

# Current Views on Insulin Receptors

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## EXAMINATION OF THE INSULIN RECEPTOR IN THE MEMBRANE BY RADIATION INACTIVATION STUDIES

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### INTRODUCTION TO RADIATION INACTIVATION

The technique of radiation inactivation has been used to determine the size of several hormone receptors *in situ* in the membrane (Houslay *et al.*, 1977; Schlegel *et al.*, 1979; Harmon *et al.*, 1980). This procedure has several advantages over other procedures. Firstly, it can be performed on the receptor *in situ*, i.e. in the membrane. Secondly, the results are independent of the purity of the preparation being examined. Finally, the procedure relies on the determination of the size of the "functional unit" which is the smallest component necessary to achieve the biological activity being measured.

When the source of radiation is high energy electrons, the ionizations resulting from the interaction of ionizing radiation with the sample are random along the path of the electron. Hence, according to classical target theory, the loss of biological activity with increasing radiation exposure can be expressed as an exponential function of radiation dose:

$$A_{\text{dose}} = A_0 e^{-V \cdot \text{dose}} \quad (1)$$

Where  $A_{\text{dose}}$  is the biological activity measured after the sample has received a given radiation dose;  $A_0$  is the biological activity of the unirradiated sample (zero

dose); and  $V$  is the "radiation sensitive volume" which is related to the size of the functional unit. Thus, the inactivation curve, for a system in which only one compound is responsible for the biological activity, will be a single exponential line (Fig. 1, left). Such is the case for the binding of the insulin-like growth factor, MSA (multiplication stimulating activity) to its receptor in rat liver membranes (Fig. 2). From the slope of this line the functional size of this receptor can be calculated to be 115,000.

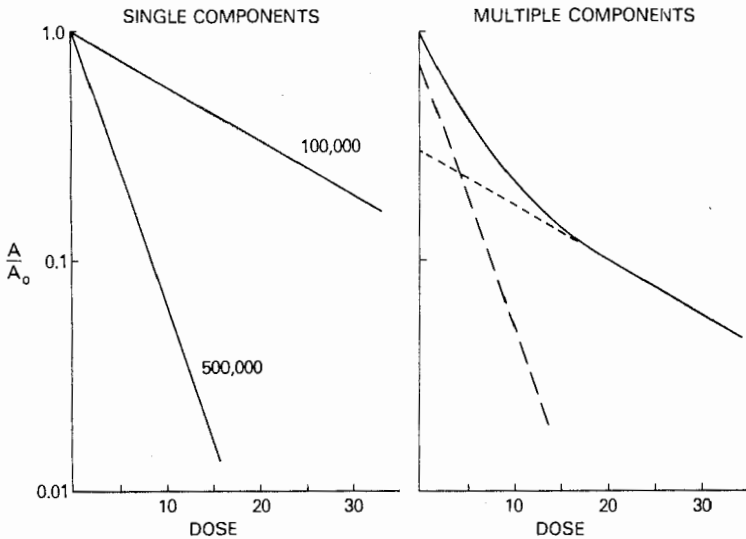


Fig. 1. Typical radiation inactivation profiles. The data are plotted as the log of the fraction of remaining biological activity ( $A$ , the activity measured after a given dose of radiation divided by  $A_0$ , the activity of the non-irradiated sample) vs dose of radiation. Radiation inactivation curves of systems, in which a single functional unit (left) or several functional units (right) are responsible for the total activity, are presented.

In a more complicated system, when the total activity is contributed to by multiple components of differing sizes, the resulting inactivation curve will be multi-exponential, composed of the sum of the individual inactivation lines (Fig. 1, right). The multi-exponential line can be resolved to obtain the functional size and relative activity contributed by each component. Epidermal growth factor (EGF) binding to its receptor on rat liver plasma membranes demonstrates a multi-exponential inactivation curve consistent with a two component system (Fig. 2). The initial rapid loss of EGF binding seen on membranes exposed to very low doses of radiation can be accounted for by a functional unit with a molecular weight of approximately 1,000,000; and the second phase of the inactivation curve would be consistent with another functional unit with a molecular weight of approximately 100,000. The larger component contributes 60-70% of the total EGF binding activity.

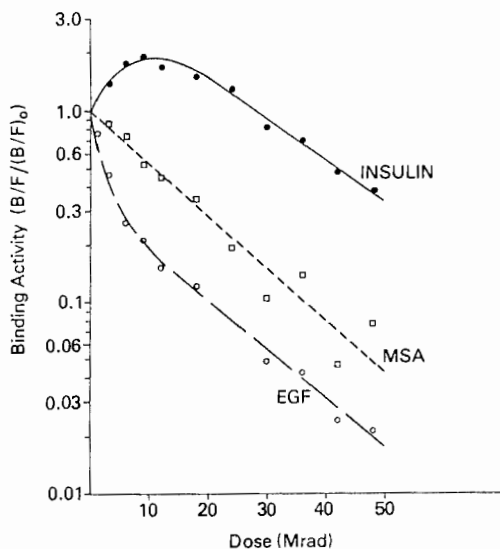


Fig. 2. Radiation inactivation profiles for the binding of several peptide hormones. Rat liver membranes which had received between 0 and 48 Mrad of ionizing radiation were used in hormone binding assays to measure specific binding of insulin, MSA or EGF. The fraction of specific binding remaining at any dose of radiation (bound/free, B/F, at a given dose divided by  $(B/F)_0$  of non-irradiated samples) was plotted vs dose of radiation received by the samples. The irradiations were performed at less than  $-100^\circ\text{C}$  as previously described (Harmon *et al.*, 1980).

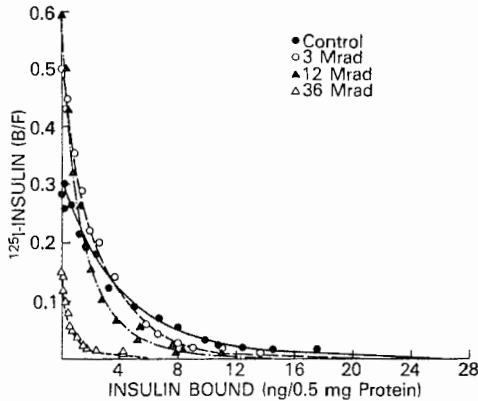
### CHARACTERIZATION OF THE INSULIN RECEPTOR BY RADIATION INACTIVATION

When insulin binding to its receptor was measured on irradiated membranes, the anticipated loss on insulin binding activity was not observed (Fig. 2). Rather there was an increase in the amount of specifically bound tracer insulin which reached a maximum at 9–12 Mrad after which the binding activity decreased. Such an increase in activity with radiation has not been previously reported.

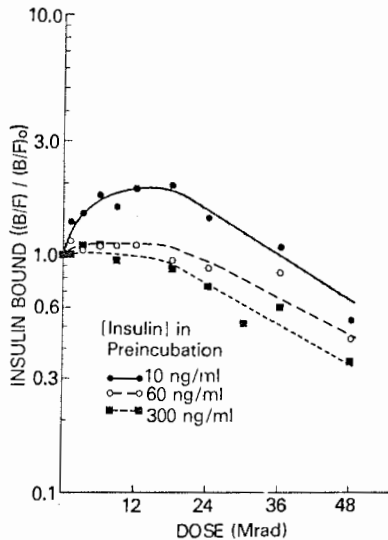
To further characterize the effect of ionizing radiation on insulin binding, we examined the effect of radiation on the apparent affinity and number of insulin binding components as determined by analysis of binding data by the method of Scatchard (Fig. 3). With increasing radiation exposure, the affinity of the insulin receptor,  $K_b$ , increased from  $0.7 \times 10^8$  to  $2.6 \times 10^8 \text{ M}^{-1}$  reaching its maximum at 12 Mrad. Receptor concentration,  $R_0$ , which was initially  $4 \times 10^{-9} \text{ M}$ , decreased at all exposure doses. Thus, the inactivation profile of the insulin receptor may also be viewed as consisting of two phenomena which are occurring concomitantly: an increase in affinity in proportion to the loss of the apparent large molecular weight component (250,000 – 300,000) and a decrease in receptor concentration in proportion to the loss of the smaller component (90,000).

## FACTORS WHICH AFFECT RECEPTOR STRUCTURE

If the membranes were preincubated in the presence of various concentrations of unlabelled insulin before irradiation; irradiated, washed to remove unbound hormone and then insulin binding activity was measured, there was a dramatic change in the inactivation profile (Fig. 4). The initial phase of the inactivation



*Fig. 3.* Analysis of competition experiments by the methods of Scatchard. Rat liver membranes were exposed to 0, 3, 12 and 36 Mrads and specific binding was measured as previously described (Harmon *et al.*, 1980). The protein concentration in all assay tubes was 0.5 mg/ml.



*Fig. 4.* Effect of insulin preincubation on the inactivation curve for  $^{125}\text{I}$ -insulin binding to rat liver membranes. Membranes were preincubated with or without insulin as previously described (Harmon *et al.*, submitted for publication). The log of the fraction of specific insulin binding has been plotted vs radiation dose. The stippled area shows a composite of 16 experiments in which membranes were preincubated without insulin and washed or used without preincubation.

curve (low doses of radiation) decreased and the decrease was proportional to the insulin concentration. This was not due to a change in the size of either of the "components" of the receptor, but some change in their interaction due to insulin binding. Membranes, preincubated in the absence of insulin and washed, demonstrated the same inactivation curve as membranes which were neither preincubated nor washed.

Other factors such as pH and ionic strength, which are known to affect the apparent affinity of the insulin receptor, also have a substantial effect on the inactivation profile. Thus, the lower the apparent affinity of the receptor, the larger the increase in binding observed after the membranes have been irradiated or the greater the interaction of binding and affinity regulatory components.

### MODELS OF THE INSULIN RECEPTOR

To ascertain if proposed models of the insulin receptor could predict our results from radiation inactivation of the insulin receptor and the results from equilibrium and kinetic experiments, we used least-square analysis to determine the best fit of various models. Several models were examined.

(1) *Inhibitor model.* The simplest model of this type is one in which the binding component binds only one hormone and the binding component can bind one inhibitor which regulates receptor affinity.

(2) *Pure site-site interaction model.* In this model there is a macromolecular receptor with at least two identical binding sites. With increasing occupancy of either site, the affinity of the empty site is altered.

(3) *Multiple independent sites model.* In this model insulin would bind to two or more different non-interacting binding components with each type of binding component having a different affinity.

(4) *Aggregation and conformation models.* The most general model of this type is one in which a protomer (smallest functional component) can reversibly self-associate to form any order of aggregation. Also the protomer and any of the aggregated forms can undergo conformational (tertiary molecular structural) changes. The resultant effect of aggregational and/or conformational changes is an alteration in the affinity of the insulin binding component.

In fitting the radiation data to any of these models, the most significant and distinguishing attribute is the increase in specific insulin binding which reaches a maximum after approximately 12 Mrad of irradiation. This phenomenon can be expressed quantitatively as the first derivative of bound/free (B/F) hormone with respect to dose of radiation being equal to zero at approximately 12 Mrad of radiation:

$$\frac{d B/F}{d \text{ dose}} = 0, \text{ dose} \approx 12 \text{ Mrad} \quad (2)$$

For a given radiation dose-response curve, the total concentration of hormone is constant, therefore, the bound hormone concentration can be expressed in terms of the free and total hormone concentration ( $B = T - F$ ). This implies that:

$$\frac{d F}{d \text{ dose}} = 0; \text{ dose} \approx 12 \text{ Mrad} \quad (3)$$

We will utilize this derivative to simplify the derivation of various molecular models for the insulin receptor. Models not satisfying this criteria can be rejected.

Having performed this derivation on the models presented above, we were able to clearly demonstrate that the only model which could predict the radiation inactivation results was the inhibitor model (Fig. 5). In this model, the insulin

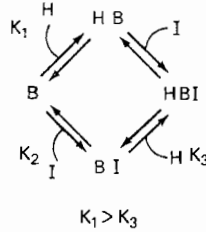


Fig. 5. Inhibitor model.

binding component (B) exists in the membrane as either a free binding component, which has a high affinity for insulin (H), or associated with an inhibitor (I). The effect of the inhibitor is to reduce the affinity of the binding component for insulin.

The easiest way to demonstrate that this type of model is capable of describing the radiation inactivation is to perform a least-square fit of the model to some typical data and then determine if the resulting "best fit" curves show the required maxima. The results of this analysis performed at various insulin concentrations is presented in Figure 6A. This general model would also predict the results of the equilibrium binding experiments (curvilinear Scatchard plots) but could not predict the observed enhanced dissociation of prebound  $^{125}\text{I}$ -insulin by unlabelled insulin (De Meyts *et al.*, 1976).

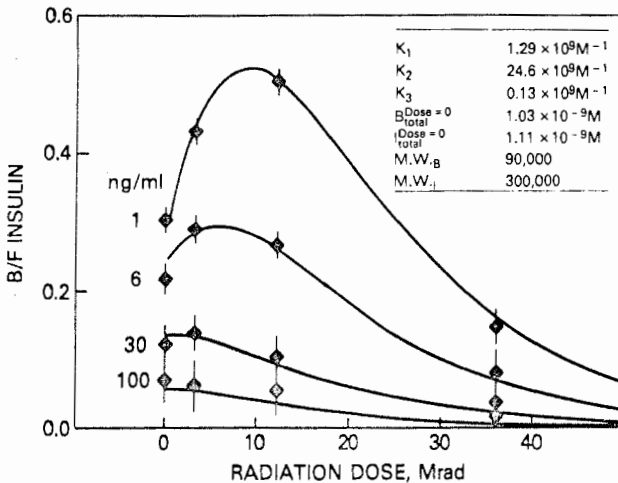


Fig. 6A. Computer analysis of radiation inactivation data in terms of the inhibitor model. The binding data (solid symbols) from competition experiments have been used to calculate the best fit lines for each insulin concentration. The values of the various parameters obtained from the curve fit are given in the insert. The molecular weights of both the binding component and inhibitor were held constant during the curve fitting procedure at the values obtained from the radiation inactivation data.

To account for this kinetic phenomenon, it is necessary to propose an oligomeric receptor which is composed of at least two binding components and two inhibitors. The curve fit of the radiation dose-response data in terms of this model is shown in Figure 6B. Other more complex models are also capable of fitting the data.

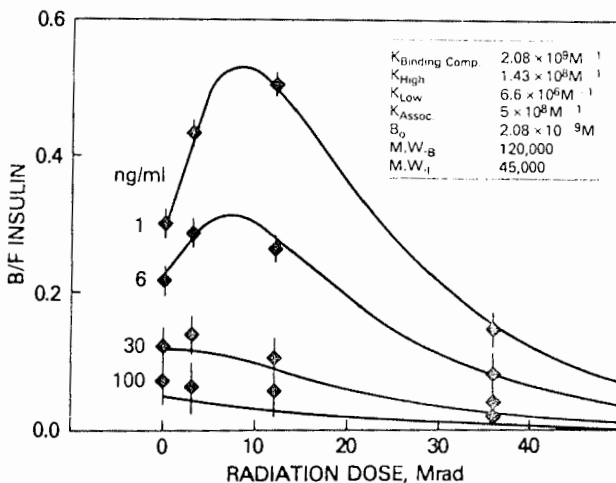


Fig. 6B. Computer analysis of proposed model for the insulin receptor which is composed of two binding components and two inhibitors. The solid lines are calculated from the constants presented in the insert.

### CONCLUSIONS

In conclusion, from the analysis of radiation inactivation data in terms of the various models presented, only a restricted subclass of possible models is allowed. At a minimum, the receptor is composed of two components. In light of the kinetic data and structural studies performed in several laboratories (Pilch and Czech, 1979; Jacobs *et al.*, 1979), we would suggest that the insulin receptor (~ 300,000) is composed of a binding component (90,000-120,000) and an inhibitor (40,000-50,000) which are tightly associated (perhaps by disulphide bonds). In addition, in order to explain the enhancement of the dissociation rate by unlabelled insulin, the native form of the receptor must have more than one binding component (Fig. 7). Occupancy of the insulin receptor with insulin causes a change in the interaction of the binding component and inhibitor. Finally, the results of radiation inactivation studies demonstrate that the insulin receptor in liver is clearly different from the receptor for the insulin-like growth factor, MSA, which has only a single component of molecular weight 115,000.

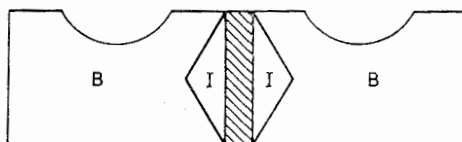


Fig. 7. Model of the insulin receptor.

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