Fluorescence Energy Transfer Studies on the Macrophage Scavenger Receptor

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ABSTRACT

The macrophage scavenger receptor is a transmembrane, trimeric glycoprotein which recognizes a number of negatively charged ligands. Cross competition studies of various ligands indicate that the scavenger receptor may bear more than one type of binding site or that there may be more than one type of receptor. In this study we employed resonance energy transfer techniques to identify the location of the binding site for maleylated bovine serum albumin. Using vesicles derived from plasma membrane, we labeled the ligand with a donor probe and labeled the membrane surface with acceptor probes to determine the distance of bound ligand from the membrane surface. Measurements were taken with three different donor-acceptor pairs. Transfer measurements for ligand labeled with dansyl and HAE (hexadecanoylaminoeosin) as the acceptor yielded a distance of 47Å from the surface of the plasma membrane. Similar measurements employing the same donors but using ORB (octadecylrhodamine B) as the acceptor produced a distance of 58Å. Assuming that the receptor extends perpendicularly from the cell surface this distance lies within the two receptor "domains" closest to the cell surface. These domains include the spacer region, with no distinct proposed structure and a region which has sequence similarity to an alpha helical coiled coil. No transfer was observed between ligand monolabeled with fluorescein and DiI in the membrane. This suggests that the orientation of mal-BSA bound to receptor places the fluorescein probe too far from acceptor on the membrane surface to experience energy transfer.

2. INTRODUCTION

The macrophage scavenger receptor is a trimeric, transmembrane glycoprotein bearing an unusually broad ligand binding specificity, recognizing certain polypurines (polyinosinic, polyguanylic), modified albumins (malondialdehyde modified, maleylated), modified-LDL (oxidized, acetylated) and other negatively charged molecules (Brown and Goldstein, 1983). The scavenger receptor is believed to play a key role in cholesterol accumulation during the formation of foam cells, one of the early lipid deposition events in atherosclerosis (Goldstein et. al., 1979; Steinberg, 1989).

Two types of receptors have been identified, type I and II. Both are predicted to be trimeric with five to six domains-- a transmembrane domain, a cytoplasmic domain, a short "spacer" region, a region of triplet repeats that highly resemble the collagen triplet repeat, a region of heptad repeats that are characteristic of alpha-helical coiled-coils (Conway and Parry, 1991) and a cysteine rich region which is absent from the type II receptor (Kodama, 1990). Both types of receptor bind ligand with similar affinity and specificity indicating

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that the cysteine rich domain is unnecessary for binding (Rohrer, 1990). This implies that the binding site is located somewhere in the remaining three extracellular domains.

In this work, resonance energy transfer is employed to find the physical location of the binding site for the ligand maleylated bovine serum albumin above the membrane surface. Transfer between donor probes located on bound ligand and acceptor probes in the membrane was measured for three different donor and acceptor pairs. Our results indicate that the bound ligand molecule lies within 47-58Å of the cell surface. Based on the proposed secondary structure of the receptor, a rigid stalk (Kodama, 1990), this places the binding site within either the alpha-helical coiled-coil region or the spacer region.

3. METHODS

Mouse macrophage cells were treated with N-ethylmaleimide which caused them to extrude plasma membrane vesicles. These vesicles were collected and dispersed to 5 samples-- a sample with donor labelled ligand bound to the receptor; controls for nonspecific binding, direct interaction between donor and acceptor, and background; and a sample from which surface density of acceptor is calculated. Donor labelled ligand is allowed to bind to receptors and acceptor molecules are titrated to the samples.

Using these samples the quenching of donor fluorescence was monitored as a function of surface density of acceptor. These curves can be fit by a single exponential of the form $\frac{Qda}{Qd}$=exp(-kc) where Q_{da} and Q_d are the quantum yields of the donor in the presence and absence of acceptor, respectively and k is a third order polynomial as follows:

$$k = B(R_o/L) = \sum_{i=0}^{3} a_i (R_o/L)^{-i}$$

The distance of closest approach between donor and acceptor, L, can be calculated from this expression, in which α_n are constants determined by numerical methods and R_o is the critical transfer distance--a characteristic of each donor-acceptor pair.

4. RESULTS AND DISCUSSION

The change in donor quantum yield as acceptor inserts into the membrane is given in figure 1 for a representative experiment with the dansyl-HAE pair. Q_{da}/Q_d versus surface density could be fit by the exponential function exp(-kC) where k was determined to be 1.237. This corresponds to a distance of closest approach of 41 Å between donor and acceptor. Five independent repeats of these measurements resulted in an average distance of 46 ± 7 Å.

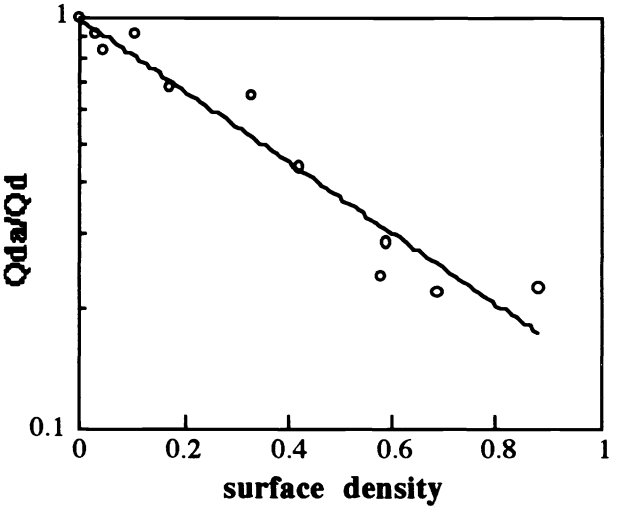

Figure 1. Relative change in donor quantum yield as a function of the surface density of acceptor for transfer between dansyl and HAE. Line represents best fit by a single exponential.

Data from a single experiment with the dansyl-ORB pair is given in figure 2. This plot could be fit to a single exponential with a decay constant of 0.782 which gives a distance value of 59 Å. Data averaged over seven independent experiments gave a distance of 58 ± 3 Å.

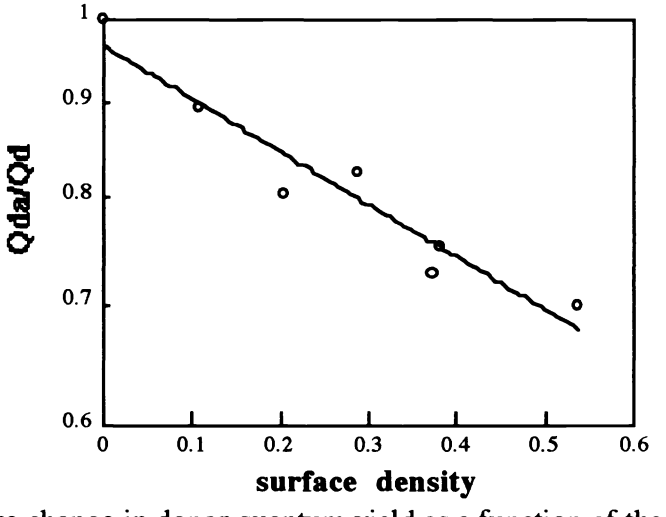

Figure 2. Relative change in donor quantum yield as a function of the surface density of acceptor for transfer between dansyl and ORB. Line represents best fit by a single exponential.

Experiments conducted with fluorescein-DiI show no evidence of energy transfer between donor and acceptor, implying that the site of labeling lies too far from the membrane surface to experience transfer. The results from the three different donor-acceptor pairs is summarized in table 1.

Donor	Qd	Acceptor	R_O (Å)	L (Å)
dansyl-lysine [a]	0.71	HAE	51	46 ± 7 [c]
dansyl-lysine [b]	0.297	ORB	46	58 ± 3 [d]
fluorescein	0.1034	DiI	44	n/a

Table 1. Summary of results derived from the three different donor-acceptor pairs. a.) experiments conducted in PBS b.) experiments conducted in 10% ethanol c.) average distance derived from five separate trials d.) average distance derived from seven separate trials.

These distance measurements give a physical location for the binding site from which the primary sequence can be deduced. The bovine macrophage scavenger receptor is proposed to be a rigid, trimeric stalk extending about 400 Å above the membrane surface. Based on this model and the results of the energy transfer experiments there would be two possible domains which contain the binding site -- the 32 amino acid spacer adjacent to the transmembrane domain or the 163 amino acid alpha-helical coiled coil domain beyond it.

5. ACKNOWLEDGMENTS

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6. REFERENCES

Brown M., & Goldstein J.L. (1983)*Ann. Rev. Biochem.* 52:223-261.

Conway, J., & Parry, D.A.D. (1991) *Int. J. Biol. Macro.* 13:14-16.

Goldstein, J.L., Ho, Y.K., Basu, K., & Brown, M.S. (1979) *PNAS* 76(1):333-337

Rohrer L., Freeman, M., Kodama, T., Penman, M., & Krieger, M. (1990) *Nature* 343:570-572.

Steinberg, D., Parthasarathy, S., Carew, T.E., Khoo, J.C., & Witztum, J.L. (1989) *New Engl. J. of Med.* 320(14):915-924.