1α,25-Dihydroxycholecalciferol and a human myeloid leukaemia cell line (HL-60)

The presence of a cytosol receptor and induction of differentiation

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Human promyelocytic leukaemia cells (HL-60) can be induced to differentiate into mature granulocytes *in vitro* by 1α,25-dihydroxycholecalciferol [1α,25(OH)$_2$D$_3$], the active form of cholecalciferol. The differentiation-associated properties, such as phagocytosis and C3 rosette formation, were induced by as little as 0.12 nM-1α,25(OH)$_2$D$_3$, and, at 12 nM, about half of the cells exhibited differentiation on day 3 of incubation. Concomitantly the viable cell number was decreased to less than half of the control. Among various derivatives of cholecalciferol examined, 1α,25(OH)$_2$D$_3$ and 1α,24R-dihydroxycholecalciferol were the most potent in inducing differentiation, followed successively by 1α,24S-dihydroxycholecalciferol, 1α-hydroxycholecalciferol, 25-hydroxycholecalciferol and 24R,25-dihydroxycholecalciferol. A cytosol protein specifically bound to 1α,25(OH)$_2$D$_3$ was found in HL-60 cells. Its physical properties closely resembled those found in such target tissues as intestine and parathyroid glands. 1α,25(OH)$_2$D$_3$ bound to the cytosol receptor was transferred quantitatively to the chromatin fraction. The specificity of various derivatives of cholecalciferol in inducing differentiation was well correlated with that of their association with the cytosol receptor. These results are compatible with the hypothesis that the active form of cholecalciferol induces differentiation of human myeloid leukaemia cells by a mechanism similar to that proposed for the classical concept of steroid hormone action.

It is well established that the biologically active metabolite of cholecalciferol, 1α,25(OH)$_2$D$_3$, first binds to a specific cytosol receptor to form a hormone–receptor complex in target tissues such as intestine and bone (Brumbaugh & Haussler, 1974a,b; Kream *et al.*, 1977b; Feldman *et al.*, 1979;

Wecksler *et al.*, 1979). The complex then moves into the nucleus by a temperature-dependent mechanism and binds to the chromatin (Brumbaugh & Haussler, 1974b). The binding of the sterol to the chromatin induces an increase in chromatin template activity (Zerwekh *et al.*, 1976). These results suggest the existence of a mechanism of action of 1α,25-(OH)$_2$D$_3$ similar to that proposed for other steroid hormones (Steggles *et al.*, 1971). Similar cytosol receptors for 1α,25(OH)$_2$D$_3$ have also been found in various tissues, including parathyroid glands (Hughes & Haussler, 1978), kidney (Chandler *et al.*, 1979; Christakos & Norman, 1979), pancreas (Christakos & Norman, 1979), pituitary (Haussler *et al.*, 1980), skin (Feldman *et al.*, 1980; Simpson & DeLuca, 1980; Eil & Marx, 1981) and eggshell gland (Takahashi *et al.*, 1980).

Some tumour cells possess the 1α,25(OH)$_2$D$_3$ receptor protein (Eisman *et al.*, 1980; Freake *et al.*,

1980; Manolagas *et al.*, 1980; Partridge *et al.*, 1980; Colston *et al.*, 1981). A biological response to $1\alpha,25(OH)_2D_3$ in tumour cells, however, was not elucidated until quite recently. We demonstrated that M1 cells, originally obtained from an SL mouse with myeloid leukaemia by Ichikawa (1969), could be induced to differentiate into macrophages *in vitro* by $1\alpha,25(OH)_2D_3$ (Abe *et al.*, 1981). The metabolite was at least 100 times more potent on a molar basis than dexamethasone, the most potent known stimulator, in suppressing cell growth and inducing cell differentiation (Abe *et al.*, 1981). More recently, we reported that HL-60 cells were also induced to differentiate by physiological plasma concentrations of $1\alpha,25(OH)_2D_3$ (Miyaura *et al.*, 1981).

The purpose of the present study was to examine whether HL-60 cells possess a cytosol protein specifically bound to $1\alpha,25(OH)_2D_3$. HL-60 cells have been reported to be induced to differentiate into mature granulocytes or macrophages by various inducers such as DMSO (Collins *et al.*, 1978), actinomycin D (Lotem & Sachs, 1979), tumour-promoting phorbol esters (Huberman & Callaham, 1979) and retinoic acid (Breitman *et al.*, 1980; Honma *et al.*, 1980). The mechanisms of differentiation of HL-60 cells by these inducers, however, are not known. We report here that the active form of cholecalciferol induces differentiation of human myeloid leukaemia cells and that the specificity of various derivatives of cholecalciferol in inducing differentiation is well correlated with that of their association with the cytosol receptor.

Materials and methods

Derivatives of cholecalciferol

$25(OH)D_3$ was purchased from Philips–Duphar, Amsterdam, The Netherlands. $1\alpha,25(OH)_2D_3$, $24R,25(OH)_2D_3$, $1\alpha,24R(OH)_2D_3$, $1\alpha,24S(OH)_2D_3$ and $1\alpha\text{-}(OH)D_3$ were the gifts of Dr. I. Matsunaga, Chugai Pharmaceutical Co., Toyko, Japan. $1\alpha,25(OH)_2[23,24\text{-}^3H]D_3$ (sp. radioactivity 91 Ci/mmol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Cells and cell culture

The human promyelocytic leukaemia cells (HL-60) isolated from the peripheral blood leucocytes of a patient with acute promyelocytic leukaemia (Collins *et al.*, 1977) were provided by Dr. T. Sugimura (National Cancer Centre Research Institute, Tokyo, Japan). Cells were cultured at 37°C in RPMI 1640 medium (Moore *et al.*, 1967) (GIBCO, Grand Island, NY, U.S.A.) supplemented with heat-inactivated 10% foetal calf serum (Flow Laboratories, Rockville, MD, U.S.A) and 100 units

of penicillin/ml and $100\mu g$ of streptomycin/ml in a humidified atmosphere of CO_2/air (1:19). Under these conditions the doubling time of HL-60 cells was 24 h. The cells (1×10^6) were inoculated in 10 ml of the medium in a 100 mm Petri dish. Each derivative of cholecalciferol dissolved in ethanol was added, keeping the final ethanol concentration at <0.1%. Control cultures were given the same volume of ethanol. The concentration of $1\alpha,25(OH)_2D_3$ in foetal calf serum used was 0.12 nM. Therefore, the endogenous concentration of the sterol in the control culture, which contained 10% foetal calf serum, appeared negligible.

Measurement of phagocytic activity and C3 receptors

Phagocytic activity was measured by the method of Collins *et al.* (1978). Cells were suspended at a concentration of 1×10^6 cells/ml in RPMI 1640 medium supplemented with heat-inactivated 10% foetal calf serum and 10% human AB serum. *Candida albicans* were washed with saline and added to the cell suspension at a final concentration of 4×10^6 fungi/ml. The suspension was incubated at 37°C for 30 min and the percentage of cells that had phagocytosed at least one fungus was determined with a haemocytometer. Cells with C3 receptors were determined by the method of Lotem & Sachs (1975) by measuring rosette formation with sheep erythrocytes coated with rabbit anti-(sheep erythrocytes) antibody and mouse complement. The percentage of rosette-forming cells with five or more erythrocytes per cell was counted with a haemocytometer. At least 200 cells were counted.

Preparation of cytosol and chromatin fractions

HL-60 cells were washed three times with PBS-($-Ca^{2+}/Mg^{2+}$) and homogenized with a Teflon/glass homogenizer in a solution containing 10 mM-Tris/HCl, pH 7.4, 2 mM-EDTA and 0.5 mM-dithiothreitol (buffer A). The homogenates were centrifuged at 4°C at 800 g for 10 min. The resulting pellet was designated a crude nuclear fraction, while the supernatant was centrifuged again at 4°C at 225 000 g for 1 h in a Hitachi 65P-7 ultracentrifuge to yield a cytosol fraction.

The crude nuclear fraction was washed three times with buffer A containing 0.25 M-sucrose and used as nuclear fraction for the cytosol–nucleus reconstitution experiment. The chromatin fraction was prepared from the nuclear fraction by washing in a solution consisting of 0.8 mM-EDTA and 25 mM-NaCl, pH 8.0, then twice in 0.1% Triton X-100 and 10 mM-Tris/HCl, pH 7.5, and finally in 10 mM-Tris/HCl, pH 7.5. The material was harvested by sedimentation at 4°C at 30 000 g for 10 min at each step.

Subcellular localization of $1\alpha,25(OH)_2[^3H]D_3$ in HL-60 cells

HL-60 cells (3×10^6) were incubated at 37°C in serum-free RPMI-1640 medium with 1 nM-$1\alpha,25(OH)_2[^3H]D_3$ in the presence or absence of a 100-fold excess of $1\alpha,25(OH)_2D_3$. After incubation, the cells were washed three times with PBS($-Ca^{2+}$/Mg^{2+}), resuspended in buffer A and homogenized. The cytosol and the chromatin fractions were prepared as described above. The radioactivity in each fraction was counted in 10 ml of an aqueous counting scintillant (ACS-II; The Radiochemical Centre) with a Packard liquid-scintillation counter (model 3255). The radioactivity in the cytosol fraction was determined after treatment with dextran-coated charcoal at a final concentration of 0.5% charcoal and 0.05% dextran at 4°C for 20 min and centrifugation at 4°C at 800 g for 10 min.

Cytosol–nucleus reconstitution experiment

The cytosol fraction prepared from HL-60 cells was pre-incubated with 1 nM-$1\alpha,25(OH)_2[^3H]D_3$ for 2 h at 25°C, and then the nuclear fraction was added and incubated at 37°C. After incubation for various times up to 60 min, the mixture was immediately chilled in an ice/water bath and centrifuged at 4°C at 800 g for 10 min. The resulting supernatant was treated with dextran-coated charcoal. The chromatin fraction was prepared as described above. The radioactivity in the supernatant and the chromatin fraction was counted as described previously.

Binding assay

Of the cytosol fraction 1 ml (0.3 mg of protein) was incubated with 0.1 nM-$1\alpha,25(OH)_2[^3H]D_3$ and graded amounts of authentic derivatives of cholecalciferol in buffer A containing 0.3 M-KCl for 1 h at 25°C. Separation of the bound and free $1\alpha,25(OH)_2[^3H]D_3$ was accomplished by the addition of dextran-coated charcoal for 20 min at 4°C. The $1\alpha,25(OH)_2[^3H]D_3$ bound to protein was counted for radioactivity after centrifugation. The dissociation constant and the specific binding sites were determined by Scatchard-plot analysis.

Sucrose-density-gradient analysis

A linear gradient (4.8 ml) of 4–20% sucrose in buffer A containing 0.3 M-KCl was prepared with a Hitachi DGF-U density-gradient former. Cytosol (0.2 ml) was incubated with 10 nM-$1\alpha,25(OH)_2$-$[^3H]D_3$ in the presence or absence of a 1000-fold excess of unlabelled $1\alpha,25(OH)_2D_3$, treated with dextran-coated charcoal as described above and layered on to the top of the gradient. Centrifugation was carried out at 4°C for 18 h at 232 500 g (average force) in a Hitachi 65P-7 ultracentrifuge using a

PRS-65T rotor. Fractions (five drops/vial) were collected from the top of the gradient into counting vials and the radioactivity in each vial was determined. Chymotrypsinogen (2.5 S) and bovine serum albumin (4.4 S) were used for the estimation of sedimentation coefficients.

Results

Suppression of cell growth and induction of differentiation by $1\alpha,25(OH)_2D_3$

When HL-60 cells were cultured with 12 nM-$1\alpha,25(OH)_2D_3$, cell growth was markedly inhibited in a time-dependent manner (Fig. 1). The inhibition of cell growth was effected by as little as 1.2 nM-$1\alpha,25(OH)_2D_3$ (Miyaura et al., 1981) and, at 12 nM, the viable cell number was reduced to 40% of the control on day 3 (Fig. 1). In addition, 12 nM-$1\alpha,25(OH)_2D_3$ significantly induced phagocytic activity and C3 rosette formation. These changes are known to be typical markers of the differentiation of HL-60 cells (Lotem & Sachs, 1979). On day 3, the effect of the $1\alpha,25(OH)_2D_3$ attained a maximum and nearly 50% of the cells exhibited the differentiation-associated changes. Morphologically, the $1\alpha,25(OH)_2D_3$ induced about half of the cells to differentiate into myeloid cells, including myelocytes, metamyelocytes or mature granulocytes (Miyaura et al., 1981).
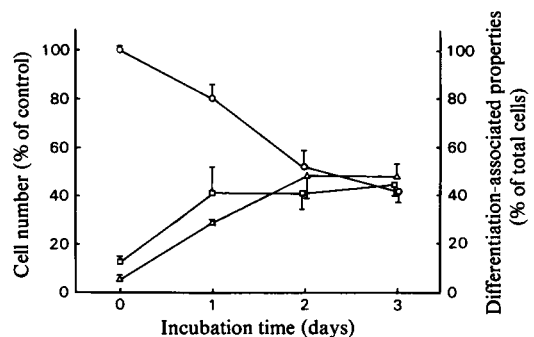

Fig. 1. *The effect of $1\alpha,25(OH)_2D_3$ on the suppression of cell growth, induction of phagocytic activity and appearance of C3 receptors*

HL-60 cells were incubated with 12 nM-$1\alpha,25(OH)_2D_3$ for 3 days. At indicated times, cells were harvested and the number of cells (O) was counted and expressed as a percentage of the non-treated control. In addition, the percentage of phagocytic cells (△) and that of cells with C3 rosette (□) within the treated culture were determined as described in the Materials and methods section. Points are means ± S.E.M. (represented by the bars) of six replicates.

Comparison of the effects of various derivatives of cholecalciferol on induction of phagocytic activity and C3 rosette formation

Fig. 2 shows the dose–response effects of various derivatives of cholecalciferol on the induction of phagocytic activity on day 3. Treatment with $0.12\,nM$-$1\alpha,25(OH)_2D_3$ or $-1\alpha,24R(OH)_2D_3$ induced phagocytic activity. $1\alpha,24S(OH)_2D_3$ also induced similar phagocytic activity with approx. 10-fold higher doses on a molar basis. Two to three orders higher concentrations of $1\alpha(OH)D_3$ and $25(OH)D_3$ than of $1\alpha,25(OH)_2D_3$ were required to induce similar differentiation.

The dose–response effects of various derivatives of cholecalciferol on the formation of C3 receptors are shown in Fig. 3. The order of the potency in inducing C3 receptors was almost identical with that in inducing phagocytic activity. These results indicate that the most potent derivatives of cholecalciferol in inducing differentiation of HL-60 cells are $1\alpha,25(OH)_2D_3$ and $1\alpha,24R(OH)_2D_3$, followed by $1\alpha,24S(OH)_2D_3$, $1\alpha(OH)D_3$, $25(OH)D_3$ and $24R,25(OH)_2D_3$ in that order.

Subcellular localization of $1\alpha,25(OH)_2[^3H]D_3$

When HL-60 cells were incubated with $1\,nM$-$1\alpha,25(OH)_2[^3H]D_3$ in serum-free culture medium at $37\,°C$, the labelled compound was incorporated very rapidly into HL-60 cells and attained a maximum at $30\,min$ (Fig. 4). Similar results were obtained even in the presence of a 100-fold excess of unlabelled

$1\alpha,25(OH)_2D_3$. Subcellular fractionation of HL-60 cells indicated that the amount of protein-bound $1\alpha,25(OH)_2[^3H]D_3$ found in the cytosol fraction was low, whereas the chromatin fraction incorporated as much as 25% of the radioactivity recovered in the whole cells. The radioactivity found in the chromatin fraction was markedly decreased by adding a 100-fold excess of $1\alpha,25(OH)_2D_3$ (Fig. 4). The remaining radioactivity was mostly detected in a free form in the cytosol fraction. Thus $1\alpha,25(OH)_2D_3$ present in HL-60 cells appears to be localized preferentially in the nuclear chromatin fraction, as has been established previously for the binding of the sterol in intestine and parathyroid glands (Brumbaugh & Haussler, 1974a,b; Hughes & Haussler, 1978).

Time-dependent transfer of $1\alpha,25(OH)_2[^3H]D_3$ from cytosol to nucleus

When the cytosol fraction was pre-incubated with $1\,nM$-$1\alpha,25(OH)_2[^3H]D_3$ at $25\,°C$ for $2\,h$ and then the nuclear fraction was added at time zero, the $1\alpha,25(OH)_2[^3H]D_3$ bound to the cytosol receptor protein was decreased in a time-dependent manner (Fig. 5). Concomitantly, the radioactivity found in the chromatin fraction was markedly increased. When specific association of $1\alpha,25(OH)_2[^3H]D_3$ to the chromatin fraction by way of the cytosol protein was calculated by subtracting the direct binding to the chromatin fraction (incubations without cytosol), the decrease in the radioactivity bound to the cytosol protein was almost equivalent quantitatively
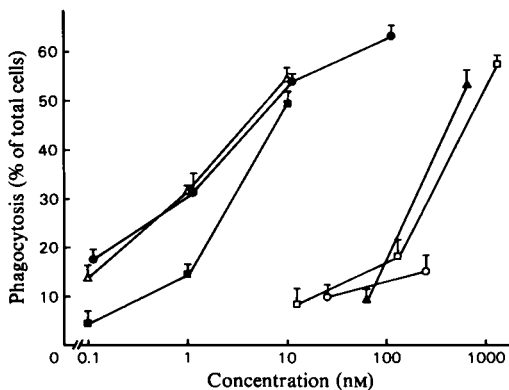

Fig. 2. *Comparative effects of various derivatives of cholecalciferol on the induction of phagocytic activity of HL-60 cells*

The cells were incubated for 3 days with each derivative of cholecalciferol: ●, $1\alpha,25(OH)_2D_3$; △, $1\alpha,24R(OH)_2D_3$; ■, $1\alpha,24S(OH)_2D_3$; □, $1\alpha(OH)D_3$; ▲, $25(OH)D_3$; ○, $24R,25(OH)_2D_3$. The control value was $5.5 \pm 0.4\%$. Points are means $\pm$ s.e.m. (represented by the bars) of six replicates.
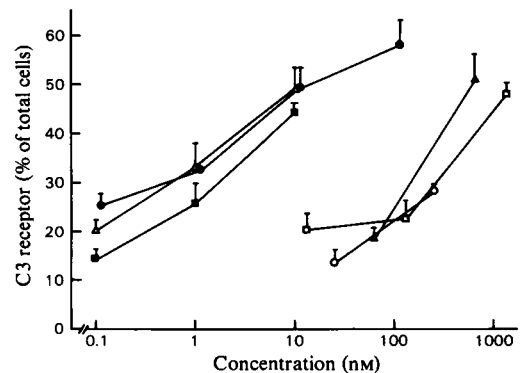

Fig. 3. *Comparison of the effects of various derivatives of cholecalciferol on the formation of C3 receptors of HL-60 cells*

The cells were incubated for 3 days with each derivative of cholecalciferol: ●, $1\alpha,25(OH)_2D_3$; △, $1\alpha,24R(OH)_2D_3$; ■, $1\alpha,24S(OH)_2D_3$; □, $1\alpha(OH)D_3$; ▲, $25(OH)D_3$; ○, $24R,25(OH)_2D_3$. The control value was $12.7 \pm 2.0\%$. Points are means $\pm$ s.e.m. (represented by the bars) of six replicates.
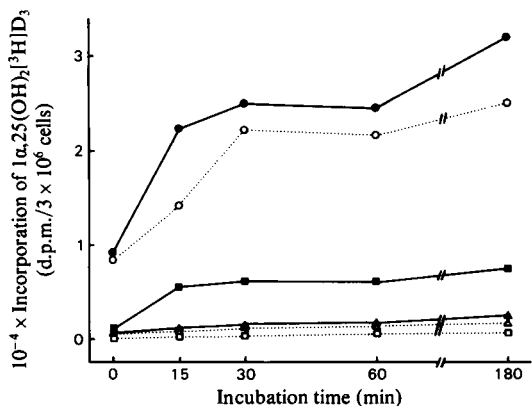
Fig. 4. *Incorporation of 1α,25(OH)₂[³H]D₃ into HL-60 cells*

HL-60 cells were incubated with 1 nM-1α,25(OH)₂[³H]D₃ in the presence or absence of a 100-fold excess of 1α,25(OH)₂D₃ at 37°C. After incubation, the cells were homogenized and fractionated. The cytosol fraction was treated with dextran-coated charcoal to separate protein-bound 1α,25(OH)₂[³H]D₃ from the free radioisotope. Results show radioactivity in whole cells (●, ○), in the chromatin fraction (■, □) and in the cytosol fraction (▲, △). The filled symbols indicate incubation with 1α,25(OH)₂[³H]D₃ and the unfilled symbols incubation with 1α,25(OH)₂[³H]D₃ plus unlabelled 1α,25(OH)₂D₃.
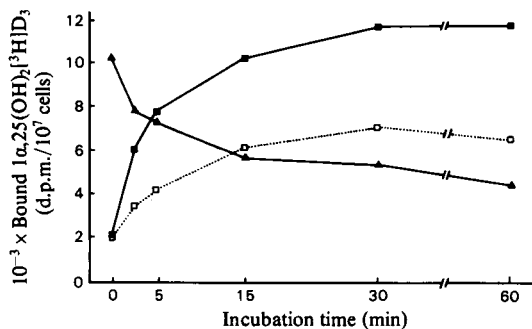
Fig. 5. *Time-dependent transfer of 1α,25(OH)₂[³H]D₃ from cytosol to nuclei*

The cytosol fraction prepared from HL-60 cells was pre-incubated with 1 nM-1α,25(OH)₂[³H]D₃ for 2 h at 25°C, and then the nuclear fraction was added. The transfer of the isotope occurred at 37°C, and the cytosol and chromatin fractions were separated. Results show radioactivity in the cytosol fraction (▲) and in the chromatin fraction (■). The radioactivity in the cytosol fraction was counted after treatment with dextran-coated charcoal. The direct binding of 1α,25(OH)₂[³H]D₃ to the chromatin fraction (□) was measured in incubations without cytosol fraction.
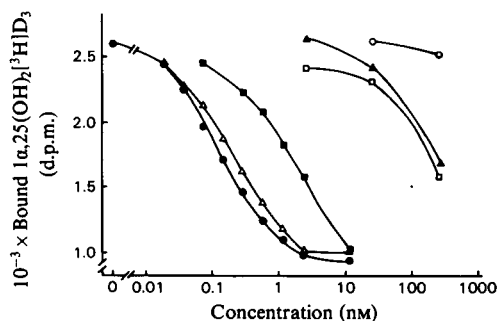

Fig. 6. *Competition of various derivatives of cholecalciferol for specific 1α,25(OH)₂[³H]D₃-binding sites in the cytosol fraction*

Cytosol (0.3 mg of protein) was incubated with 0.1 nM-1α,25(OH)₂[³H]D₃ plus graded concentrations of each derivative of cholecalciferol. Bound and free hormone were separated by using dextran-coated charcoal. Data are means of duplicate assays. Symbols: ●, 1α,25(OH)₂D₃; △, 1α,24R(OH)₂D₃; ■, 1α,24S(OH)₂D₃; □, 1α(OH)D₃; ▲, 25(OH)D₃; ○, 24R,25(OH)₂D₃.

to the increase in the radioactivity specifically associated with the chromatin fraction.

Cytosol binding of 1α,25(OH)₂[³H]D₃

To further examine the specificity of the association with the cytosol protein, the cytosol fraction was incubated with various derivatives of cholecalciferol for 60 min at 25°C and the protein-bound 1α,25(OH)₂[³H]D₃ was determined (Fig. 6). The displacement curve of 1α,25(OH)₂D₃ was almost identical with that of 1α,24R(OH)₂D₃. The equilibrium dissociation constant (K_d) was 23 pM, and the value for specific 1α,25(OH)₂D₃-binding sites was 2000 per cell (71 fmol/mg of protein). The concentrations required to displace 50% of the bound 1α,25(OH)₂[³H]D₃ were 1 nM-1α,24S(OH)₂D₃ and 100 nM-1α(OH)D₃ and -25(OH)D₃. 24R,25(OH)₂D₃ was a very poor binding competitor; a 10³ molar excess of 24R,25(OH)₂D₃ resulted in little competitive binding.

Sucrose-density-gradient analysis

Fig. 7 shows a sucrose-density-gradient analysis of the cytosol fraction incubated with 1α,25(OH)₂[³H]D₃ in the presence or absence of a 1000-fold excess of 1α,25(OH)₂D₃ for 1 h at 25°C. A protein-bound radioactive peak was found in the 4S region of the gradient with a shoulder at 3.2S. The peak disappeared in the presence of a 1000-fold excess of unlabelled 1α,25(OH)₂D₃; excess 1α,25(OH)₂D₃ had little effect on the displacement of the shoulder.
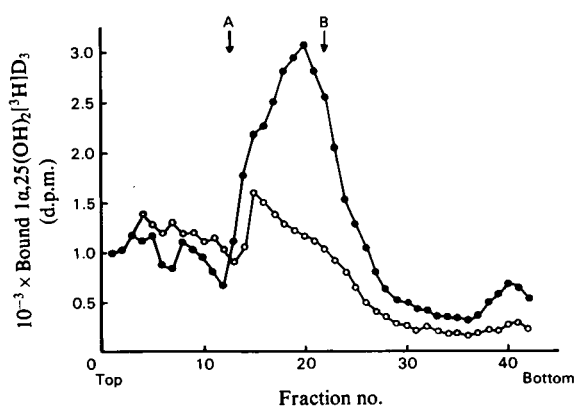
Fig. 7. *Sucrose-density-gradient analysis of $1\alpha,25(OH)_2[^3H]D_3$ binding by cytosol preparations* Cytosol protein (approx. 5 mg) was incubated with $10\,\text{nM-}1\alpha,25(OH)_2[^3H]D_3$ (●) or the same concentration of $1\alpha,25(OH)_2[^3H]D_3$ plus a 1000-fold excess of $1\alpha,25(OH)_2D_3$ (○) and analysed on 4–20% sucrose gradients. Arrow A indicates the elution position of chymotrypsinogen (2.5 S) and arrow B shows that of bovine serum albumin (4.4 S).

Discussion

Recently, Colston et al. (1981) demonstrated that the active form of cholecalciferol increases the doubling time of human malignant melanoma cells. The present study, together with our previous report (Miyaura et al., 1981), clearly indicate that $1\alpha,25(OH)_2D_3$, besides its activity in suppressing cell growth, is capable of inducing differentiation of human promyelocytic leukaemia cells (HL-60) into mature myeloid cells (myelocytes, metamyelocytes and mature granulocytes). Like murine myeloid leukaemia cells (M1 cells) (Abe et al., 1981), HL-60 cells were induced to differentiate by physiological plasma concentrations (0.12 nM) of $1\alpha,25(OH)_2D_3$. It should be noted that M1 cells were induced to differentiate into macrophages (Abe et al., 1981), whereas HL-60 cells were induced into granulocytes by $1\alpha,25(OH)_2D_3$. The differentiation was determined by phagocytosis and C3 rosette formation. Both activities attained maximal levels within 2 days of adding $12\,\text{nM-}1\alpha,25(OH)_2D_3$. Concomitantly, the viable cell number was decreased to 40% of the control on day 3 (Fig. 1). The potency of $12\,\text{nM-}1\alpha,25(OH)_2D_3$ in suppressing cell growth and inducing differentiation was almost equivalent to that of either 1% DMSO, 4 nM-actinomycin D or 1 nM-TPA (Miyaura et al., 1981). These compounds are capable of inducing differentiation of HL-60 cells, but have little effect in inducing differentiation of M1 cells (Sachs, 1978; Lotem & Sachs, 1979). On the contrary, dexamethasone,

which is the most potent stimulator in M1 cells, failed to induce differentiation of HL-60 cells, as previously reported (Lotem & Sachs, 1979). It is therefore noteworthy that $1\alpha,25(OH)_2D_3$ at physiological plasma concentrations is capable of inducing differentiation of both murine and human myeloid leukaemia cells.

The mechanisms of differentiation in human myeloid leukaemia cells by various inducers such as DMSO and TPA are still unknown. Induction of differentiation by phorbol esters occurs in parallel with their tumour-promoting activities in mouse skin (Huberman & Callaham, 1979).

Induction of differentiation of HL-60 cells by $1\alpha,25(OH)_2D_3$ appears to occur by a mechanism similar to that proposed for steroid hormone action. First, HL-60 cells possess a specific cytosol receptor for $1\alpha,25(OH)_2D_3$. Binding affinity is most specific for $1\alpha,25(OH)_2D_3$ and $1\alpha,24R(OH)_2D_3$, followed successively by $1\alpha,24S(OH)_2D_3$, $1\alpha(OH)D_3$, $25(OH)D_3$ and $24R,25(OH)_2D_3$. The specificity of their binding to the cytosol receptor is well correlated with that in such target tissues as intestine and parathyroid glands (Brumbaugh & Haussler, 1974b; Kream et al., 1977a; Hughes & Haussler, 1978; Siebert et al., 1979; Ishizuka et al., 1981). The physical properties of the cytosol receptor found in HL-60 cells also resemble those in the target tissues. Its dissociation constant (K_d) was identical with that reported in intestine and parathyroid glands (Hughes & Haussler, 1978; Feldman et al., 1979; Colston et al., 1980; Wecksler et al., 1980). The specific binding sites for $1\alpha,25(OH)_2D_3$ in HL-60 cells appear to be less than those in intestine (Feldman et al., 1979; Colston et al., 1980). It should be noted that some tumour cell lines derived from breast cancer (Eisman et al., 1980; Freake et al., 1981), osteogenic sarcoma (Manolagas et al., 1980; Partridge et al., 1980), VX_2 carcinoma (Freake et al., 1980) and malignant melanoma (Colston et al., 1981) also possess cytosol receptors similar to those found in intestine. It is interesting that the 4 S peak found in the cytosol fraction of HL-60 cells in the sucrose-density-gradient analysis was mostly displaced by adding a 1000-fold excess of unlabelled $1\alpha,25(OH)_2D_3$, whereas the 3.2 S shoulder was not subject to competition. The significance of this shoulder remains to be elucidated.

Secondly, the $1\alpha,25(OH)_2D_3$ bound to the cytosol receptor is transferred quantitatively to the chromatin fraction (Fig. 5). Considerable amounts of $1\alpha,25(OH)_2[^3H]D_3$ were incorporated into the chromatin fraction in the incubations without cytosol, suggesting the possibility that unoccupied $1\alpha,25(OH)_2D_3$ receptors may also be located in the nuclear fraction that was prepared with low ionic buffers, as reported by Walters et al. (1980, 1981).

Finally, the specificity of various derivatives of cholecalciferol in inducing differentiation (Figs. 2 and 3) is well correlated with that of their association with the cytosol receptor (Fig. 6). These results strongly suggest that the metabolically active form of cholecalciferol, $1\alpha,25(OH)_2D_3$, first binds to the cytosol receptor to form a $1\alpha,25(OH)_2D_3$–receptor complex and then moves to chromatin before suppressing cell growth and inducing differentiation of HL-60 cells. Whether $1\alpha,25(OH)_2D_3$ is involved in the differentiation of normal bone marrow cells remains to be elucidated.

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