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Chiral Recognition of D-Kyotorphin by Lipidic Membranes: Relevance Toward Improved Analgesic Efficiency

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D-Kyotorphin (D-KTP), the most potent isomer of the endorphin-like dipeptide kyotorphin (KTP), is a good drug candidate for the treatment of chronic pain and is thought to be involved in receptor-mediated processes. According to the "membrane catalysis" model, ligands interact with membrane lipids to attain high local concentrations in the receptor vicinity and to adopt the necessary conformation for docking. Therefore, the interaction and recognition of D-KTP by membranes is potentially important to its increased analgesic effect. In spite of the neutral net charge of D-KTP at pH 7.4, fluorescence spectroscopy reveals that the interaction with large unilamellar vesicles is more extensive than was observed for KTP. The tyrosine residue interacts extensively with rigid membranes, with a location and well-defined orientation in the bilayer. This suggests not only that D-KTP meets the structural constraints needed for receptor–ligand interaction in a manner similar to that of KTP, but also that the stronger membrane interaction and ability to discriminate rigid membrane domains might contribute to its improved analgesic effect.

Introduction

D-Kyotorphin (D-KTP, L-Tyr-D-Arg, shown in Figure 1) is the most powerful optical isomer[1] of the natural analgesic dipeptide Kyotorphin (KTP, L-Tyr-L-Arg). KTP was initially isolated from bovine brain[2] and acts as a neurotransmitter and neuromodulator in nociceptive responses in the central nervous system[3] with a 4.2-fold higher analgesic effect than endogenous opioid peptides such as Met-enkephalins.[4,5] However, its analogue D-KTP shows enhanced analgesic activity 5.6-fold higher than that observed with KTP.[3] Takagi and co-workers[4] suggested that the enhanced analgesic effect of D-KTP is the result of protease resistance conferred by the substitution of L-arginine with a D-arginine residue, but this might not be the only factor behind the enhanced analgesic effect. An action mediated by specific receptors (non-opioid) for KTP and D-KTP has been suggested by several authors.[6,7] The peptides have shown a non-opioid analgesic effect in the peripheral nervous system,[8] which makes them quite appealing for the treatment of chronic pain. Moreover, these peptides inhibit cell proliferation, indicating that peripheral tissue cells also contain KTP-specific receptors.[9] D-KTP has a phenolic group, which is common and essential for the interaction of biologically active peptides with cell-surface receptors.[10,11] Moreover, it has already been shown for related peptides[12,13] that different membrane properties can modulate the exposure and orientation of this critical group, which is most likely involved in receptor interaction.

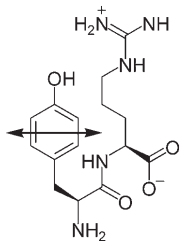

Figure 1. D-KTP (L-Tyr-D-Arg); the ^1L_b transition in the phenolic ring is represented by the double arrow.

A study of D-KTP based on modifications in the lipid bilayer–water interface is important, as Sargent and Schwyzer[14] proposed that peptides interact with membrane lipids prior to receptor binding, and this interaction allows them to adopt an appropriate conformation for docking cell receptors. This hypothesis has gained experimental support,[12–15] which highlights the fact that critical groups, such as the phenolic ring of tyrosine, may indeed be exposed and oriented by lipid membranes.

The goal of the study reported herein was twofold: to report structural information on the tyrosine moiety of D-KTP both in aqueous solution and in interaction with a model system of biological membranes, and to evaluate the impact of different structural and chemical changes at the phospholipid–water interface on the binding, location, and exposure of the dipeptide's critical group thought to be involved in receptor docking.

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Results and Discussion

Studies in aqueous solution

Prior to the studies of peptide–membrane interactions, we determined the net charge of D-KTP at physiological pH. Formal net charges may not be realistic for small peptides. The experimental titration curve showed that at pH 7.4 all D-KTP is in the supernatant and is therefore globally neutral. The N terminus is the group that presents a titration point closer to physiological pH and is therefore predominantly deprotonated at pH 7.4 in the case of D-KTP, as was observed for KTP.[12]

Absorption and emission spectra of D-KTP in aqueous solution, ethanol, and in multi-bilayers of dipalmitoyl phosphatidylcholine (DPPC) are shown in Figure 2. The spectrum of D-KTP

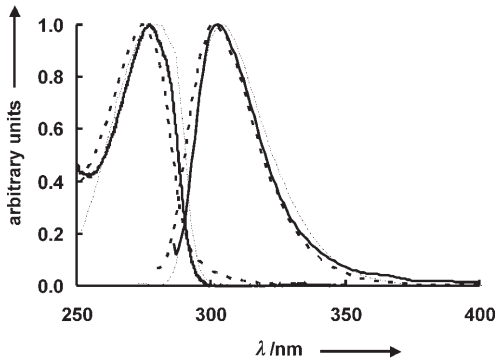

Figure 2. Normalized absorption and fluorescence emission spectra of D-KTP in Bis-Tris propane buffer (20 mM, pH 7.4; -----), ethanol (••••), and DPPC multi-bilayers (——) at room temperature (λ_{ex} = 277 nm). The mole ratio of DPPC/D-KTP was 2.5:1, and the concentration of D-KTP in a solution of buffer and ethanol was 70 μM.

in buffer solution is similar to that observed for free L-Tyr in solution. The photophysical parameters summarized in Table 1 show that although the peptide has maximum absorption and emission wavelengths equal to those of L-Tyr, the fluorescence quantum yield (Φ_F) and mean lifetime ($\bar{\tau}$) values are smaller.

Table 1. Photophysical parameters of L-Tyr and D-KTP.[a]

Parameters	L-Tyr	D-KTP
λ_{abs} [nm][b]	275[f]	275
λ_{em} [nm][c]	303[f]	303
$\Phi_{F,W}$[d]	0.14[f]	0.028
$\Phi_{F,L}$[d]	n.d.[g]	0.040
$\bar{\tau}_W$ [ns][e]	3.6[f]	1.7
$\bar{\tau}_L$ [ns][e]	n.d.[g]	3.0

[a] Determined in Bis-Tris propane buffer (20 mM) and in large unilamellar vesicles (5 mM) at pH 7.4; [L-Tyr] = [D-KTP] = 70 μM. [b] Maximum absorption wavelength. [c] Maximum emission wavelength. [d] Fluorescence quantum yield. [e] Mean fluorescence lifetime for which subscripts W and L denote aqueous and lipidic environment, respectively. [f] Determined at 23 °C in aqueous solution at neutral pH as reported in Ref. [18]. [g] Not determined.

Several authors[16,17] have suggested a mechanism in which the fluorescence of an aromatic amino acid side chain can be quenched by the peptide group as a result of charge transfer between the excited chromophore (phenol ring) acting as a donor, and electrophilic units in the peptide backbone acting as an acceptor. Moreover, Ross and co-workers[18] observed that if the phenol side chain is shielded from the solvent and the local environment contains no proton acceptors, many intra- and intermolecular interactions result in a decrease in the quantum yield.

Interaction with model systems of biomembranes

The fluorescence quantum yield and mean excited state lifetime of D-KTP increases in the presence of large unilamellar vesicles (LUVs) relative to homogeneous aqueous solution (Table 1). This is a clear indication that the phenolic ring interacts with the lipidic vesicles. However, two major factors seem to govern the partition of D-KTP to lipidic systems: lipid charge and lipid phase (Table 2).

Table 2. Partition coefficient (K_p) and fractional fluorescence intensity (f) values for D-KTP.[a]

System	Cholesterol[b]	$K_p \times 10^3$	f_L[c]	f_B[d]
POPC[e]	−	0.14 ± 0.04	0.58 ± 0.02	0.99 ± 0.03
	+	4.20 ± 1.7	0.97 ± 0.01	0.49 ± 0.02
DPPC	−	5.86 ± 0.7	0.98 ± 0.01	n.d.
POPG[f]	−	0.38 ± 0.05	0.66 ± 0.01	0.99 ± 0.02
	+	6.10 ± 0.05	0.99 ± 0.01	0.47 ± 0.01

[a] Determined at room temperature and pH 7.4. [b] The absence (−) or presence (+) of cholesterol (33 molar percent relative to the total lipid concentration) is denoted. [c] Fraction of fluorescence intensity emitted from peptides in the lipidic environment (calculated with [lipid] = 3 mM). [d] Fraction of fluorescence intensity accessible to each quencher. Iodide (in the presence of cholesterol) and cholesteryl bromide (in the absence of cholesterol) were used as quenchers in the systems. [e] 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine. [f] 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidyl-DL-glycerol.

Higher partition coefficients (K_p) were obtained for D-KTP in gel crystalline phase systems (DPPC) than in liquid crystalline systems (POPC and POPG; Table 2 and Figure 3). However, similar K_p values were observed upon comparison of the several rigid systems studied: DPPC (gel crystalline phase), and POPC+cholesterol and POPG+cholesterol (both 33 molar percent cholesterol and in liquid ordered phase). The addition of cholesterol (33 molar percent) to liquid crystalline phase systems such as POPC and POPG increases the partition coefficient (Table 2). This is expected from its condensation effect, in light of the results obtained with DPPC. Condensation is mainly achieved by an enhancement of the van der Waals interaction between adjacent lipid molecules and a consequent decrease in the average molecular area of the lipid in the bilayer surface. Lipidic acyl chains become more ordered, a consequence of the transition from the liquid crystalline phase to a liquid ordered phase, which causes a drying effect in the lipid–

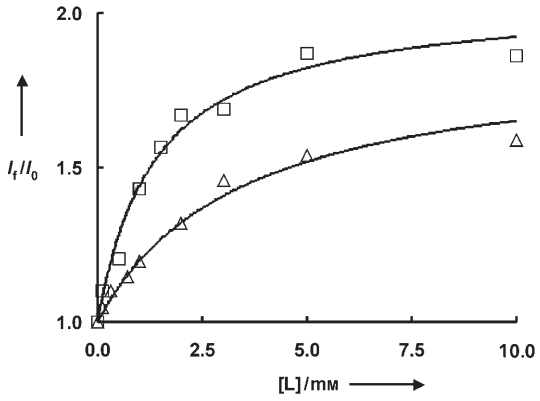

Figure 3. The dependence of D-KTP fluorescence intensity ($\lambda_{ex}=277$ nm; $\lambda_{em}=303$ nm) on the concentration of DPPC (□) and POPG (△) forming LUVs at pH 7.4 ([L] = lipid concentration). K_p was obtained by nonlinear regression fit of Eq. (2) (Experimental Section) to the data points. Mole ratio DPPC/D-KTP = 2.5:1.

water interface. These more rigid membrane areas are particularly important, because the membrane receptors are usually anchored to membranes by lipidic rafts.[19–21]

An increase in the negative surface charge does not seem to have a pronounced effect in the binding of D-KTP to lipidic vesicles, and similar K_p values are obtained in POPC and POPG systems, with or without cholesterol (see Table 2). This is not surprising, because at physiological pH D-KTP is globally neutral. Nevertheless, in comparison with KTP,[12] D-KTP shows an enhanced capacity to discriminate between patches of membranes that have different rigidities; this may be one of the key features and one of the biophysical foundations of its action, namely in "finding the way" toward receptors.

Localization in model systems of biomembranes

Fluorescence quenching studies were performed to investigate the location of the phenolic ring of D-KTP in the different systems. Cholesteryl bromide and iodide anions were used as quenchers of D-KTP fluorescence in the presence of bilayers with or without cholesterol, respectively. The ability of the quenchers to decrease tyrosine fluorescence can be used to determine the degree to which these amino acids are exposed to the aqueous phase or lipidic interface. The I^- anion is able to quench tyrosine residues that are exposed to the aqueous phase. The bromine group (quencher) of cholesteryl bromide replaces the OH group at position 3 of cholesterol, and putatively penetrates to the same depth in the lipidic bilayer interface, being thus able to quench phenolic rings at this depth. If the fluorophore is exposed to the aqueous medium, a considerable fraction of the emitted fluorescence will be quenched by I^-; on the other hand, if it is buried in the lipidic head group interface (that is, in the vicinity of quencher) then quenching by cholesteryl bromide will be effective. Insertion deep into the core of the membrane would lead to no quenching by either species. The fraction of fluorescence intensity accessible to each quencher (f_B) was determined from application of the Lerher equation [Eq. (5)] (Experimental Section and Figure 4), while the fraction of fluorescence intensity emitted from the lipidic environment (f_L) was determined from K_p [Eq. (3)], with a lipid concentration of 3 mM. It is clear that

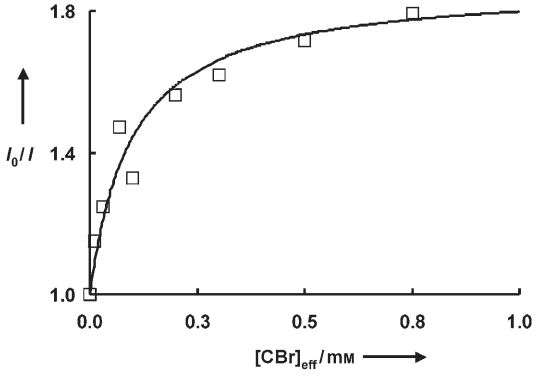

Figure 4. Stern–Volmer plot for the fluorescence quenching ($\lambda_{ex}=277$ nm; $\lambda_{em}=303$ nm) of D-KTP by cholesteryl bromide (CBr) in POPG+cholesterol LUVs (33 molar percent total sterol/(cholesterol+cholesteryl bromide)) at pH 7.4.

almost all the fluorescence signal comes from peptides that interact with the lipidic bilayer; f_L is always ≥ 0.58 (Table 2). In systems without cholesterol, $f_B \cong 1$ is observed, showing that the tyrosine ring in D-KTP is fully exposed to the aqueous phase. Despite the fact that the condensation effect of cholesterol promotes peptide interaction with lipidic palisades (increase in K_p and f_L), its addition to both POPC and POPG causes 50% of the tyrosine fluorescence intensity to be quenched by cholesteryl bromide (Figure 4). This means that under these conditions, there are two dipeptide populations: one that is more deeply buried in the lipidic head group interface and the other that remains in a superficial location in the membrane. The condensation effect of cholesterol would not promote by itself a deeper insertion of the dipeptide in the lipidic palisades. This points to a specific interaction with the sterol group which is known to occur between phenolic chromophores and cholesterol.[15] The shallow location of one of the dipeptide populations in interaction with the membrane surface exposes the phenolic ring of tyrosine, making it accessible for molecular recognition through cellular receptor-mediated processes. The effect of an increased local concentration of peptide with a corresponding increase in biological efficiency in cholesterol-rich membranes has already been reported.[22] Moreover, it has already been shown that cholesterol is able to constrain opioid receptors in the active form,[23] so the presence of a dipeptide in a cholesterol-rich region of the membrane would be quite meaningful to improve the efficiency of the dipeptide.

Orientation of the tyrosine side chain in different lipidic multi-bilayers

The lowest-energy singlet transition of tyrosine results from the ^1L_b transition oriented across the phenyl ring (Figure 1) with an absorption maximum at approximately 277 nm.[18] If

the excitation radiation wavelength is >260 nm, absorption and emission occur from the same ^1L_b state, and the orientation of the phenolic ring of the peptide can be obtained from UV/Vis linear dichroism methodologies. We used UV/Vis linear dichroism to determine the orientation of the tyrosine ring system in membrane-inserted D-KTP. The second-rank order parameter ($\langle P_2 \rangle$) values obtained (Table 3) report the orientation of the ^1L_b moment (arrow in Figure 1) relative to the bilayer normal. The second-rank order parameters show that the phenolic ring orientation is rather insensitive to membrane properties, as demonstrated by the small range of values obtained for the mean angle: $30^\circ < \langle \Psi \rangle < 37^\circ$. Nevertheless, a slight increase in the mean angle ($\approx 16\%$) is observed in the case of rigid systems relative to those of fluid systems.

Table 3. Second-rank order parameters ($\langle P_2 \rangle$) for D-KTP in various lipid systems.[a]

System	Cholesterol[b]	$\langle P_2 \rangle$
DPPC	−	0.598
	−	0.467
POPC	+	0.620
	−	0.497
POPG	+	0.592

[a] Obtained from UV/Vis linear dichroism at room temperature and pH 7.4. [b] The absence (−) or presence (+) of cholesterol (33 molar percent relative to the total lipid concentration) is denoted.

Conclusions

D-KTP is globally neutral at pH 7.4 and interacts strongly with the model systems of biological membranes. It has already been shown that L,D enantiomers of dipeptides are not only able to interact more strongly with lipidic membranes than L,L enantiomers, but are also able to associate in a more internal/hydrophobic region of the lipidic membranes.[24] Gel crystalline phase systems are preferred, and in this manner, D-KTP shows an increased capability to discriminate between rigid and fluid regions in the lipidic surface. It was concluded that the phenolic ring of tyrosine is superficially located in cholesterol-free membranes. Phenolic groups are common and essential in many biologically active peptides and are involved in the interaction with cell receptors.[10] The exposure and/or orientation of such groups is therefore crucial for molecular recognition through cellular receptor-mediated processes. In cholesterol-rich membranes, the phenolic ring has been shown to be more deeply buried on average; nevertheless, 50% of the population of D-KTP molecules remains in a shallow position. This can help to increase the local concentration of D-KTP, which might be necessary to effectively increase its analgesic effect. The present study also demonstrates that the phenolic ring of D-KTP has a tilted mean orientation relative to the bilayer plane and is rather insensitive to membrane phase, which lends further evidence to the "membrane catalyst" model. This model states that the role of the membrane is to concentrate ligands and to allow them to adopt the necessary docking constraints to interact with cell receptors. In addition to the fact that D-KTP has a D-arginine residue, which might make its recognition by degrading enzymes difficult, this dipeptide shows an enhanced capacity to discriminate in favor of rigid lipid patches, which are known to keep opioid receptors in the active form.[23] An increased local concentration proximal to the receptor helps to enhance the biological effect of this dipeptide. Moreover, D-KTP is able to interact with these areas without altering the orientation of the potential critical group (the phenolic ring of tyrosine). The sum of all these factors represents a more consistent explanation for the observation that D-KTP has a greater analgesic effect than KTP.

Experimental Section

Materials and reagents: D-KTP (Bachem, Switzerland), L-tyrosine, cholesterol, cholesteryl bromide, and 5-methoxyindole (Sigma, USA) were 99% pure.

Q-Sepharose and SP-Sepharose resins used for the determination of the net charge of D-KTP at pH 7.4 (Amersham Biosciences, Germany) had operational pH ranges of 3–11 and 4–13, respectively. Bis-Tris propane buffer (20 mM) was used in the pH range 6.3–9.3 in the batch titration studies; pH 7.4 was used in all other studies. All lipids were obtained from Avanti Polar Lipids (USA) and the solvents (Merck, Germany) were spectroscopic grade.

UV/Vis absorption measurements were carried out in a Shimadzu spectrophotometer (model UV-3101 PC). A spectrofluorimeter (SLM-Aminco 8100) equipped with a Xe lamp (450 W), double monochromators in both excitation and emission, and a quantum counter was also used. D-KTP excitation and emission wavelengths used were 277 and 303 nm, respectively.

Quantum yield: The relative quantum yield of D-KTP was determined by using 5-methoxyindole in aqueous solution as a reference ($\Phi_F = 0.28 \pm 0.01$).[25] Polarizers at the magic angle orientation were used with lipidic (vesicle) suspensions to avoid anisotropic effects. All measurements were carried out at room temperature.

Ionization state of D-KTP: Typical acid–base titration techniques were not sensitive enough to determine the ionization state of D-KTP. Instead, a batch technique was used with SP-Sepharose Fast Flow (anionic propylsulfonyl: $-CH_2CH_2CH_2SO_3^-$) and Q-Sepharose Fast Flow (cationic quaternary ammonium: $-CH_2N^+(CH_3)_3$) resins. The rationale of the technique is that if D-KTP is positively or negatively charged at a given pH (which has to be within the operational pH range of the resins), it will bind to one of the two resins. On the other hand, if D-KTP is neutral, it will not bind to any resin. The resins were left to equilibrate overnight at each of the selected pH values. D-KTP was added afterward to a final concentration of 4×10^{-4} M. After incubation for 60 min, the samples with different concentrations of the resins were centrifuged for 5 min at 2000 *g*. The supernatant was separated for electronic absorption measurements. The two resins were pre-tested with fluorescein as reported by Lopes et al.[12]

Fluorescence decays: Fluorescence decay measurements were carried out with a time-correlated single-photon counting system. For excitation of the systems with D-KTP at 285 nm, a frequency-doubled cavity-pumped dye laser of rhodamine, synchronously pumped by a mode-locked Ar^+ laser (514.5 nm, Coherent Innova 400-10) was used. The emission wavelength was 308 nm, and a photomultiplier (Hamamatsu R-2809 MCP) was used for detection.

A filter (Corion W-305-S) was used to avoid excitation light scattering interference with the measurements.
Large unilamellar vesicles (LUVs, 5 mM) were obtained by the extrusion method.[26] A solution of D-KTP was added afterward to obtain a final concentration of 70 μM. Fluorescence decays were complex and are described by a sum of three exponentials. The mean lifetime, $\overline{\tau}$, was obtained from Equation (1):[27]

$$\overline{\tau} = \frac{\sum a_i \tau_i^2}{\sum a_i \tau_i} \quad (1)$$

The extent of incorporation of D-KTP in lipidic membranes: Partition studies were carried out in LUVs prepared as described in the work by Hope and co-workers,[26] by using various lipid (or lipid+cholesterol at 33 molar percent relative to the total lipid concentration) concentrations up to a final concentration of 10 mM. Although small unilamellar vesicles (SUVs) have the advantage of low scattering, the curvature caused by small radii may introduce artifacts and lead to biased measurements. Moreover, this tight curvature renders SUVs inadequate as biomembrane models. The larger radii of LUVs means that there is no significant curvature at the molecular scale; they can be considered planar for addressing problems at the molecular scale. Multilamellar vesicles (MLVs) can also be considered planar at the molecular level. However, not all the lipid is exposed to the bulk solvent, and this hinders quantitative studies on the interaction between solutes and lipids. Moreover, MLVs scatter light efficiently. LUV size depends on the size of the pores used in the final step of the extrusion method.[28,29] For the work reported herein, the pore size was 0.1 μm, giving rise to LUVs ≈100 nm in diameter. The dipeptide concentration was kept constant (70 μM). Fluorescence intensity was measured with λ_{ex}= 277 nm and λ_{em}=303 nm. The measured fluorescence intensity, I_f, is a balance between the fluorescence intensities of the peptide in bulk aqueous phase (I_W) and those of the peptide inserted in the lipidic matrix (I_L) [Eq. (2)].[30] The weight factors in this balance depend on the partition coefficient, K_p, which was calculated as a fit parameter in a nonlinear regression methodology and is dimensionless (Figure 3). γ_L denotes the molar volume of the lipid used and has the reciprocal units used for the lipid concentration:

$$\frac{I_f}{I_W} = \frac{1 + K_p \gamma_L [L](I_L/I_W)}{1 + K_p \gamma_L [L]}; \; K_p = \frac{[KTP]_L}{[KTP]_W} \quad (2)$$

for which [L] is the lipid concentration; the subscripts L and W refer to the lipidic and bulk aqueous media, respectively.
The fraction of the fluorescence intensity emitted by the peptide incorporated in the membrane (f_L) can be calculated:[30]

$$f_L = \frac{\left(I_L/I_W\right) K_p \gamma_L [L]}{1 + \left(I_L/I_W\right) K_p \gamma_L [L]} \quad (3)$$

Details regarding this methodology can be found elsewhere.[30]
Herein, we studied the lipid-phase influence on D-KTP partitioning to membranes. POPC and POPG bilayers are in liquid crystalline phase, DPPC is in gel phase, and both POPC+cholesterol (33%) and POPG+cholesterol (33%) are in the liquid ordered (l_o) phase. Phosphatidylcholine is the most abundant lipid class in cell membranes, and 33% is the approximate maximum limit for obtaining homogeneous and orderly packed bilayer vesicles with high cholesterol content.

Fluorescence quenching studies: Differential quenching studies were performed to study the location (depth) of D-KTP in LUVs of phospholipids with or without cholesterol (cholesterol was added until a final 33 molar percent relative to the total lipid concentration was achieved). In systems without cholesterol, quenching studies were performed by adding small aliquots of KI (0.1 M) to the LUV suspension with previously added D-KTP. For systems in which cholesterol was present, D-KTP fluorescence (final dipeptide concentration = 70 μM) was quenched by cholesteryl bromide in LUVs of lipid+cholesterol with increasing concentrations of cholesteryl bromide. The bromine atom replaces the OH group at position 3 in cholesterol and is putatively located at the same depth in the lipidic bilayer interface. Data treatment was made as described in the work by Castanho and Prieto.[31] Fluorescence intensity was measured with excitation and emission wavelengths of 277 and 303 nm, respectively.
The simplest model that describes dynamic or static fluorescence quenching leads to linear Stern–Volmer plots:

$$\frac{I_0}{I} = 1 + K_{SV}[Q] \quad (4)$$

for which I_0 and I denote the fluorescence intensities without and with quencher, respectively; K_{SV} is the Stern–Volmer constant and [Q] is the quencher concentration. However, nonlinear Stern–Volmer plots are observed for cases in which there are multiple fluorophore classes in solution (Figure 4).[32] Considering that there is a fluorophore population protected from the contact of the quencher (A) and a population that is accessible to it (B), the Lehrer equation can be used to fit quenching data:[33]

$$\frac{I_0}{I} = \frac{1 + K_{SV}[Q]}{(1 + K_{SV}[Q]) \times (1 - f_B) + f_B} \quad (5)$$

for which f_B is the fraction of total emitted light due to sub-population B:

$$f_B = \frac{I_0^B}{I_0} \quad (6)$$

A more detailed description for the interpretation of quenching results can be found in the work by Castanho and Prieto.[31]

UV/Vis linear dichroism studies: Samples of aligned lipid multilayers (with or without cholesterol) were obtained by slow evaporation of the solvent as described by Castanho and co-workers.[34] The final mole ratios of lipid/D-KTP and lipid/cholesterol/D-KTP were 2.5:1 and 1.5:1:1, respectively.
UV/Vis absorption and fluorescence measurements were carried out as described elsewhere.[34] Namely, second-rank order parameters ($\langle P_2 \rangle$) were obtained from electronic absorption in aligned multi-bilayers. $\langle P_2 \rangle$ is obtained through the dichroic ratio from Equation (7):

$$\frac{\sin(\psi) A_\psi}{A_{\frac{\pi}{2}}} = 1 + \frac{3\langle P_2 \rangle}{(1 - \langle P_2 \rangle) n^2} \cos^2(\psi) \quad (7)$$

$\langle P_2 \rangle$ values obtained from UV/Vis linear dichroism report the orientation of the ^1L_b moment (Figure 1) relative to the bilayer normal.

Acknowledgments

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Keywords: biophysics · fluorescence · lipids · membranes · peptides · vesicles

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