Characterization of the Binding Domain of the β-Adrenergic Receptor with the Fluorescent Antagonist Carazolol

EVIDENCE FOR A BURIED LIGAND BINDING SITE*

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The antagonist carazolol has been used as a fluorescent probe for the binding site of the β-adrenergic receptor (βAR). The fluorescence properties of carazolol are dominated by the emission of the carbazole group, with the fine structure of the spectrum, but not the quantum yield, sensitive to the environment of the probe. The fluorescence emission spectrum of the bound probe is consistent with an extremely hydrophobic environment in the binding site of the receptor. Binding of carazolol to the purified βAR increases the polarization of the fluorophore. Exposure to collisional quenchers has demonstrated the bound carazolol to be completely inaccessible to the solvent. Furthermore, the fluorescence of bound carazolol is not quenched by exposure to sodium nitrite, a Förster energy acceptor which has an Rf value of 11.7 Å with carazolol. Thus, physical analysis of the binding site of the βAR by carazolol fluorescence indicates that the antagonist binds to the βAR in a rigid hydrophobic environment which is buried deep within the core of the protein.

The β-adrenergic receptor (βAR) is one of the best characterized members of a family of receptors which mediate their actions through signal transduction pathways involving guanine nucleotide binding regulatory proteins (G-proteins). Binding of agonists to these receptors results in the activation of specific G-proteins, leading to the stimulation or inhibition of effector enzymes and modulation of the levels of intracellular second messengers (1). The cloning of several G-protein coupled receptors has shown them to share common structural features, which presumably reflect their similar mechanisms of action (2). The model which has been proposed for these receptors consist of seven transmembrane helices, connected by hydrophilic loops of varying lengths. The amino terminus, which contains two sites of N-linked glycosylation, is proposed to be exposed extracellularly, thereby dictating the alternating internal and external exposure of the remaining loops. This proposed orientation of the cytoplasmic and extracellular loops of the βAR has been confirmed by immunological analysis using anti-peptide antibodies (3, 4).

The majority of primary sequence homology among G-protein coupled receptors is concentrated within the putative transmembrane domains, with the hydrophilic loop regions being more divergent. Genetic analysis of the βAR has revealed that the ligand binding domain of the receptor involves residues within this conserved hydrophobic core of the protein (5, 6). Asp113 in transmembrane helix 3 has been determined to be essential for both agonist and antagonist binding to the receptor, suggesting the formation of an ion pair between the protonated amine group of the adrenergic ligand and the carboxylate side chain of the aspartate residue (7, 8). In addition, a combined genetic and biochemical approach suggests that the catechol groups of catecholamine agonists bind and activate the βAR through the formation of hydrogen bonds to serine residues at positions 204 and 207 in transmembrane helix 5 (9). Additional mutagenesis studies have implicated residues in helices 2, 6, and 7 in ligand binding to the receptor (2). These mutagenesis data are in agreement with the results of photoaffinity labeling studies, which suggest the involvement of regions within transmembrane helices 2 and 7 in antagonist binding to the βAR (10, 11). Similarly, biochemical and genetic analysis of rhodopsin has revealed that the ligand retinal binds to the opsin protein via formation of a Schiff base with Lys296 in the seventh transmembrane helix (12, 13). Gln113 in the third transmembrane helix has recently been shown to contribute the counter-ion for this base (14).

The model which emerges from these genetic and biochemical experiments is of a ligand binding site buried within the hydrophobic core of the receptor protein, formed by contributions from residues on several of the transmembrane helices (2, 13). Biophysical data to support the model of a buried retinal binding site in rhodopsin has arisen in part from fluorescence quenching studies using retinal as an energy acceptor (15) and from electron spin resonance studies using a spin-labeled retinal analog which forms a stable complex with opsin (16, 17). Although the genetic and photoaffinity labeling data suggest that the ligand binding site of the βAR also resides within the hydrophobic domain, there is no direct biophysical or crystallographic evidence to support the assignment of this region to the membrane bilayer. β-Adrenergic ligands are protonated aromatic amines and, as such, are considerably more polar than retinal. The concept of a protonated amine ligand penetrating deep into the transmembrane core of the receptor protein has been challenged with the proposal that the ligand could bind to a more external domain (18). An alternative structure has been proposed for the βAR which provides for a larger extracellular domain with increased secondary structure to accommodate high affinity stereoselective ligand binding (19). Direct physical analysis of the orientation of the ligand in the binding pocket of the βAR would address the validity of using rhodopsin as a model for...
the structure of other G-protein coupled receptors. In the present study, we have utilized the high affinity β-adrenergic antagonist carazolol as a fluorescent probe for the ligand binding site of the βAR. Examination of the fine structure and polarization of carazolol fluorescence indicates that the ligand is bound to the receptor in a rigid hydrophobic environment. Furthermore, quenching studies indicate that this ligand binding pocket is buried deep within the core of the receptor protein.

**EXPERIMENTAL PROCEDURES AND RESULTS**

**DISCUSSION**

Although the interactions of the βAR with adrenergic ligands have been analyzed by genetic, biochemical, and pharmacological approaches, direct physical information about the structure of the ligand binding site has been lacking. In the present study, we have used the antagonist carazolol as a specific fluorescent probe for the binding site of the βAR. Because of the high affinity of this antagonist for the receptor, it was possible to isolate a βAR-carazolol complex, thus treating carazolol as a pseudo-covalent probe for the active site. The fluorescence properties of this ligand allowed observation of the βAR-carazolol complex without any substantial interference from protein tryptophan fluorescence. Therefore, carazolol could be used as a fluorescent reporter to analyze the environment of the ligand binding site of the βAR.

Binding of carazolol to the βAR resulted in a large increase in polarization of the carazolol fluorescence. This increase in polarization upon binding to the βAR is indicative of a decrease in the rotation of the carbazole group in the receptor, and suggests the ligand is immobilized in the binding site. Whereas the quantum yield of carazolol was independent of the composition of the solvent, the fine structure of the absorption and fluorescence emission spectra was sensitive to the dielectric constant of the solvent. The structured emission spectrum of carazolol consisted of a high energy peak (varying from 341 to 343 nm) and a lower energy peak (355–358 nm). The ratio of the short:long wavelength peak increased with increasing polarity of the solvent, providing a convenient measure of the environment of the probe. These comparisons indicated that the binding site of the βAR is very hydrophobic.

The environment of carazolol in the receptor binding pocket was observed to be more hydrophobic than 90% ethylene glycol and similar to 90% dioxane.

In addition to altering the relative intensities of the fluorescence peaks, solvents of different polarity were also observed to affect the positions of the peaks. The short wavelength peak displayed a slight blue shift, the magnitude of which correlated with the hydrophobicity of the solvent. However, whereas the peak ratio for carazolol bound to the receptor was similar to that measured in 90% dioxane, the position of the short wavelength peak was more consistent with that of carazolol measured in a dodecyl-β-D-maltoside solution. It therefore seems likely that the solvent effects on peak position and peak intensity may arise from different mechanisms. One interpretation is that carazolol experiences both specific and general solvent effects. Such divergent solvent effects have been observed for compounds having indole ring systems (25). Like the indole structure, the carbazole ring system contains a polar nitrogen atom (30). Therefore, it is conceivable that, while the overall binding pocket of the βAR is hydrophobic, the nitrogen atom is involved in a specific localized polar environment (for example, a hydrogen bond). Further experiments involving a combination of genetic and biophysical approaches will be necessary to establish the source of this putative ligand-receptor interaction.

The receptor-bound carazolol was exposed to a variety of quenchers in order to investigate the accessibility of the binding site of the βAR to the solvent. The compounds which were used had previously been demonstrated to quench the fluorescence of tryptophan. Because of the similar fluorescence properties of carazolol and tryptophan, it was expected that these compounds would be equally effective in quenching carazolol fluorescence. The collisional quenchers KI, acrylamide, NaNO₃, and methyl ethyl ketone were able to quench the fluorescence of free carazolol as shown in Fig. 6 and Table 3. In contrast, these compounds did not appreciably quench the fluorescence of carazolol bound to the βAR. These data suggest that the bound carazolol is not exposed to the solvent to any significant degree.

Since carazolol appears to be shielded from solvent in the binding site of the βAR, it is of interest to compare the parameters of quenching of receptor-bound carazolol with the quenching of internal tryptophans in proteins. Acrylamide quenching has been used to localize buried tryptophan residues and to explore protein flexibility. Examination of a variety of single-tryptophan containing proteins has revealed an inverse correlation between the degree of acrylamide quenching of tryptophan fluorescence and the extent to which the tryptophan residue is buried in the protein (31–33). Of the proteins examined in these studies, ribonuclease T₁ had the lowest accurately measurable acrylamide quenching rate (0.2–0.3 M⁻¹ ns⁻¹). The tryptophan residue in ribonuclease T₁ is almost completely isolated from the solvent, as determined by the position of the fluorescence emission peak and crystallographic measurements (32, 33). The fluorescence of the tryptophan residue of azurin was not measurably quenched by acrylamide (kₒ < 0.05 M⁻¹ ns⁻¹); this tryptophan residue is completely sequestered from the solvent (32). In the present study, the biomolecular quenching rate of βAR-bound carazolol by acrylamide was also determined to be below the experimental limits of detection (kₒ = 0.1 M⁻¹ ns⁻¹ would be an extreme upper limit). By comparison to the quench constants determined for the occluded tryptophan residues of ribonuclease T₁ and azurin, this low quenching rate for βAR-bound carazolol implies a completely buried ligand binding site for the receptor.

In addition to serving as a collisional quencher, NaNO₃ has a significant spectral overlap with tryptophan (33, 34) and carazolol (present study). Therefore, this compound could also quench the fluorescence of either tryptophan or carazolol by acting as a resonance energy acceptor. As expected, enhanced quenching of free carazolol by this compound was observed when compared to NaNO₃ (Fig. 6 and Table 3), which does not exhibit any significant spectral overlap. This enhanced quenching was similar to that which has been observed for NaNO₃ quenching of N-acetyltryptophanamide (33, 34). Because it could quench by energy transfer, NaNO₃ was able to effectively quench the fluorescence of the buried tryptophan in ribonuclease T₁, while NaNO₃ was not (34). However, in the present study we observed that NaNO₃ was not able to quench the fluorescence of carazolol in the binding site of the βAR, again suggesting that carazolol bound to the receptor is buried more deeply than the tryptophan in ribonuclease T₁.
In a similar study, the quenching of Tb$^{3+}$ fluorescence by energy transfer from retinal in the binding site of rhodopsin was used as a measure of the depth to which retinal was buried in the opsin protein. The $R_0$ for retinal and Tb$^{3+}$ was determined to be 46.7 Å. Assuming that Tb$^{3+}$ was in the rapid diffusion limit, the distance of closest approach between bound retinal and Tb$^{3+}$ was calculated to be 22 Å (15). In the present study, the $R_0$ value for NaNO$_2$ and carazolol was determined to be 11.7 Å. If the distance of closest approach between bound carazolol and NaNO$_2$ was also 22 Å, and assuming that NaNO$_2$ was in the rapid diffusion limit (see “Appendix”), then the quenching rate of the fluorescence of βAR-bound carazolol by NaNO$_2$ would have been 0.07 M$^{-1}$ ns$^{-1}$. This value is clearly below the limits of detection for NaNO$_2$ quenching in the present study. For the model compound 11-9 (carbazole) undeconic acid in dodecyl-$\beta$-D-maltoside micelles, we detected a rate constant of 0.644 M$^{-1}$ ns$^{-1}$ for NaNO$_2$ quenching. This rate constant corresponds to a distance of closest approach of 10.3 Å, demonstrating that the fluorescence of the carbazole group could be quenched if it were within 10 Å of the surface of the receptor. A detection limit for NaNO$_2$ quenching of carazolol fluorescence of 0.6 M$^{-1}$ ns$^{-1}$ can be assigned on the basis of the error measurement (Table 3) and verified by comparison to 11-9 (carbazole) undeconic acid. This limit corresponds to a distance of closest approach of 10.9 Å. Thus, the failure to detect any quenching of bound carazolol fluorescence by NaNO$_2$ indicates that the carazolol molecule is buried in the βAR at a depth of >11 Å.

The demonstration by collisional and energy transfer quenching that the carazolol is not bound near the extracellular surface of the βAR but, rather, resides in a deeply buried hydrophobic domain, agrees with the results of deletion mutagenesis studies (5,6), which indicate that the ligand binding domain of the βAR involves residues within the hydrophobic core of the protein. According to the model which has been proposed (2), the amino acid residues which have been demonstrated to be important for ligand binding to the βAR, including Asp$^{113}$, Ser$^{204}$, Ser$^{207}$, and Phe$^{296}$ (hamster β$_2$-adrenergic receptor nomenclature), would be located on various transmembrane helices, all approximately 30–40% of the way into the membrane bilayer. The determination that the carbazole fluorophore is buried at least 10.9 Å into the protein provides direct biophysical evidence to support such a model, and is consistent with the hypothesis that this region of the protein forms a membrane-spanning bundle.

The parameters of the carazolol binding site which have been determined in the present study are similar to those previously determined for the retinal binding site in mammalian opsin. Using similar fluorescence techniques, retinal was estimated to be 22 Å from the membrane surface (15). In ESR studies, a spin-labeled retinal analog was found to be highly immobilized in the binding site of rhodopsin and inaccessible to water-soluble reagents (16), as well as being sequestered from the phospholipid bilayer (17). The observation that the ligand binding domains of rhodopsin and the βAR share these physical characteristics is consistent with the previous observation of the similar hydropathy profiles and primary structures of these two proteins (20). The results of the present study suggest that, despite the structural differences in the ligands which bind to the βAR and rhodopsin, the structures of the ligand binding sites of these proteins may be similar. The fluorescence properties of bound carazolol provide physical evidence that, like rhodopsin, the ligand binding domain of the βAR is in a constrained, hydrophobic
environment sequestered away from the surface of the protein.

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Procedures

**Experiments**

β-Adrenergic Receptor Fluorescence

**Results**

**Purification of βAR**

βAR was purified from *A. niger*, a yeast typically grown in media containing high levels of glucose. The purification process involved several steps, including solubilization of the receptor, detergent removal, and affinity chromatography. The purified receptor was then subjected to gel filtration chromatography to obtain a homogeneous preparation.

**Characterization of the Binding Domain**

The binding domain of the purified βAR was characterized using a variety of techniques, including fluorescence spectroscopy. The binding affinity of various ligands was determined using fluorescence polarization assays. The results showed a high degree of specificity for the binding domain, with the IC50 values ranging from 10 to 50 nM.

**Steady State Fluorescence**

Steady state fluorescence was used to measure the conformational changes in the receptor upon ligand binding. The fluorescence intensity was found to increase upon addition of the agonist, indicating a conformational change in the receptor.

**Time-resolved Fluorescence**

Time-resolved fluorescence was used to measure the dynamics of the receptor-ligand complex. The results showed a significant increase in the fluorescence lifetime upon ligand binding, suggesting a conformational change in the receptor.

**Temperature and pH Effects**

The effects of temperature and pH on the receptor-ligand complex were studied. The fluorescence intensity was found to increase with increasing temperature and pH, indicating a stabilization of the receptor-ligand complex.

**Conclusions**

The study provided insights into the structural and functional aspects of the βAR. The results have implications for the development of new drugs targeting the βAR, which could be used to treat various diseases.
quenched than bound carazolol, this would lead to an apparent time-dependent quenching of the PAR-
carazolol complex. The addition of a high concentration of acrylamide to a fresh sample of PAR-carazolol
caused an immediate decrease in fluorescence. However, after 19 minutes, there was a 1.2-fold decrease in fluorescence. The magnitude of this fluorescence decrease is
consistent with the reported dissociation rate constant of 0.0040 min⁻¹ for PAR-carazolol (29), which would
result in a 1.3-fold decrease in bound carazolol after 19 minutes. Therefore, the small amount of quenching
of the carazolol-PAR by these compounds observed in Fig. 6 appears to result from the quenching of
bound free carazolol.

Whereas the quenchers described above act as collisional quenchers and require direct contact with
the ligand, NaNO₂ can quench the fluorescence of carazolol by energy transfer, as well. Because NaNO₂
has a significant spectral overlap with carazolol, it may act as a Förster energy acceptor for carazolol
fluorescence, providing a means for quenching through space, without contact between the molecules.
The degree of quenching by energy transfer is a function of the Förster distance Rₐ (defined as the distance
over which half-maximal energy transfer occurs), which is dependent on the overlap between the emission
spectrum of the fluorescent donor and the absorption spectrum of the quencher. The spectral overlap (I)
for the fluorescence of carazolol and the absorption of NaNO₂ was determined to be 2.9 x 10⁻¹⁷ m³ M⁻¹
from equation 4. Using equation 5, the Rₐ between nitric and carazolol was then calculated to be 11.7 Å. A
Scalar-Vector plot for the quenching of carazolol fluorescence by NaNO₂ is shown in Fig. 6, and the
quenching constants given in Table 3. Although NaNO₂ quenched the fluorescence of free carazolol, no
significant quenching of bound carazolol by this compound was observed (Kₐ = 8.6  x  10⁻³ M⁻¹ min⁻¹). In
these experiments, NaNO₂ contributed a significant fluorescence background which was not linear with
concentration, and there was a significant inner filter effect at both the emission and excitation wavelengths
of carazolol, contributing to the relatively large error for this small number (Table 3). Because nitric has a
substantial spectral overlap with carazolol, the small negative value of Kₐ may also be attributed to an
close increase in the fluorescence of carazolol upon re-absorption of a photon from NaNO₂ fluorescence.

In order to further characterize the interaction of these quenchers with the fluorescent carbonyl group
of carazolol in detergent, a model system was established using 11-CU incorporated into DM micelles.
Table 4 shows the bimolecular quench rates of 11-CU by the quenchers used in the present study.
The collisional quenchers, I₂, acrylamide, NaNO₂, and MEM all showed a very limited degree of quenching
of 11-CU in DM micelles, confirming that the carbonyl group of the lipid is buried in the micelle and not
exposed to the solvent. However, the energy transfer quencher NaNO₂ showed a substantial rate of
quenching in this model system, confirming that longer-range interactions are involved. The Rₐ value
determined for 11-CU and NaNO₂ in DM at 25 °C was 12.5 Å (2.5 x 10⁻¹³ m³ M⁻¹), corresponding to a
distance of closest approach between the fluorescent donor and the acceptor of 10.3 Å (see Appendix). This
value appears to be a reasonable estimation for the depth of the carbonyl group in the micelle; the value
considered for the length of an idealized 11-carbon chain would be approximately 14 Å.

Figure 2: Binding of carazolol to purified PAR. Carazolol binding to solubilized purified PAR was
determined by competition with [³H]carazolol in 10 mM Tris, 0.1 mM EDTA, 0.05% DM at room
temperature as described in Experimental Procedures. The PAR concentration was 0.7 pmol and the
concentration of PAR-carazolol complex was determined by competition with [³H]carazolol in
solubilized purified PAR. The PAR concentration was 4.7 pmol as determined by comparison with
molecular weight markers as shown with arrow.

Figure 3: Absorption and fluorescence spectra of carazolol. Absorption of 10 μM carazolol (---)
was measured in 10 mM Tris, 0.1 mM EDTA, 0.05% DM, pH 7.3. The fluorescence emission spectrum of
2 μM carazolol (-----) in the same buffer, using an excitation wavelength of 325 nm, is shown after solvent
subtraction. The structure of carazolol is shown in the inset.

Figure 4: Solvent perturbation of the absorption and fluorescence spectra of carazolol. Absorption
and fluorescence spectra of 5 μM carazolol in 90% diethanolamine (---), 45% ethylene glycol (-----),
or 10 mM Tris 7.3, 0.1 mM EDTA, 0.05% DM (----) are shown. The fluorescence is given in arbitrary units,
normalized to the fluorescence in 90% diethanolamine. Carazolol was dissolved as a 30% stock in 10 mM
Tris 7.3, 0.1 mM EDTA, 0.05% DM, and diluted into the appropriate solvent.

Table 1: Solvent Perturbation of Carazolol Fluorescence

| Solvent                  | Peak 1 (nm) | Fluorescence Peaks | Fluorescence Peaks | Ratio |
|--------------------------|-------------|--------------------|--------------------|-------|
| 0.05% DM¹                 | 333         | 335                | 0.976              |
| 45% Ethylene glycol²     | 333         | 335                | 1.022              |
| 90% Ethylene glycol      | 334         | 335                | 1.087              |
| 90% Dioxane¹             | 341         | 336                | 1.125              |
| pAR Bound                | 333         | 335                | 1.182              |

¹These fluorescence spectra are presented in Fig. 3.
²The peak ratio represents the fluorescence of Peak 2/Peak 1, after background subtraction.

Figure 5: Fluorescence of carazolol bound to the AAR, AAR (7 picomoles) in 1.5 ml of 10 mM Tris, 0.1 mM
EDTA, 0.05% DM, pH 7.3 was incubated with 90 μM carazolol at room temperature for 20 min. This
solution was then chilled on ice and concentrated to 250 μl using a Centricon 30 (Amicon). The
concentrated AAR was separated from uncharged on a G-15 spin columns equivalent to 10 ml Tris, 0.1
mM EDTA, 0.05% DM, pH 7.3. (A) Emission of the PAR-carazolol complex (top), or PAR which was
incubated with 10 μM alprazolam prior to exposure to carazolol (bottom). The excitation wavelength was
325 nm. The peak at 335 nm observed in the presence of alprazolam is the water Raman vibrational band. (B)
Spectrum of PAR-carazolol with the alprazolam background subtracted.
### Table 2

| Fluorophore             | Substrate           | Polarization |
|-------------------------|---------------------|--------------|
| Carazolol (50 nM)       | 10 mM Tris 7.3, 0.1 mM EDTA | 0.005 ± 0.004 |
| Carazolol (50 nM)       | 10 mM Tris 7.3, 0.1 mM EDTA, 0.05% DMS | 0.038 ± 0.026 |
| αAR-Carazolol complex (80 nM) | 10 mM Tris 7.3, 0.1 mM EDTA, 0.05% DMS | 0.275 ± 0.011 |
| II-CU (5 μM)            | 50% Ethanol         | 0.001 ± 0.002 |
| II-CU (5 μM)            | 10 mM Tris 7.3, 0.1 mM EDTA, 0.05% DMS | 0.038 ± 0.001 |

### Table 4

| Quencher | kq (M⁻¹ ns⁻¹) ± SD |
|----------|--------------------|
| KI       | 0.074              |
| Acrylamide | 0.121              |
| NaN02    | 0.004              |
| MEK      | 0.047              |

### APPENDIX

A lower limit for the distance of closest approach between carazolol in the binding site of the αAR and NaN02 may be estimated from the available data. To understand NaN02 may be estimated from the results approach, nitrite must be in the fast diffusion time (100), as it has previously been shown to occur for NaN02 quenching of tryptophan fluorescence (34). Birk and Georgius (30) determined that Stern-Volmer kinetics apply and diffusion need not be considered for determining energy transfer rates when r > 3R0, where R0 is the mean distance travelled by the quencher during the excitation lifetime of the fluorophore and can be described as:

\[ r > (2D0)^{1/2} \]

where \( D \) is the sum of the diffusion coefficients of the donor and the acceptor. \( D \) may be calculated from the reciprocal quenching constant averages for nitric quenching of free carazolol (Table 2). A diffusion-controlled quenching reaction may be described by equation 6 and 7:

\[ k_q = 5.62 \times 10^4 \text{cm}^3 \text{molecule}^{-1} \text{s}^{-1} \]

where \( k_q \) is the sum of the diffusion rate, \( p \) is the quenching efficiency, and \( N \) is Angstrom’s number. As \( R = R_0 \), then p = 0.35 (35-38), and the equation can be solved for \( D \).

For NaN02 quenching of free carazolol, \( k_q = 5.25 \times 10^4 \text{M}^{-1} \text{s}^{-1} \) (Table 2) and D, the sum of diffusion coefficients for nitric and carazolol is, as described, 2A × 10⁻⁶ cm² s⁻¹. If the assumption is made that the diffusion rates of NaN02 and carazolol are equal, then the diffusion coefficients for nitric are \( 1.5 \times 10^{-6} \text{cm}^2 \text{s}^{-1} \). From equation 7, for nitric 48.4 X. Therefore \( r > 3R_0 \) (7.7 X), and Stern-Volmer kinetics should apply.

A lower limit for the distance of closest approach between nitric and αAR-bound carazolol may then be approximated by the method of Thoma et al. (31) or \( k_q = 5.25 \times 10^4 \text{M}^{-1} \text{s}^{-1} \) where the rate of energy transfer (kq) is given as:

\[ k_q = 5.62 \times 10^4 \text{cm}^3 \text{molecule}^{-1} \text{s}^{-1} \]

In this equation, \( D \) is the distance of closest approach between the donor and the acceptor. The upper limit of kq for nitric, as determined from the standard error (Table 3), would be approximately 5.25 × 10⁴ M⁻¹ s⁻¹. As a concentration of 0.04 M NaN02 (resulting in a 10% decrease in fluorescence), \( k_q = 9.8 \times 10^4 \text{M}^{-1} \text{s}^{-1} \) (2.02 M) = 0.812 s⁻¹, from the Stern-Volmer equation. At this concentration, the density of acceptors (d) would be 1.2 × 10⁵ nitric molecules per cubic angstrom. Therefore, equation 9 can be given a limit for the closest approach of nitric in carazolol bound to the αAR of 10³ X.
Characterization of the binding domain of the beta-adrenergic receptor with the fluorescent antagonist carazolol. Evidence for a buried ligand binding site.
M R Tota and C D Strader

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