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Ortho-aminobenzoic acid as a fluorescent probe for the interaction between peptides and micelles

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Abstract

Ortho-aminobenzoic acid (*o*-Abz) has been used as a fluorescent probe in internally quenched fluorescent peptides for continuous protease assays. We investigated the fluorescent properties of the probe in order to verify if it can be used to monitor the interaction of peptides with micelles. Abz-aminoacyl-monomethyl amides (Abz-Xaa-$NHCH_3$, where Xaa = Arg, Phe, Leu and Glu) were synthesized. Quantum yield, spectral position, anisotropy and lifetime decay were analyzed in the presence and absence of sodium dodecyl sulfate (SDS) micelles. Significant changes in the fluorescence parameters were observed for Abz-Arg-$NHCH_3$ in comparison to Abz-Glu-$NHCH_3$, indicating a strong electrostatic component in the compound's interaction with the negative charged micelles. The change in fluorescence parameters, observed when the probe is bound to hydrophobic amino acids Abz-Phe-$NHCH_3$ and Abz-Leu-$NHCH_3$, is probably due to insertion of those compounds into micelles. Abz-$NHCH_3$ fluorescence is less affected by the presence of micelles, indicating that the occurrence of interaction is dependent on the properties of the amino acid to which the fluorophore is attached. The quenching data with acrylamide confirmed these results. Titration curves allowed the estimation of association constants between Abz compounds and SDS, according to a single partition model. Although the results cannot be strictly applied to the titration with charged compounds, it was verified that the association constant for the isolated Abz-$NHCH_3$ is significantly lower than those for Abz-Phe-$NHCH_3$ and Abz-Leu-$NHCH_3$. It is concluded that the Abz group is a sensitive and convenient fluorescent probe to monitor peptide binding to amphiphilic aggregates. That conclusion is supported by measurements with the peptide Abz-Leu-Arg-Phe-NH_2.

Keywords: Peptide fluorescent probe; Peptide synthesis; Peptide-lipid interaction; *Ortho*-aminobenzoyl amino acids

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1. Introduction

Ortho-aminobenzoic acid (Abz) is a fluorescent molecule that has been used in internally quenched fluorescent peptides, which are employed for continuous protease assays. Placed in the amino terminal of these peptides, the Abz fluorescence is quenched by an acceptor group bound to the carboxyl end. The cleavage of these peptides increases the fluorescence due to the separation of the fragments containing the donor and acceptor groups [1–8]. Besides the use in enzymatic assays, the fluorescent properties of Abz could give information about the local environment in which the peptide is inserted. Such an approach has been applied to tryptophan-containing peptides and is very useful in verifying whether these peptides partition in vesicles and membranes [9,10]. Monitoring the tryptophan fluorescence parameters is used to detect the interaction of peptides with model membranes, as well as the structural changes in peptides accompanying their insertion into the lipid phase [11]. Those studies provide information about the possible modulation effects of the lipid phase on the peptides biological activity [12].

The aim of the present work is to check whether *o*-Abz is a convenient probe to be employed in the study of the interaction between peptides and amphiphilic aggregates. It could be used in cases where an intrinsic fluorophore like tryptophan is not present in the peptide. Its small size is likely to affect less the structure and the function of the peptide, compared to larger probes, including the naturally occurring tryptophan. Furthermore, its high quantum yield, long lifetime and the possibility of being excited at wavelengths in the near UV (see Table 1) make it easy to handle in fluorescence experiments. We performed a study of the fluorescent properties of Abz bound to the α-amino group of α-carboxyl monomethyl-amidated Arg, Glu, Leu and Phe, as well as in the model peptide Abz-Leu-Arg-Phe-NH_2 (Abz-LRF-NH_2), in the presence of anionic micelles of sodium dodecyl sulfate (SDS). The Abz-monomethyl amide (Abz-$NHCH_3$) was studied as a reference for the behavior of the probe itself in the presence of a water-membrane interface. We measured the fluorescence intensities, anisotropies, spectral position and lifetimes of the compounds in phosphate buffer, both in the absence and in the presence of SDS micelles. To provide more information about the affinity of the Abz-aminoacyl-monomethyl amides to micelles, experiments of fluorescence quenching by acrylamide were also performed.

2. Materials and methods

2.1. Synthesis

2.1.1. Abz-Leu-$NHCH_3$ and Abz-Phe-$NHCH_3$

Leu (or Phe) (1 g) was dissolved in 20 ml of 10% $NaCO_3$ and the temperature adjusted to 4°C. Boc-Abz-*N*-hydroxysuccinimide [6] (10% molar excess) dissolved in 10 ml of dioxane was added to the amino acid solution. After 24 h the reaction mixture was concentrated, dissolved in water, the pH adjusted to 2.5–3 and extracted with ethyl acetate. The organic phase was washed with water, the solvent evaporated and the desired compounds precipitated from petroleum ether. Boc-Abz-Leu-OH (or Boc-Abz-Phe-OH) was coupled to monomethyl amine by the mixed anhydride procedure using isobutyloxycarbonyl-chloride [13]. The resulting Boc-Abz-Leu-$NHCH_3$ (or Boc-Abz-Phe-$NHCH_3$) was treated with TFA in order to remove the Boc protective group.

2.1.2. Abz-Arg-$NHCH_3$ and Abz-Glu-$NHCH_3$

Boc-Arg(Tosyl)-$NHCH_3$ and Boc-Glu(OBzl)-$NHCH_3$ were obtained by mixed anhydride procedure. After Boc was removed by treatment with TFA, Boc-Abz was introduced using Boc-Abz-*N*-hydroxysuccinimide, as above. The resulting Boc-Abz-Arg(Tosyl)-$NHCH_3$ and Boc-Abz-Glu(OBzl)-$NHCH_3$ were treated with anhydrous HF, resulting in the desired compounds.

2.1.3. Abz-LRF-NH_2

The peptide Abz-LRF-NH_2 was synthesized by standard solid phase peptide synthesis procedure. Abz-$NHCH_3$ was obtained by treatment of Boc-Abz-*N*-hydroxysuccinimide with 10-times excess of aqueous solution of monomethyl amine. If necessary the compounds were purified by chro-

matography on silica-gel till more than 97% purity by HPLC.

2.2. Measurements

For the optical absorption measurements a HP spectrophotometer 8452 A was used. Steady state fluorescence experiments were performed in a Hitachi 3010 spectrofluorometer. For time-resolved fluorescence experiments a PTI spectrometer which operates based on the stroboscopic optical boxcar technique was used [14]. Light pulses were provided by an N_2 laser pumping a dye emitting at 636 nm. A frequency doubling crystal generated light at 318 nm used for the excitation of Abz compounds. Decay profiles were fitted to exponential curves, and the quality of the fit was judged by the analysis of the statistical parameters reduced-χ^2 and Durbin-Watson, and by the inspection of the residuals distribution.

Samples contained Abz-aminoacyl-monomethyl amide or Abz-$NHCH_3$ at the concentration of 10^{-5} M, in phosphate buffer 0.01 M (pH 7.4). Experiments were performed at a temperature of 22°C. Excitation wavelength was set at 310 nm in steady state measurements. For lifetime measurements the excitation and emission wavelengths were 318 nm and 420 nm, respectively.

SDS was obtained from Sigma Chemical Co. (St. Louis, MO) and used without additional purification. Micelle stock samples were prepared suspending the amphiphilic at the concentration of 1.0 M in phosphate buffer 0.01 M (pH 7.4). In the titration experiments, small aliquots of the SDS stock suspension were added to the sample solution containing the Abz compounds.

A 2.0 M stock solution of acrylamide (Sigma; used without further purification) was prepared in phosphate buffer 0.01 M (pH 7.4). In the quenching experiments, aliquots of this solution were added to the samples containing Abz-aminoacyl-monomethyl amide. Whenever necessary, corrections due to dilution, and blank and micelles scattering were applied.

3. Results and discussion

It is known [1] that Abz in water has an absorption band with maximum centered at 310 nm, and an emission band with a maximum at 398 nm. The reference compound Abz-$NHCH_3$ presents a more pronounced Stokes shift and the emission maximum is located at 414 nm (Table 1). The wavelengths of maximum emission of the Abz-aminoacyl-monomethyl amides in water are around 415–418 nm and the absorption maxima are red shifted to 314 nm. The kinetics of the fluorescence decay of all compounds obey a monoexponential process and the lifetime values showed a dispersion around 8.0 ns. The errors in the lifetime for each compound, obtained from the fits of the experimental curves, are possibly underestimated and the lifetime values for the different compounds are relatively constant, within a 10% variation range. The quantum yields of Abz-$NHCH_3$ and of *o*-Abz are similar. Compared to these, the changes in quantum yields for the other compounds are more pronounced, decreasing by 20–40% (Table 1). An interesting question is raised about the mechanisms responsible for a decrease in quantum yield without affecting the lifetimes of the compounds. At the present, we are not able to offer an explanation for that fact and further studies are necessary.

In the presence of excess concentration of SDS, compared to those from the Abz compounds, we observed changes in the Abz fluorescence intensity, wavelength of maximum emission, lifetime and anisotropy. This general trend, however, was not observed for Abz-Glu-$NHCH_3$. Fig. 1 is representative of the spectral changes that occurred in the fluorescence of all compounds, but Abz-Glu-$NHCH_3$, due to the presence of SDS mi-

Table 1
Optical parameters (spectral position of absorption and emission bands, quantum yield and lifetime) for Abz compounds in phosphate buffer 0.01 M (pH 7.4)

Compounds	λ_{max}^{abs} (nm)	λ_{max}^{em} (nm)	Q.Y.	τ (ns)
Abz	310	396	0.60	8.4 ± 0.1
Abz-$NHCH_3$	310	414	0.58	7.5 ± 0.1
Abz-Leu-$NHCH_3$	314	416	0.48	8.4 ± 0.1
Abz-Phe-$NHCH_3$	314	418	0.47	7.5 ± 0.1
Abz-Glu-$NHCH_3$	314	416	0.44	8.0 ± 0.2
Abz-Arg-$NHCH_3$	316	415	0.35	8.1 ± 0.3

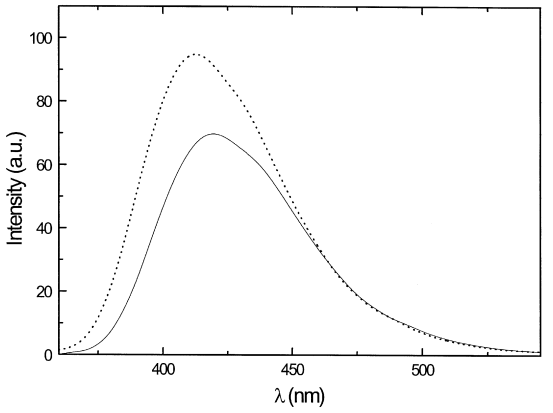

Fig. 1. Emission spectrum of Abz-Phe in phosphate buffer 0.01 M (pH 7.4) (continuous line) and in the presence of SDS (dotted line). Concentration of SDS 50 mM, excitation wavelength 310 nm.

celles. The results agree with a previous report [15] where it was shown that the fluorescence of *o*-Abz increases in intensity and is blue shifted in the presence of cetyltrimethylammonium bromide (CTAB) micelles. Changes in the fluorescence of the Abz-compounds are similar to those observed for the tryptophan fluorescence when the residue moves from aqueous to lipid phase [9–11,16,17]. The blue shift of fluorescence emission is not so evident as that observed for tryptophan, meaning that the electronic states involved in the fluorescence of the Abz-compounds are less affected by the polarity of the medium, compared to those of tryptophan.

The increase in intensity results from a decrease in the non-radiative rate of decay of the excited state. Concomitant to that, we observed higher values for the experimental lifetimes, meaning that a new environment is being sensed by the Abz group. The results can be interpreted as originating from the passage of the fluorophore to the non-polar phase of the SDS aggregates, away from the aqueous medium. The increase in the compounds' fluorescence anisotropies in the presence of SDS, is consistent with the interpretation that the insertion of the fluorescent group into the micelles causes a restriction to the movement of the Abz derivatives.

The modifications in the fluorescence parameters are not the same for the different Abz compounds (Table 2). Greater changes were observed for Abz-Arg-$NHCH_3$ and minor modifications were detected for Abz-Glu-$NHCH_3$. Furthermore, the interaction of Abz-$NHCH_3$ with SDS micelles is much less strong than that verified for the neutral and positive amino acids attached to Abz. In what follows we try to get a better understanding of the different behavior of the Abz-compounds in the presence of SDS micelles.

3.1. Acrylamide quenching

Other evidence of the distinct behavior of the different Abz-aminoacyl-monomethyl amides in the presence of SDS micelles came from the experiments of fluorescence quenching by acrylamide. We monitored both the intensity and the decay profile of Abz fluorescence upon addition of acrylamide, both in aqueous medium and in the presence of SDS micelles.

In aqueous medium we observed that acrylamide quenches the fluorescence of all compounds, and that the Stern-Volmer plots, obtained from the fluorescence intensities as a function of the concentration of acrylamide, are linear (Fig. 2a). Time-resolved experiments showed that in the presence of the quencher, the fluorescence decay curves of the Abz compounds are still fitted to monoexponential curves. The Stern-Volmer plots obtained from lifetime values, like the plots from intensities, are linear (Fig. 2b), and, for each compound, the angular coefficients from both plots are roughly similar, suggesting that the quenching is dynamic in nature (Table 3). The Stern-Volmer constants obtained from static flu-

Table 2
Variation in fluorescence intensity (I), anisotropy (A) and lifetime (τ) for Abz compounds in the presence of SDS (ratio [SDS]/[Abz-aminoacyl-monomethyl amides] = 4000/1, I_o is the fluorescence intensity in the absence of SDS)

Compounds	I/I_o	A	τ (ns)
Abz-$NHCH_3$	1.23	0.004	8.5 ± 0.2
Abz-Leu-$NHCH_3$	1.68	0.017	9.3 ± 0.1
Abz-Phe-$NHCH_3$	1.63	0.017	8.9 ± 0.2
Abz-Glu-$NHCH_3$	1.03	0.005	7.6 ± 0.2
Abz-Arg-$NHCH_3$	1.86	0.022	10.3 ± 0.3
Abz-LRF-NH_2	2.45	0.028	ND

Table 3
Constants (K_{SV}) from Stern-Volmer plots for the quenching of Abz fluorescence by acrylamide, in phosphate buffer 0.01 M (pH 7.4) and in the presence of SDS (ratio [SDS]/[Abz-aminoacyl-monomethyl amides] = 4000/1)

Compounds	Static $(M)^{-1}$			Dynamic $(M)^{-1}$			k_q $(Ms)^{-1}$
	Buffer	With SDS	Ratio	Buffer	With SDS	Ratio	
Abz-$NHCH_3$	45.1 ± 0.8	32.3 ± 0.3	1.4	30 ± 2	30 ± 2	1.0	4.0×10^9
Abz-Leu-$NHCH_3$	28.7 ± 0.2	13.5 ± 0.3	2.1	26 ± 2	13.7 ± 0.2	1.9	3.1×10^9
Abz-Phe-$NHCH_3$	26.1 ± 0.2	14.0 ± 0.2	1.9	19.5 ± 0.8	10 ± 2	2.0	2.6×10^9
Abz-Glu-$NHCH_3$	28.2 ± 0.3	24.3 ± 0.3	1.2	25 ± 2	23.7 ± 0.9	1.1	3.1×10^9
Abz-Arg-$NHCH_3$	31.3 ± 0.5	15.8 ± 0.1	2.0	25 ± 2	12 ± 2	2.1	3.1×10^9
Abz-LRF-NH_2	26.9 ± 0.4	10.2 ± 0.1	2.6	–	–	–	–

Static and dynamic constants obtained from intensity and lifetime measurements, respectively. Column ratio obtained from Stern-Volmer constants in buffer and in the presence of SDS.
Last column refers to bimolecular constant k_q.

orescence are slightly higher than the dynamic ones, suggesting that some static quenching may be occurring, except in the cases of Abz-Leu-$NHCH_3$ and Abz-Glu-$NHCH_3$. It has to be noted, however, that the errors involved in lifetime experiments are higher than those yielded by intensity measurements, which may preclude a definite conclusion about the occurrence of static quenching.

Results of the K_{SV} constants, obtained from intensity and lifetime measurements (Table 3), indicate a higher rate of quenching for Abz-$NHCH_3$ compared to those of the Abz-aminoacyl-monomethyl amides. That is in agreement with a quenching mechanism dependent on the collisions between fluorophore and quencher, which are regulated by the diffusion of the molecules in the medium. A bimolecular rate constant, k_q, proportional to the diffusion coefficient, can be derived from the lifetimes measured in the absence of the quencher (τ_o), and the Stern-Volmer constants obtained from the lifetime plots ($k_q = K_{SV}/\tau_o$). The highest value of k_q was obtained for the smallest compound, Abz-$NHCH_3$ (Table 3), in accord with its larger diffusion coefficient compared to Abz-aminoacyl-monomethyl amides, resulting in a higher rate of collision between quencher and fluorophore. A comparison with tryptophan can be done. It was observed [18] that the bimolecular constant for the pair tryptophan-acrylamide was 5.9×10^9 M^{-1} s^{-1}, a value larger than that obtained for the pair Abz-

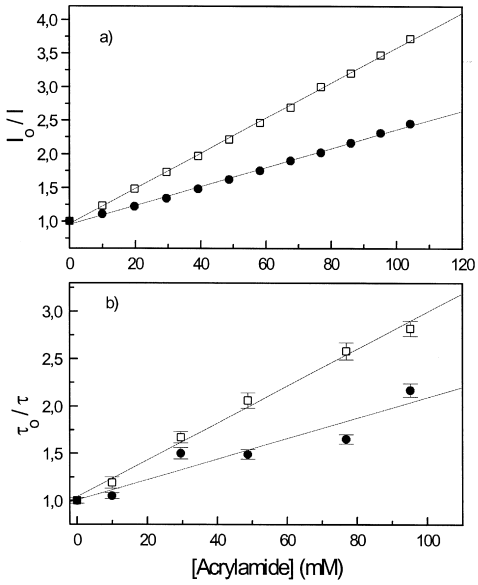

Fig. 2. Stern-Volmer plot for the quenching of Abz-Phe fluorescence by acrylamide in phosphate buffer 0.01 M (pH 7.4) (□) and in the presence of SDS (●). Abz-Phe concentration 1×10^{-5} M, SDS concentration 40 mM. (a) Plot from intensities measurements. (b) Plot from lifetime measurements.

$NHCH_3$-acrylamide. If the diffusion coefficient of both fluorophores are comparable, the difference in bimolecular constants could be originated by the lower efficiency of Abz fluorescence quenching by acrylamide.

When SDS micelles were present in the medium, the extent of quenching decreased. The Stern-Volmer plots were still linear, and the low-

ering in quenching indicates a diminution in the rate of collisions between the fluorophore and quencher. The ratio between the Stern-Volmer constants in water and in the presence of SDS, calculated as a mean value between those obtained from the intensities and lifetime plots, is equal to 2.0 for Abz-Arg-$NHCH_3$, Abz-Phe-$NHCH_3$ and Abz-Leu-$NHCH_3$ (Table 3). For Abz-Glu-$NHCH_3$ and Abz-$NHCH_3$, this ratio was only 1.15 and 1.2, respectively. The above results can be interpreted as an indication that the accessibility of the quencher to the fluorophore decreases due to the insertion of the Abz-aminoacyl-monomethyl amides into the non-polar region of the micelles. The effect is higher for Abz-Arg-$NHCH_3$, Abz-Phe-$NHCH_3$ and Abz-Leu-$NHCH_3$ and is very small for Abz-$NHCH_3$ and Abz-Glu-$NHCH_3$. The results from the quenching experiments are in agreement with the qualitative differences between the compounds presented previously in Table 2.

3.2. Titration curves

Changes in Abz fluorescence intensity were followed at increasing concentrations of SDS. The intensity continuously increases with the addition of SDS (Fig. 3). A plateau is reached for Abz-aminoacyl-monomethyl amides, indicating a saturation, representative of the occurrence of interaction between the compounds and the micelles. Association constants were obtained according to a model in which we consider the interaction between Abz-aminoacyl-monomethyl amides and SDS on a molecule to molecule basis. As a result the association constant (K_a) comes from the equation

$$K_a = \frac{[P_b]}{[P_f][M_f]} \quad (1)$$

where P_b and P_f are the concentrations of Abz-compound bound and free, respectively, and M_f is the concentration of free micelles. The concentration of bound compound was determined from the fluorescence spectra, measuring the intensity at 400 nm at different concentrations of amphiphilic. From Eq. (1), in the approximation that

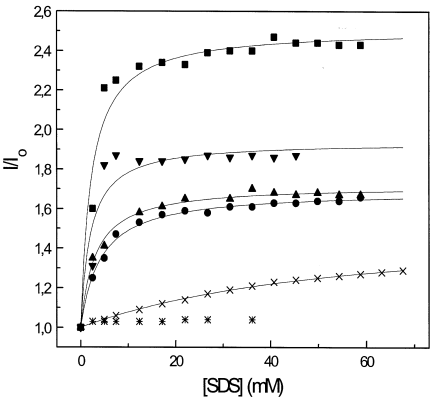

Fig. 3. Titration curves for Abz compounds. Phosphate buffer 0.01 M (pH 7.4). Concentration of Abz compounds: 1×10^{-5} M. Solid curves obtained from fit to Eq. (2). (■) Abz-LRF-NH_2; (▼) Abz-Arg-$NHCH_3$; (▲) Abz-Leu-$NHCH_3$; (●) Abz-Phe-$NHCH_3$; (×) Abz-$NHCH_2$; (∗) Abz-Glu-$NHCH_3$.

the concentration of free SDS is the total concentration, the intensities (I) at 400 nm are related to the total concentration [M] of SDS by

$$\frac{I}{I_0} = 1 + \frac{K_a[M]}{1 + K_a[M]}\left(\frac{I_{max}}{I_0} - 1\right) \quad (2)$$

where I_0 is Abz fluorescence intensity without micelles and I_{max} is the maximum Abz intensity of fluorescence, corresponding to a very high concentration of micelles. The titration curves were fitted to this expression by a non-linear least square method, where K_a and I_{max} were the adjustable parameters.

Although a reasonable fit was obtained for Abz bound to Phe and Leu, and for Abz-$NHCH_3$, the data for Abz-Arg-$NHCH_3$ could not be well fitted with Eq. (2). At the very low initial concentrations of SDS, below the critical micellar concentration (in the conditions of our experiments, a value of 8 mM for the CMC was obtained, from ESR measurements, with spin labeled lipids probing the SDS aggregates, data not shown here), the changes in the fluorescence intensity of Abz-Arg-$NHCH_3$ are noticeably higher than those predicted by Eq. (2). The bad quality of the fit, in this case, can be due to a strong electrostatic contribution to the interaction, as the micelles are negatively charged and the compound has a net positive charge. As a

result of the electrostatic interaction, the concentration of Abz-Arg-$NHCH_3$ close to the SDS aggregate surface is much higher than the bulk concentration. Those effects were not taken into consideration in the derivation of Eq. (2), as the concentration $[P_f]$ is the bulk and not the surface concentration. The results for Abz-Glu-$NHCH_3$ can also be understood in the light of the electrostatic effects: there are only small changes in the fluorescence parameters of the compound in the presence of SDS micelles, and this can be due to the electrostatic repulsion that prevents the approximation of the negative residue to the micelles, avoiding the insertion of the compound into the non-polar core of the micelles.

Despite the poor adjustment for Abz-Arg-$NHCH_3$, determination of the equilibrium constant K_a, from Eq. (2), shows that the extent of SDS interaction is quite low for Abz-$NHCH_3$ compared to that of Abz-Phe-$NHCH_3$ and Abz-Leu-$NHCH_3$ (Table 4). For the neutral molecules, the values of the association constants are realistic, because there are no electrostatic effects contributing to the interaction. In general terms, one may say that the strength of the interaction follows the order: Abz-Arg-$NHCH_3$ > Abz-Leu-$NHCH_3$ > Abz-Phe-$NHCH_3$ > Abz-$NHCH_3$ > Abz-Glu-$NHCH_3$.

It was previously observed [15] that the interaction of Abz with micelles was dependent on the charge of the surfactant. The interaction was strong with the cationic CTAB, weak with the neutral *p-tert*-octylphenoxy-polyoxyethylene ether (TX100) and non-existent with the anionic SDS. As Abz was in the acidic form, those results could be understood in terms of the electrostatic effects present in the interaction. As the aim of this work is to check the use of Abz as a probe for the study of peptides, we adopted the electrically neutral compound Abz-$NHCH_3$ as a reference for the properties of the chromophore alone, eliminating the electrostatic effects present with Abz. We could then observe that the interaction between Abz-$NHCH_3$ and SDS micelles is weak, due to the small hydrophobicity of the compound. In the Abz-aminoacyl-monomethyl amides examined here, the α-carboxyl of the amino acids bound to Abz was also amidated, and electrostatic effects that could be attributed to the acidic terminal group were eliminated. Thus, the ionic, hydrophobic or hydrophilic character of the compounds came from the aminoacyl residue, which is ultimately responsible for the existence or not of peptide-SDS micelles interaction.

Table 4
Equilibrium constants for the association of Abz compounds with SDS micelles

Compounds	K_a $(mM)^{-1}$
Abz-$NHCH_3$	0.016 ± 0.001
Abz-Leu-$NHCH_3$	0.33 ± 0.03
Abz-Phe-$NHCH_3$	0.24 ± 0.01
Abz-Arg-$NHCH_3$	0.49 ± 0.18
Abz-LRF-NH_2	0.44 ± 0.10

3.3. The tripeptide Abz-Leu-Arg-Phe-NH_2

The fluorescence parameters of the tripeptide Abz-L-R-F-NH_2 in aqueous medium were similar to those for Abz-aminoacyl-monomethyl amides. When SDS micelles are present in the medium, the fluorescence of Abz bound to the tripeptide follows the same trend demonstrated by Abz-aminoacyl-monomethyl amides (Table 2). The anisotropy obtained in the presence of excess SDS was higher than those yielded by the other Abz-compounds, reflecting the higher restriction to movement of the larger tripeptide, in the interior of the micelles. Experiments with acrylamide showed that the extent of quenching in water was similar to those presented by the Abz-amino acid compounds. The ratio of the Stern-Volmer constant in water to that in the presence of SDS is equal to 2.6, meaning a more pronounced decrease in quenching due to the presence of SDS, than those verified for the other compounds. It suggests a stronger interaction between the peptide and the micelles compared to Abz-aminoacyl-monomethyl amides.

The titration curve obtained with the addition of SDS micelles shows some characteristics that were present in the titrations, both with the charged Abz-Arg-$NHCH_3$ and with the neutral compounds Abz-Phe-$NHCH_3$ and Abz-Leu-$NHCH_3$ (Fig. 3). The behavior of the tripeptide

seems to result from the combined effects of the component residues. There are large changes in the Abz fluorescence in the pre-micellar concentrations of SDS, as observed with Abz-Arg-$NHCH_3$, due to the electrostatic effects. In the micellar phase, the electrostatic interaction increases the concentration of peptide near to the surface of the micelle, followed by the insertion of the peptide into the inner core of the micelle, promoted by the hydrophobic residues Leu and Phe. Due to the electrostatic effects, there was no exact fit of the experimental data to the curve describing the interaction with SDS, according to Eq. (2). The association constant obtained is equal to 0.44 mM^{-1}, a value higher than those for the neutral Abz-aminoacyl-monomethyl amides. However the values cannot be directly compared because, similar to the case of Abz-Arg-$NHCH_3$, the concentration $[P_f]$ in Eq. (1) is the bulk and not the surface concentration of the peptide.

4. Conclusions

When considering the possibility of the use of an extrinsic probe to monitor the interaction of a peptide with model membranes, one must take into account, among other factors, the extent to which the probe itself contributes to the interaction. For example, it has been recently shown that steady state and time resolved fluorescence of indole and methylindole are strongly affected by the presence of the surfactant Brij-35 (poly(oxyethylene)23lauryl ether), which forms micelles in aqueous media [19]. Modifications of the fluorescence parameters, as a function of the surfactant concentration, allowed the determination of an equilibrium constant equal to 2.6×10^4 M^{-1} for the association between the micelles and 3-methylindole molecules. As indole is the aromatic group of tryptophan, this result shows that the residue plays an important role in the interaction of peptides with lipids, giving a positive contribution to the insertion of peptides into the non-polar region of micelles and vesicles.

In the present work we focus on the fluorescent properties of the probe aminobenzoic acid attached to amino acids, in the presence of acidic SDS micelles. The increase in the fluorescence intensity and anisotropy, and the blue shift of the maximum of fluorescence emission, indicates that the compounds Abz-Arg-$NHCH_3$, Abz-Phe-$NHCH_3$ and Abz-Leu-$NHCH_3$ interact with micelles. No such changes were observed for Abz-Glu-$NHCH_3$, meaning that this compound does not interact with the SDS aggregates. The experiments in the presence of acrylamide allowed the identification of collisional dynamics as the predominant process of quenching, and clearly confirmed the difference between the various compounds used here, concerning their interaction with the micelles. Furthermore, all the results indicated that the Abz-$NHCH_3$ molecule has small affinity for the SDS micelles.

Additional information was obtained from the titration curves. The results allowed the discrimination between Arg and the neutral amino acids Phe and Leu. It was possible to verify that there is a strong electrostatic component in the interaction. Being positively charged, Abz-Arg-$NHCH_3$ is attracted to the micelles, resulting in a high association constant due to the large peptide concentration close to the micelle. On the other hand, Abz-Glu and SDS presents a repulsive interaction, resulting in a very low Abz-micellar association constant. When the electrostatic effects are absent, as in Abz-Phe-$NHCH_3$ and Abz-Leu-$NHCH_3$, intermediate values of K_a were registered. Those values should be representative of the hydrophobic effects which drive these residues from water to the less polar environment of the micelles core.

It should be stressed that the Abz-$NHCH_3$ molecule shows small affinity to the non-polar phase, its association constant to SDS micelles being equal to 16 M^{-1}, one order of magnitude lower than those observed for Abz-Phe-$NHCH_3$ and Abz-Leu-$NHCH_3$. Furthermore, the value of the constant is two orders of magnitude lower than that obtained for indole interaction with Brij-35 micelles [19]. Hence, contrary to the tryptophan residue, the fluorescent group Abz attached to a peptide will not be the group responsible for the peptide-amphiphilic aggregate interaction, if it occurs.

Parallel to the low affinity of Abz-$NHCH_3$ to the less polar region of micelles, we found that, in

the Abz-aminoacyl compounds, the fluorescence parameters of the bound Abz are sensitive to the insertion of amino acids into that medium. Thus, monitoring these parameters we can obtain information about the peptides' partition into the hydrophobic micelle core, driven by the amino acid residues. We can then conclude that the fluorescent group Abz is a very convenient probe for the study of peptides–hydrophobic aggregates interactions. The results for Abz-Leu-Arg-Phe-NH_2 are illustrative of the use of Abz as a fluorescent probe for the interaction of a tripeptide with SDS micelles. An extension can be conjectured, in which the probe can be used to study the interaction of peptides with model and natural membranes.

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