

INSULIN BINDING TO THE HL-60 HUMAN PROMYELOCYTIC CELL LINE

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The HL-60 cell is a human leukemic promyelocyte that can be grown in liquid suspension culture. This cell line binds radiolabeled insulin in a rapid, reversible specific manner indicating that it contains cell surface insulin-binding sites. These sites show a pH optimum of 7.8–8.0.

Maximal binding was achieved in approx. 60 min at 22°C and was linear with cell number between 5×10^6 and 10^8 cells per ml. Displacement of labeled insulin by unlabeled hormone was consistent with a 'two site' model with a high affinity site of $2.71 \pm 0.55 \times 10^8 \text{ M}^{-1}$ and 8700 sites per cell. Release of labeled insulin from its binding site was accelerated by unlabeled insulin at temperatures above 22°C, but not at 4 or 15°C.

We conclude that the HL-60 cell contains cell surface binding sites for insulin with properties similar to other well-described insulin receptors of other tissues.

Keywords: HL-60 cells; insulin receptors; negative cooperativity.

Specific binding sites for insulin have been found in a wide variety of tissues (Goldfine, 1978). Circulating leukocytes, more specifically, monocytes (Schwartz et al., 1975) and polymorphonuclear neutrophils (PMN) (Fussganger et al., 1976) have been shown to bind [^{125}I]iodoinsulin in a highly specific, rapid, reversible, temperature- and pH-dependent manner.

The normal promyelocyte is an early myeloid precursor which matures to the polymorphonuclear (PMN) granulocyte. The HL-60 cell is a leukemic promyelocyte which may also be induced to mature to later myeloid forms; these induced cells share functional and morphologic characteristics with normal polymorphs. This cell line was derived from an adult female with acute promyelocytic leukemia (Collins et al., 1977) and can be grown in liquid suspension media supplemented with fetal calf serum or in defined media plus insulin (5 $\mu\text{g}/\text{ml}$) and transferrin (5 $\mu\text{g}/\text{ml}$) in the absence of serum (Breitman et al., 1980a). Dimethylsulfoxide, dimethylformamide and retinoic acid (Collins et al., 1978) induce myeloid maturation of HL-60 cells, resulting in a spectrum of more mature forms including about 30% PMN's (Collins et al., 1978; Breitman et al., 1980b). Tumor promoters such as phorbol esters cause cells to adhere to plastic and mature to a form sharing functional characteristics with normal monocytic phagocytes (Rovera et al., 1979).

MATERIALS AND METHODS

(1) *HL-60 cell line*

The HL-60 cell line was grown in RPMI 1640 medium plus 10% heat inactivated bovine fetal serum (Gibco Laboratories, Grand Island, NY) in 75-cm² Falcon culture flasks at a density of 2×10^5 per ml at 37°C in an atmosphere of 95% air, 5% CO₂ and 100% humidity. The cells were passed every 4–5 days when they reached a density of $0.8\text{--}1.0 \times 10^6$ per ml. They were washed twice in binding buffer before studies were performed.

(2) [¹²⁵I]Iodoinsulin

Insulin was iodinated to a specific activity of 200–300 μCi/μg by a modification of the Hunter and Greenwood technique (Hunter and Greenwood, 1962). Monomeric [¹²⁵I]iodoinsulin was initially separated from higher molecular weight labeled aggregates and from unreacted ¹²⁵I by chromatography on a 0.9 × 90 cm column of Sephadex G-50 (Pharmacia Fine Chemicals, Piscataway, NJ). Repurification of aliquots of the originally purified monomeric iodoinsulin was performed weekly using the same gel filtration technique.

(3) *Insulin-binding assay*

HL-60 cells in a 25 mM Tris buffer, pH 7.8, at a density of approx. 1×10^7 cells/0.5 ml were incubated in triplicate with 30–60 pM [¹²⁵I]iodoinsulin and $0\text{--}3.3 \times 10^{-6}$ M unlabeled insulin for 60–90 min at 22°C in the presence of 5×10^{-4} M phenylmethylsulfonyl fluoride (PMSF) to prevent insulin degradation (Fussganger et al., 1976). At the completion of binding studies two 200-μl aliquots of the cell suspension were layered on top of 200 μl ice cold binding buffer in 500 μl capacity microfuge tubes. The cell pellet was collected by centrifugation for 1 min in a Beckman Model B microcentrifuge. The supernatant solution was removed by vacuum aspiration and the cell pellet excised for gamma radiation counting.

For dissociation studies the cells were incubated for 90 min at 22°C followed by 100-fold dilution in binding buffer or binding buffer containing 3.33×10^{-6} M unlabeled insulin at 4, 15, 22, 30 and 37°C for 5–180 min (DeMeyts et al., 1976).

The binding isotherms and displacement curves were analyzed by a least-squares analysis using a modification of the Gauss–Newton technique (Johnson et al., 1976). The data were also analyzed by the method of Scatchard (1949).

(4) *Specificity of insulin binding*

The specificity of insulin binding to the HL-60 cell was examined using various insulin preparations, insulin analogues, and other peptide hormones. The following specific reagents were employed: human growth hormone (HS 2243 E), human luteinizing hormone (LER 960), human follicle stimulating hormone (HS-1). They were obtained from the Hormone Distribution Office of NIAMDD. Human prolactin was a gift from Dr. H. Friesen, multiplication stimulating factor a gift from

Dr. S.P. Nissley and angiotensin II a gift from Dr. M. Peach. Oxytocin (Grade II) and vasopressin (Grade VI arginine vasopressin) were purchased from Sigma Chemical Company. Each was employed in a binding assay and the displacement of [125 I]iodoinsulin from the HL-60 cell was recorded.

RESULTS

(1) pH profile

The pH optimum is between pH 7.8 and 8.0. At these hydrogen ion concentrations approx. 11% of 60 pg [125 I]iodoinsulin binds specifically to 3.5×10^6 cells after incubation for 90 min at 22°C.

(2) Binding isotherm

The time course of binding of [125 I]iodoinsulin to HL-60 cells at 22°C is shown in Fig. 1. The middle curve, the specifically bound [125 I]iodoinsulin, is derived from the difference between total binding (upper curve) and non-specific binding (residual binding in the presence of 3.3×10^{-6} M unlabeled insulin; lower curve).

(3) Binding of [125 I]iodoinsulin versus cell number

The total, specific and non-specific binding of [125 I]iodoinsulin to increasing concentrations of HL-60 cells at 22°C after 90 min is shown in Fig. 2. Between 10^6 and 10^7 cells the binding is linear with more than 90% of the bound insulin being specifically bound.

(4) Displacement of [125 I]iodoinsulin by unlabeled insulin

The displacement of specifically bound [125 I]iodoinsulin by increasing amounts

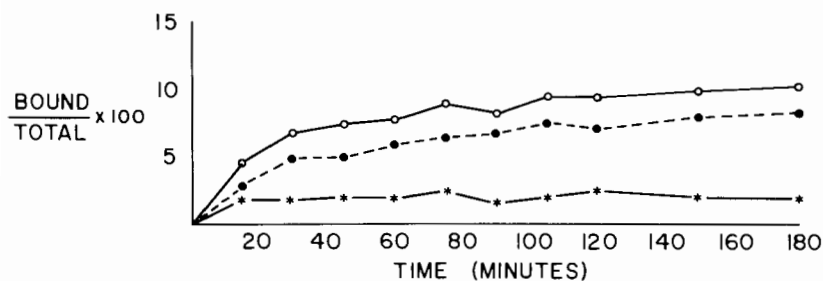


Fig. 1. Bound [125 I]iodoinsulin by HL-60 cells as a function of time of incubation at 22°C. ○, total binding; ●, specific binding; *, non-specific binding-residual binding in the presence of 10 000 ng/ml (1.67×10^{-6} M) unlabeled insulin. The specific binding is the difference between total binding and non-specific binding. 5.9×10^6 cells per incubation tube.

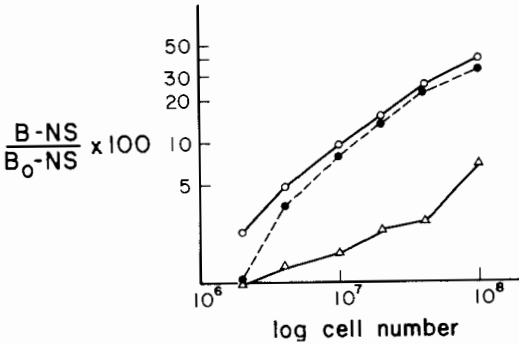


Fig. 2. Bound [¹²⁵I]iodoinsulin by HL-60 cells as a function of cell number at 22°C. The symbols are the same as in Fig. 1. Note the logarithmic scale for both coordinates.

of unlabeled insulin is shown in Fig. 3. The curve is drawn using the equation

$$\frac{n_1 k_1 x}{1 + k_1 x} + \frac{n_2 k_2 x}{1 + k_2 x}$$

where the parameters n_i and k_i are shown in Table 1. This 2-site model fits the experimental data more closely than the 1-site model $(nkx)/(1 + kx)$. The Scatchard plot bound/free versus bound is curvilinear and is shown as the inset to Fig. 3.

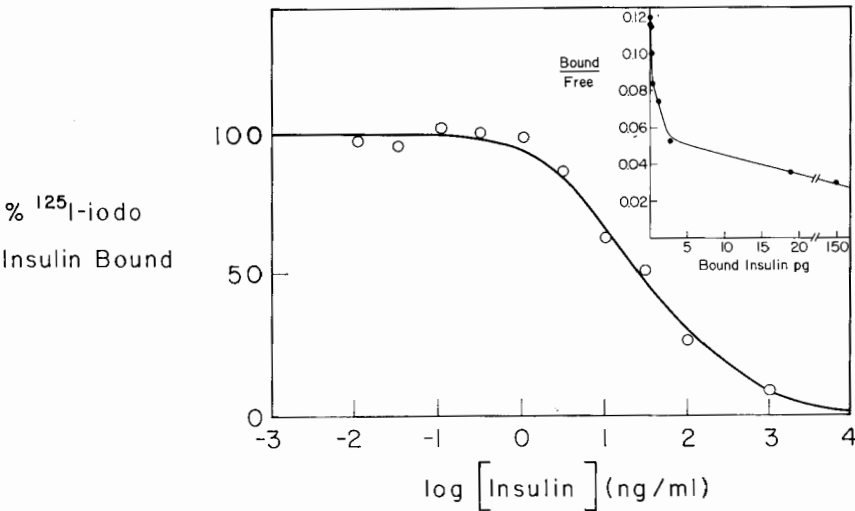


Fig. 3. Displacement of [¹²⁵I]iodoinsulin by unlabeled insulin. The curve is that calculated as described in the text and represents only the specifically bound fraction. 7.6×10^6 cells per incubation tube. The inset shows the analysis by the method of Scatchard.

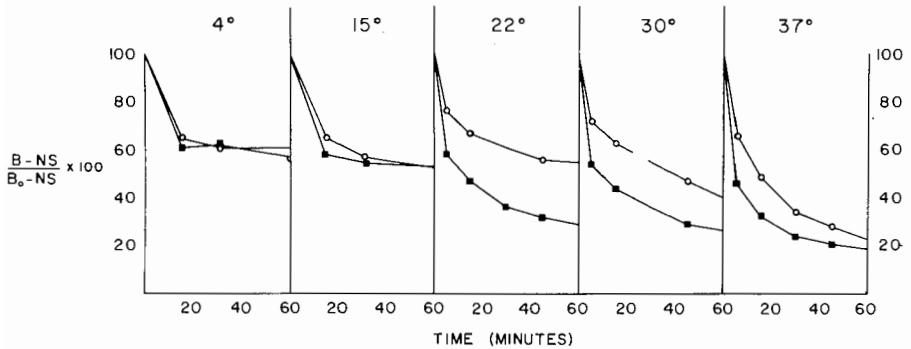


Fig. 4. Displacement of specifically bound [^{125}I]iodoinsulin by 100-fold dilution (\circ) or by 100-fold dilution in the presence of 10 000 ng/ml (1.67×10^{-6} M) unlabeled insulin \blacksquare . Cells were incubated at 22°C for 80–90 min before dilution at the various temperatures.

(5) Release of bound [^{125}I]iodoinsulin by dilution or dilution in the presence of unlabeled insulin

Cells were incubated for 90 min at 22°C followed by 100-fold dilution in binding buffer or binding buffer containing 3.3×10^{-6} M unlabeled insulin at 4, 15, 22, 30 and 37°C for 5 to 180 min. The data for the first 60 min are shown in Fig. 4. At 4 and 15°C , the 2 curves are indistinguishable, but at the higher temperatures unlabeled insulin accelerates the release of bound [^{125}I]iodoinsulin (Fig. 4).

(6) Specificity of binding of [^{125}I]iodoinsulin to HL-60 cells

The insulin preparations and analogues were employed in a binding assay to assess their relative potency compared to porcine insulin to displace labeled insulin from its binding site on the HL-60 cell. The results are calculated from the amount of insulin or analogue required to displace 50% of the labeled insulin (Table 2) as

$$\frac{\text{molar concentration of porcine insulin to displace 50\% of } [^{125}\text{I}]\text{iodoinsulin}}{\text{molar concentration of analogue to displace 50\% of } [^{125}\text{I}]\text{iodoinsulin}}$$

Less than 10% of labeled insulin was displaced by the following hormone (maximum amount in parentheses): hGH (4.5×10^{-8} M); hPrI (4.5×10^{-8} M); angiotensin II (1×10^{-5} M); oxytocin (4×10^{-7} M); vasopressin (2.2×10^{-7} M); LH and FSH (250 IU/l).

DISCUSSION

These data indicate that there are insulin-binding sites on the surface membrane of the HL-60 cell. We have noted increasing insulin degradation with time of incuba-

Table 1

Binding parameters for [125 I]iodoinsulin to HL-60 cells ^a

Parameter ^b
n_1 8.73 ± 2.6 (SD) 10^3 sites/cell
k_1 $2.71 \pm 0.55 \times 10^8$ M ⁻¹
n_2 $1.12 \pm 0.32 \times 10^5$ sites/cell
k_2 $7.85 \pm 3.6 \times 10^6$ M ⁻¹

^a Cells incubated at a density of 6×10^7 per ml for 90 min at 22°C; [125 I]iodoinsulin, 40 pM.^b Derived from the equation

$$\text{Bound} = \frac{n_1 k_1 x}{1 + k_1 x} + \frac{n_2 k_2 x}{1 + k_2 x}$$

where n_i , site number; k_i , affinity; x , free insulin concentration.

tion as Fussganger and co-workers have found for circulating granulocytes (Fussganger et al., 1976). They had noted that 5×10^{-4} M phenylmethylsulfonyl fluoride (a serine protease inhibitor) would inhibit the breakdown by more than 90%. Destruction of insulin was not as great in the HL-60 cell as compared to the more mature granulocyte and the same concentration of PMSF reduced insulin breakdown to virtually nil during the incubation period. Maximal binding occurred between pH 7.8 and 8.0 although no sharp peak was noted. This range has been noted for insulin receptors in a number of tissues (Gavin III et al., 1973).

Maximal binding was achieved in approx. 60 min at 22°C. The nonspecific binding represented less than 20% of the total and was relatively constant with time of incubation. The binding was linear with cell number between 5×10^6 and 10^8 cells per ml and we usually chose $0.8-1.0 \times 10^7$ cells for convenience. There was no significant displacement of labeled insulin by other small peptides (angiotensin II, oxytocin and vasopressin) or by larger peptide hormones (growth hormone, prolactin, luteinizing hormone or follicle-stimulating hormone). The various insulins

Table 2

Potency of insulin and insulin analogues relative to porcine insulin ^a

Insulin analogue	Receptor binding: HL-60 cells (%)
Porcine insulin	100
Chicken insulin	100
Bovine desalanine, desasparagine insulin	4.1
Guinea pig insulin	3.5
Multiplication stimulating activity	0.3
Bovine desactapeptide insulin	0.1

^a Calculated as

$$\frac{\text{molar concentration of porcine insulin to displace 50\% of } [^{125}\text{I}]\text{iodoinsulin}}{\text{molar concentration of analogue to displace 50\% of } [^{125}\text{I}]\text{iodoinsulin}}$$

and insulin analogues show a rank order of potency in the range noted for circulating lymphocytes (Kahn, 1975). These data suggest that the insulin binding is to a specific 'insulin' receptor rather than to a 'growth factor' receptor (King et al., 1980).

The displacement of labeled insulin by unlabeled hormone was analyzed by several methods. Using the classical method of Scatchard, a curvilinear relationship was noted (Scatchard, 1949). We then analyzed the binding and displacement by an equation of the type

$$B = \frac{n_1 k_1 x}{1 + k_1 x} + \frac{n_2 k_2 x}{1 + k_2 x}$$

(see methods).

The data fit best to the 2-site model yielding 2 'binding' sites with affinities and number per cell as noted in the table. The constant for the 'high' affinity site falls within the range noted for insulin receptors defined for other tissues (10^8 – 10^{10} M^{-1}) as reviewed by Kahn (1975). There are $8.73 \pm 2.6 \times 10^3$ sites per cell compared to approx. 1×10^3 sites per cell for the smaller mature granulocyte (Fussganger et al., 1976). Since the HL-60 promyelocyte can be made to 'mature' to more normal granulocytes, we can follow the binding of insulin throughout the transition.

Above 22°C, the release of labeled insulin from its binding site by dilution was accelerated by the addition of a large excess of unlabeled insulin. Although some investigators have considered the enhanced dissociation of a ligand from its receptor to indicate 'negative cooperativity' (DeMeyts et al., 1976; DeMeyts, 1976) others have analyzed similar data without implicating negative cooperativity (Saltiel et al., 1980). Suffice it to say that the binding and the dissociation data may fit several models (e.g. several classes of binding sites of differing affinities and receptor number or a single class of varying affinity dependent upon occupancy).

Below 22°C, a single curve of release of insulin from its binding site was noted whether performed in the presence or absence of unlabeled insulin. The data speak to the point of a temperature-dependent hormone–receptor interaction, but do not specify a unique model.

In summary, we have shown rapid, reversible, specific binding of insulin to the HL-60 cell.

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