eluates were sterile filtered and bioassayed directly. For autoradiography, gels were fixed for 1 hr in 10% (vol/vol) trichloroacetic acid, equilibrated for 2.5 hr in 10% (vol/vol) methanol, 8% (vol/vol) acetic acid, and dried on a slab gel dryer (Hoefer, San Francisco). Autoradiography was carried out by exposing the dried gel to x-ray film (Kodak SB-5) using an intensifying screen (Picker, Highland Heights, OH). Molecular masses were assessed by comparison of the mobilities of H-1 activity and ^{125}I peaks with the mobilities of reduced low molecular mass marker proteins (Bio-Rad).

RESULTS

Standardization of the H-1 Bioassay. Dilutions of a stable H-1 reference preparation were included in every H-1 assay. The assay was standardized by comparison of the results obtained for unknown preparations with the dose–response curve of the reference preparation. A typical dose–response curve for the reference preparation is shown in Fig. 1. The maximum response was observed at ≈0.25 unit/ml and was not decreased at a 4-fold higher concentration of the preparation.

General Properties of H-1 in Partially Purified Preparations. Prior to purification, preliminary studies were carried out on partially purified (stage 2) preparations. H-1 activity was not reduced by heating at 55°C for 30 min but was completely lost by heating to 70°C for 30 min. Activity was stable to incubation (20°C, 1 hr) in aqueous solutions in the pH range 4–9 or in 40% (vol/vol) methanol or 15% (vol/vol) 1-propanol

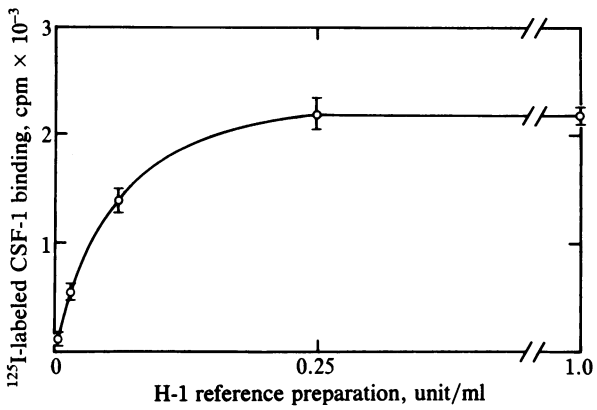

FIG. 1. Dose–response curve for the H-1 reference preparation in the H-1 bioassay. Results represent means of quadruplicates ± SD.

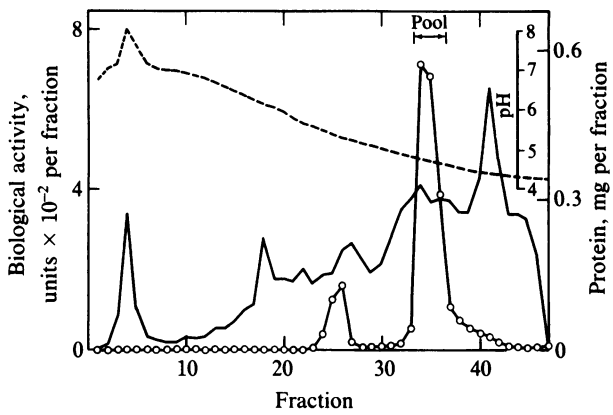

FIG. 2. H-1 purification: stage 2, chromatofocusing on PBE-94. o–o, Biological activity; —, protein; ---, pH.

at pH 7.4. However activity was lost following incubation at lower pH values or higher concentrations of these alcohols. H-1 bound to calcium phosphate gel in 1 mM Na phosphate, pH 6.5. However, it did not bind to phenyl-boronate (in 50 mM Hepes/10 mM $MnCl_2$, pH 8.5) or concanavalin A-Sepharose (in 1.0 M NaCl/100 mM Na acetate/10 mM $MgCl_2$/10 mM $MnCl_2$/10 mM $CaCl_2$, pH 6.0).

Purification of H-1. The procedure developed for the purification of H-1 is summarized in Table 1. The first stage in the procedure provided a relatively rapid means of concentrating the large starting volume with a good recovery of activity (73–99%) and purification (5.1- to 16.6-fold). The behavior of stage 1 material on chromatofocusing on PBE-94 is shown in Fig. 2. Two peaks of activity were observed to elute consistently at pH ≈5.3 and pH ≈4.8. The material in each of these peaks possessed the same apparent molecular weight as assessed by gel filtration on Sephadex G-75 (data not shown). The material in the minor peak (pH ≈5.3) had dose–response characteristics similar to those in the major peak but it was not studied further. The material in the major peak (pH ≈4.8) was filtered through a Sephadex G-50 column. In Fig. 3 the elution profile of proteins with H-1 activity on Sephadex G-50 is shown against the elution profile of a trace amount of ^{125}I-labeled stage 2 protein. The ^{125}I-labeled stage 2 material was added to the sample prior to chromatography as a means of following the very small amount of protein in the fractions (ref. 21, see *Materials and Methods*). A single peak of H-1 activity was observed to have a molecular mass of ≈18 kDa. The final stage of the purification procedure, chromatography of the material in the pooled, active, Sephadex G-50 fractions on phenyl-Sepharose, is shown in Fig. 4. The single peak of activity was not associated with a particular peak of ^{125}I. However, as iodinated proteins are more hydrophobic than their uniodinated counterparts, and because the stage 4 H-1 was shown to be pure by several criteria (see below), it is likely that the peak of ^{125}I eluted in fractions 28–32 contained the ^{125}I-labeled H-1. An alternative stage 4 procedure, reversed-phase HPLC utilizing a Brownlee RP-300 column and elution with 0–60% gradients of acetonitrile in 0.1% trifluoroacetic acid, yielded a similar degree of purification but with a significantly lower recovery of activity.

The results of a typical purification are summarized in Table 1. Similar results were obtained for two other purifications. With the exception of the final step, the recoveries of activity at each stage were generally high. Although the degree of purification of stage 4 material varied somewhat from purification to purification (30,000- to 72,000-fold) its specific activity was relatively constant (≈10^6 units per mg protein). Stage 4 H-1 was stable on storage at −20°C for up to 8 months.

Analysis of Purity of Stage 4 H-1. Stained bands were completely absent following silver staining of 15% $NaDodSO_4$/PAGE lanes loaded with as much as 60 ng of the purified H-1. Thus, to examine the purity of the very small amounts of stage 4 H-1, the protein was first iodinated with carrier-free ^{125}I (≈8 × 10^5 cpm/ng of protein), some ^{125}I-labeled H-1 was added to unlabeled stage 4 material as a tracer, and the mixture was run on 17.5% $NaDodSO_4$/PAGE either unreduced or following reduction in sample buffer containing β-mercaptoethanol (0.1 M, 37°C, 30 min). The ^{125}I-labeled native preparation migrated as a broad band with a molecular mass of 23.2 kDa that coelectrophoresed with the biological activity (Fig. 5*a*). The ^{125}I-labeled reduced preparation migrated more rapidly and as a sharper band (22.2 kDa) that was also coincident with the band of activity (Fig. 5*b*). The molecular masses for H-1 were somewhat higher and the banding of native H-1 was broader in this experiment than in others, probably because of the presence of Triton X-100 in the samples (see below).

Molecular Mass of H-1. The molecular mass of H-1 was determined by 15% $NaDodSO_4$/PAGE of ^{125}I-labeled H-1 prepared in the absence of Triton X-100. Radioautographs of gels of reduced and unreduced ^{125}I-labeled stage 4 H-1 are

Table 1. Summary of purification of H-1 from serum-free human 5637 CM

Stage	Total protein, mg	Total activity, units	Specific activity, units/mg	Purification, fold	Yield, %
Serum-free 5637 CM	222.0	3346	15	1	100
1. Batch DEAE-cellulose	20.3	3076	152	10	92
2. Chromatofocusing (PBE-94)	0.472	2830	5,996	400	85
3. Sephadex G-50	0.0175	2855	163,143	10,876	85
4. Phenyl-Sepharose	0.0011*	1083	984,545	65,636	32

*Calculated from the recovery of ^{125}I radioactivity in the peak believed to be ^{125}I-labeled H-1 (fractions 28–32, Fig. 4) and the protein concentration at stage 3.

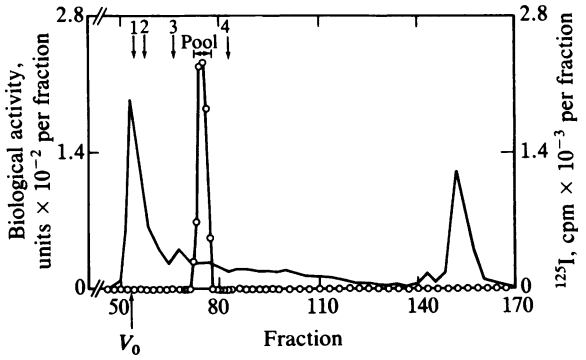

FIG. 3. H-1 purification: stage 3, gel filtration on Sephadex G-50. V_0, void volume. o—o, Biological activity; —, ^{125}I. Arrows indicate elution volumes of marker proteins: 1, bovine serum albumin (67 kDa); 2, ovalbumin (43 kDa); 3, chymotrypsinogen (25 kDa); 4, ribonuclease (13.7 kDa).

shown in Fig. 6. The molecular mass of H-1 is ≈17 kDa as determined from the migration of reduced H-1. Of interest is the slight but reproducible decrease in molecular mass of H-1 following reduction.

DISCUSSION

The H-1 activity of 5637 CM is composed of two charged species of similar size that are clearly resolved in the chromatofocusing step. Further studies are required to determine whether these two species have different biological properties. They either represent two distinct proteins or two forms of the same protein. The failure of the H-1 activity of 5637 CM to be adsorbed by either phenyl-boronate or concanavalin A-Sepharose suggests that H-1 is a protein rather than a glycoprotein. Thus it is unlikely that the charge heterogeneity results from variation in glycosylation of the molecule.

The purification scheme described is for the more highly charged H-1, which represents ≈85% of the starting activity of 5637 CM. The scheme is relatively efficient in that purification at each stage is substantial (≥ 6.5-fold) with recoveries of >85% at each of the first three stages. Recoveries at the final stage, though lower (≈40%), are reasonable considering the extremely small amounts of protein involved. Losses at this stage appear to be due to adsorption to surfaces. Although the yield of purified H-1 from 7 liters of serum-free 5637 CM is low (≈1 μg), the purification scheme

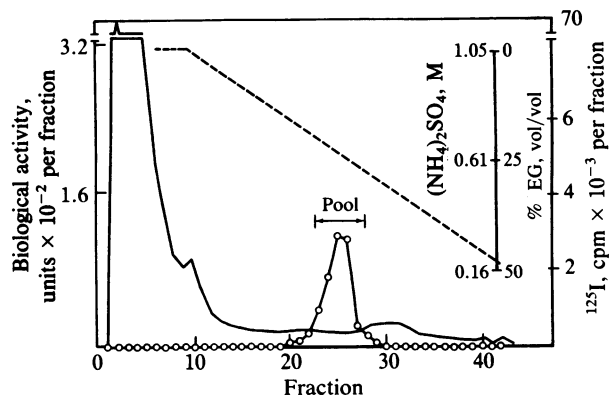

FIG. 4. H-1 purification of stage 4 by hydrophobic chromatography on phenyl-Sepharose. o—o, biological activity; —, ^{125}I; ----, $(NH_4)_2SO_2$/ethylene glycol (EG).

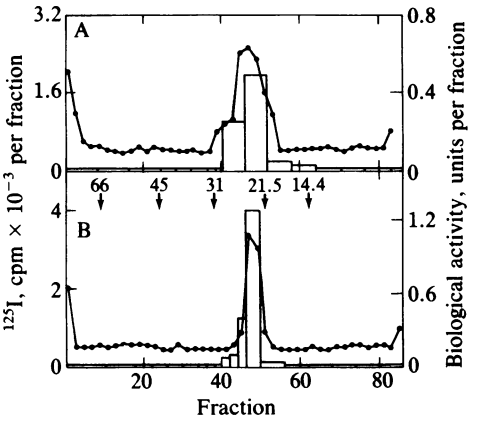

FIG. 5. Analysis of the purity of stage 4 H-1: 17.5% NaDodSO$_4$/PAGE of stage 4 H-1. *(A)* Native. *(B)* Reduced (0.1 M 2-mercaptoethanol, 37°C, 30 min). ●, ^{125}I; □, biological activity. Markers are indicated in kDa.

can be easily scaled-up to handle much larger volumes. In a scaled-up version it may be more convenient to replace the first stage with ultrafiltration followed by ion exchange column chromatography to avoid handling very large volumes of diluted 5637 CM.

The ^{125}I-labeled purified material behaved as a single homogeneous band on NaDodSO$_4$/PAGE before and after reduction with 2-mercaptoethanol. The biological activity of the purified material co-electrophoresed with this band despite the slight decrease in its molecular weight following reduction. These observations are consistent with the stage 4 H-1 being both pure and homogeneous.

The slight decrease in molecular mass of ^{125}I-labeled H-1 following reduction was highly reproducible. In contrast, a slight increase in the molecular mass is usually observed following reduction of single polypeptide chains that contain disulfide bonds. The results may reflect the existence of a small disulfide-bonded subunit of ≈1000 Da that was not iodinated under the conditions used. However, should such

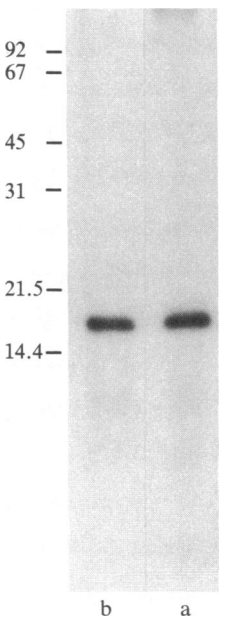

FIG. 6. Molecular mass of stage 4 H-1. Autoradiograph of 15% NaDodSO$_4$/PAGE of native (lane a) and reduced (lane b) (0.1 M β-mercaptoethanol, 100°C, 5 min) ^{125}I-labeled H-1.

a subunit exist, it is clearly not required for the biological activity of the molecule since the biological activity of the reduced ^{125}I-labeled H-1 was retained following NaDodSO$_4$/PAGE. The alternative explanation is that H-1 is comprised of a single polypeptide chain and that reduced H-1 exhibits anomalous behavior on NaDodSO$_4$/PAGE.

The biological activities of the purified H-1 are of particular interest and remain to be characterized in detail. Partially purified H-1 has been shown to be clearly distinct from erythroid burst-promoting activity and IL-3 (13). However, it is similar in properties to the factor with synergistic activity described by Kreigler *et al.* (6). As with H-1, this hematopoietic growth factor requires the presence of CSF-1-containing preparations for its action on developmentally early hemopoietic cells. Detailed studies of the effects of H-1 on the formation of "mixed colonies" (containing megakaryocytic, erythroid, granulocytic, and mononuclear phagocytic elements) by murine bone marrow cells cultured in semisolid culture medium have recently been completed. They indicate that H-1 is a multilineage hematopoietic growth factor that can synergize with another hematopoietic growth factor besides CSF-1. However, it has no detectable colony stimulating activity by itself (unpublished work). The relationship of H-1 to the factor(s) in 5637 CM with human bone marrow colony-stimulating activity (25, 26) remains to be elucidated.

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1. Stanley, E. R. & Jubinsky, P. T. (1984) *Clin. Haematol.* **13,** 329–348.
2. Iscove, N. N. (1978) in *Hematopoietic Cell Differentiation,* ICN–UCLA Symposia on Molecular and Cellular Biology, eds. Golde, D. W., Cline, M. J., Metcalf, D. & Fox, F. C. (Academic, New York), Vol. 10, pp. 37–52.
3. Iscove, N. N., Roitsch, C. A., Williams, N. & Guilbert, L. J. (1982) *J. Cell. Physiol.* **111,** Suppl. 1, 65–78.
4. Bradley, T. R. & Hodgson, G. S. (1979) *Blood* **54,** 1446–1450.
5. Bradley, T. R., Hodgson, G. S. & Bertoncello, I. (1980) in *Experimental Hematology Today,* eds. Baum, S. G., Ledney, G. D. & Van Bekkum, D. W. (Springer, Berlin), pp. 284–297.
6. Kreigler, A. B., Bradley, T. R., Januszewics, E., Hodgson, G. S. & Elms, E. F. (1982) *Blood* **60,** 503–508.
7. McNeice, I. K., Bradley, T. R., Kriegler, A. B. & Hodgson, G. S. (1982) *Cell Biol. Int. Rep.* **6,** 243–251.
8. Stanley, E. R. & Guilbert, L. J. (1981) *J. Immunol. Methods* **42,** 253–284.
9. Guilbert, L. J. & Stanley, E. R. (1980) *J. Cell Biol.* **85,** 253–259.
10. Byrne, P. V., Guilbert, L. J. & Stanley, E. R. (1981) *J. Cell Biol.* **91,** 848–853.
11. Guilbert, L. J. & Stanley, E. R. (1984) *J. Immunol. Methods* **73,** 17–28.
12. Bartelmez, S. H., Sacca, R. & Stanley, E. R. (1985) *J. Cell. Physiol.* **122,** 362–369.
13. Bartelmez, S. H. & Stanley, E. R. (1985) *J. Cell. Physiol.* **122,** 370–378.
14. Ihle, J. N., Keller, J., Henderson, L., Klein, F. & Palaszynski, E. (1982) *J. Immunol.* **129,** 2431–2436.
15. Schrader, J. W., Lewis, S. J., Clark-Lewis, I. & Culvenor, J. A. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 323–327.
16. Dexter, T. M., Allen, T. D. & Lajtha, L. G. (1977) *J. Cell. Physiol.* **91,** 335–344.
17. Fogh, J. (1978) *Natl. Cancer Inst. Monogr.* **49,** 5–9.
18. Stanley, E. R., Hansen, G., Woodcock, J. & Metcalf, D. (1975) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34,** 2272–2278.
19. Burgess, A. W., Camakaris, J. & Metcalf, D. (1977) *J. Biol. Chem.* **252,** 1998–2003.
20. Bradford, M. (1976) *Anal. Biochem.* **72,** 248–254.
21. Stanley, E. R. & Heard, P. M. (1977) *J. Biol. Chem.* **252,** 4305–4312.
22. Greenwood, F. C., Hunter, W. M. & Glover, J. S. (1963) *Biochem. J.* **193,** 265–275.
23. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
24. Das, S. K. & Stanley, E. R. (1982) *J. Biol. Chem.* **257,** 13679–13684.
25. Svet-Moldavsky, G. J., Zinzar, S. N., Svet-Moldavskaya, I. A., Mann, P. E., Holland, J. R., Fogh, J., Arlin, Z. & Clarkson, B. D. (1980) *Exp. Hematol* **8** (7), 76, (abstr. 133).
26. Myers, C. D., Katz, F. E., Joshi, G. & Millar, J. L. (1984) *Blood* **64,** 152–155.