Research article

Optical properties of (3-(acetamidomethyl)phenyl)boronic acid and its interactions with selected sugars

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Abstract: *(3-(Acetamidomethyl)phenyl)boronic acid (3AAPBA)has at pH 7 absorbance maximum at 270 nm with molar absorbance coefficient 516 $M^{-1}cm^{-1}$. 3AAPBA exhibits weak fluorescence with maximum at 297 nm and quantum yield 0.062 ± 0.001. Fluorescence decay is monoexponential and the lifetime is 2.05 ± 0.01 ns. Interactions of 3AAPBA with selected sugars were studied by absorbance, steady-state and time-resolved fluorescence measurements. At pH 7 fluorescence of 3AAPBA is quenched only by fructose (with quenching constant 67.9 M^{-1}) and to some extend by galactose. Addition of these two monosaccharides causes also changes of absorbance spectra of 3AAPBA. Acid-base dissociation of free 3AAPBA and its esters with sugars was studied by absorbance and steady-state fluorescence measurements in pH range from 4.5 to 11.00. Esterification of phenylboronic acid derivatives by sugars leads to increased acidity of them. In case of 3AAPBA the obtained values of pK indicate that affinity of studied sugars towards it can be ordered as follows: fructose > galactose > glucose > maltose > lactose > sucrose. At pHs higher than pK the fluorescence decays turn to biexponential with additional shorter component in lifetime which we propose to attribute to anionic form of 3AAPBA or its esters.*

Keywords: *(3-(Acetamidomethyl)phenyl)boronic acid, quenching constant, pK, lifetime.*

Introduction

There is a great interest in phenylboronic acid (PBA) derivatives since more than two decades because of their ability to bind diols. Phenylboronic acids form quickly and reversibly five or six membered cyclic esters with cis-1,2-diol or 1,3-diol compounds respectively in aqueous solutions [1,2]. Sugars like glucose or fructose are diols and therefore they can form esters with phenylboronic acids derivatives. As PBA derivatives are fluorophores the interactions of them with sugars induce changes in their UV-VIS and fluorescence spectra. This creates

a possibility to use PBA derivatives as recognition element in the construction of optical sensors for sugars.

PBA derivatives could be used as a recognition element in optical glucose sensors offering possibility of continuous measurements by semi-invasive or non-invasive methods [1, 2, 3]. One of the main problems of using PBA derivatives for glucose sensing is their selectivity since other diols are present in blood (fructose, L-lactate) [1]. The affinity of fructose and L-lactate to bind with monoboronic acid derivatives is much greater as compared with that of glucose [4, 5].

Sugar sensing can be carried out by measuring fluorescence emission intensity, fluorescence lifetime, color changes and other. Usually fluorescence emission of boronic acid derivatives is quenched by sugar presence [6-8]. To describe the mechanism of quenching time-resolved measurements ought to be done to evaluate the influence of quencher on receptor fluorescence lifetime [8]. For 3-amino phenylboronic acid it was found that fluorescence lifetime is independent on glucose concentration indicating static quenching [9,10]. For N-phenylboronic acid derivatives of 1,8-napthalimide the fluorescence lifetime increases upon sugar binding although the fluorescence of the probe is quenched [8] indicating that more than one process is involved in quenching mechanism. The changes of fluorescence lifetimes of such PBA derivatives offers possibility to use them for lifetime-based sensing system [8]. The advantage of such sensing mode is that fluorescence lifetime is mostly independent on the probe concentration [11]. For PBA derivatives in which signal is occurring due to photoinduced electron transfer or intramolecular energy transfer the increase of fluorescence is observed upon sugar binding [6,7,11]. PBA derivatives connected with some dyes exhibit change in absorbance spectra in VIS range after binding sugar by boronic group [12]. They can be used as colorimetric sensors for saccharides [1, 3].

Main difficulty with PBA derivatives is to obtain a sugar-dependent spectral change at physiological pH. Many of them show sugar-dependent spectral changes only at pH 8 or higher [1, 3]. At acidic and neutral pH boronic group is in neutral form and the boron atom is in trigonal planar sp^2-hybridization and is an electron-deficient Lewis acid. At alkaline pH after binding of OH^- ion, an electron-rich Lewis base is formed and boron atom is in tetrahedral sp^3-hybridization stronger binding sugar molecule as compared to the neutral form [13]. Typically, PBA derivatives have pK_a (K_a – dissociation constant) values between 8.7 and 8.9 [13]. At physiological pH they are mostly in neutral form with low affinity towards sugars. The acid-base dissociation of PBA derivatives causes the changes of optical properties of them [14]. For many PBA derivatives absorbance and fluorescence intensity is decreasing with increasing pH [8, 14] and significant decrease is observed when pH is greater than pK_a. Esterification of PBA derivatives leads to increased acidity of boronic group and increase of apparent acid-base dissociation constant (decrease of pK_a) [4]. The decrease of pK_a is correlated with affinity of particular sugar towards boronic group [15].

In our laboratory we are concerned on studying photophysical properties of simple, commercially available PBA derivatives by optical methods [9, 10, 17]. This work presents a study of optical properties of (3-(acetamidomethyl)phenyl)boronic acid and its interactions with sugars.

Experimental

Materials

(3-(acetamidomethyl)phenyl)boronic acid (3AAPBA) ($C_9H_{12}BNO_3$) of 95% purity (Fig. 1) was purchased from Fluorochem (United Kingdom) and was used as received. Glucose and D-fructose (anhydrous, pure p.a.) were purchased from Chempur (Poland), lactose monohydrate (pure) and sucrose (anhydrous, pure p.a.) were purchased from POCH (Poland), D-galactose (anhydrous, pure) and D(+)-maltose monohydrate (pure) were purchased from Sigma-Aldrich (Germany). L-tyrosine (BioUltra, > 99%) was purchased from Sigma-Aldrich (Germany). All other reagents used were of most possible purity. Distilled water was used throughout.

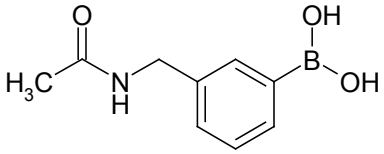

Figure 1. Chemical structure of (3-(acetamidomethyl)phenyl)boronic acid (3AAPBA)

Apparatus and measurements

Absorbance spectra were collected using a Nicolet Evolution 300 spectrophotometer (Thermo Scientific, USA) with 10-mm path-length quartz cells. Steady-state fluorescence measurements were carried out using Fluoromax-4 spectrofluorometer (Jobin Yvon-Spex Instruments S.A., Edison, New Jersey, USA). Fluorescence spectra were measured with 10-mm path-length closed quartz cells. The excitation and emission slits were set at 5 nm each. The increment was set at 1 nm and integration time at 0.5 second.

Fluorescence emission decays were measured with a time-correlated single photon counting apparatus from Edinburgh Instruments Co (UK), equipped with pulsed diode EPLED 280 (Edinburgh Instruments Co, UK) with pulse duration 870 ps, as an excitation light source. Peak wavelength of used diode was 280 nm. Emission wavelength was set at 297 nm. The measurements were carried out with the emission monitored at 90° angle to the excitation. The instrument profile was obtained by replacing the sample with Ludox as a scatter. Data were collected in 1023 channels to 10,000 counts in, and the calibration time was 53 ps per channel.

Fluorescence quantum yield of 3AAPBA (FF) was determined by comparison with standard of known quantum yield [18] using following equation:

$$\Phi_F = \Phi_{st} \cdot \frac{I_F}{I_{st}} \cdot \frac{A_{st}}{A_F} \quad (1)$$

where: Φ_{st} – quantum yield of standard, I_F – integrated fluorescence intensity of 3AAPBA, I_{st} – integrated fluorescence intensity of standard, A_{st} – absorbance of standard solution, A_F – absorbance of 3AAPBA solution.

Tyrosine dissolved in phosphate buffer, pH 7, was used as a standard (Φ_{st} = 0.14, excitation at 275 nm) [19]. Fluorescence was integrated in range 285-350 nm.

All experiments were carried out at 25°C.

Titration of 3AAPBA with sugars

Fluorescence titration of 3AAPBA dissolved in 0.05 M phosphate buffer, pH 7, was carried out as follows. 3 mL of 10^{-4} M 3AAPBA solution was poured into cuvette and titrated with 5 μL portions of 1 M glucose, fructose or sucrose, and with 10 μL 0.5 M of lactose, galactose or maltose. After each addition the cuvette was shaken for a minute and the spectra were collected. The final sugar concentration was about 0.02 M in all series. Absorbance titration was done in the same way but for 10^{-3} M 3AAPBA solution.

Time-resolved fluorescence measurements were carried out for $5 \cdot 10^{-4}$ M 3AAPBA. Titration with sugars was performed in similar way as in case of steady-state measurements. Stock solutions of glucose, fructose or sucrose was added in 10 μL portions, and lactose, galactose or maltose in 20 μL portions to obtain the final concentration of sugar about 0.02 M.

pH titration of 3AAPBA

To study acid-base properties of 3AAPBA, titration curves against pH were measured in 0.05 M buffer solutions: acetate buffer for pH 4.0-5.5, phosphate buffer for pH 6.0-8.5, and carbonate one for pH 9.0-11.0. Concentration of 3AAPBA was 10^{-3} M for absorbance measurements and 10^{-4} M for fluorescence emission measurements. Time-resolved measurements were done for $5 \cdot 10^{-4}$ M 3AAPBA solution. To assay the influence of sugars on acid-base dissociation of 3AAPBA, pH titrations were done for each sugar of concentration 0.05 M.

Results and Discussion

Characteristics of (3-(acetamidomethyl)phenyl)boronic acid

There is no data in literature about optical properties of 3AAPBA in aqueous solution. Basic characteristics of studied compound was done at pH 7. The absorbance spectrum of 3AAPBA is shown on Fig. 2. The absorption band is settled in UV range typical for Π→Π* transition in phenylboronic acid derivatives [20] and shows some vibrational structure with maximum at 270 nm. The dependence of absorbance at the maximum on 3AAPBA concentration is shown on the insert in Fig. 2. It obeys the Beer-Lambert's law up to $4 \cdot 10^{-3}$ M. Molar absorbance coefficient was estimated as 516 $M^{-1}cm^{-1}$ at 270 nm at pH 7, and this value is consistent with that reported for PBA derivatives [20].

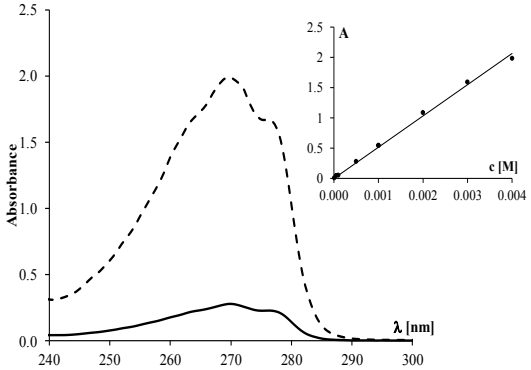

Figure 2. Absorbance spectra of 3AAPBA (solid line – $c = 5 \cdot 10^{-4}$ M, dashed line – $c = 4 \cdot 10^{-3}$ M) at pH 7; insert – dependence of absorbance at 270 nm on concentration

3AAPBA shows narrow emission band with maximum at 297 nm (Fig .3). The excitation spectrum of 3AAPBA is different from its absorbance spectrum and the Stokes shift is low, about 27 nm. The half-width of emission band is about 38 nm (0.514 eV). Fluorescence depends linearly on concentration up to 10^{-4} M (insert on Fig. 3). Quantum yield of 3AAPBA fluorescence was estimated as 0.062 ± 0.001 at pH 7. Fluorescence decay of 3AAPBA is monoexponential at pH 7 with lifetime 2.05 ± 0.01 ns. This value is lower than reported for PBA – 2.84 ns [17] and 3-amino phenylboronic acid – 8.40 ns [10].

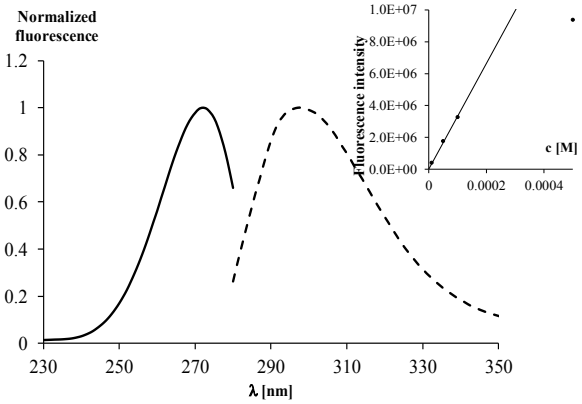

Figure 3. Normalized excitation (left; λ_{em} = 297 nm) and emission (right; λ_{exc} = 270 nm) of 10^{-4} M 3AAPBA at pH 7; insert – dependence of fluorescence emission intensity at 297 nm on concentration

Quenching of (3-(acetamidomethyl)phenyl)boronic acid fluorescence by sugars

Phenylboronic acid and its derivatives are known to bind diols, among them sugars, by esterification of boronic group [1-3]. The esterification of PBA derivatives usually leads to decrease of their fluorescence intensity [1, 8-10, 17].

Quenching of 3-amino phenylboronic acid fluorescence by sugars can be described by Stern-Volmer equation (2) [12]:

$$\frac{I_o}{I} = 1 + K_{SV}[Q] \tag{2}$$

where: I_o – initial fluorescence intensity in the absence of a quencher, I – fluorescence intensity in the presence of a quencher, K_{SV} – Stern-Volmer constant [M^{-1}], [Q] – quencher concentration [M].

The Stern-Volmer equation describes dynamic quenching. It could be also adopted for static quenching when a fluorophore and quencher form a non-fluorescent complex but the constant denoted as K_S has the meaning of an equilibrium constant of complex formation [3, 7]. Application of such model showed good results in case of 3-aminophenylboronic acid [9, 10].

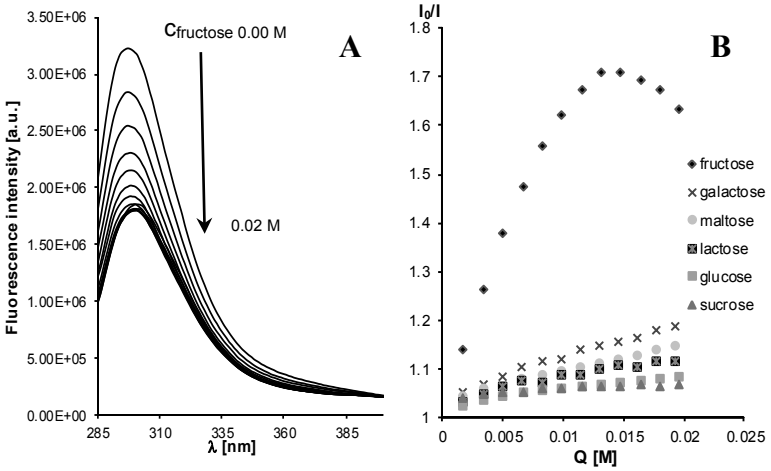

Figure 4. **A** – Fluorimetric titration of 10^{-4} M 3AAPBA by fructose at pH 7, **B** – Results of fluorimetric titration in Stern-Volmer coordinates

Figure 4A shows the results of fluorimetric titration of 3AAPBA by fructose at pH 7. The fluorescence is decreasing with increasing sugar concentration as it can be predicted. The maximum of fluorescence emission is slightly shifted from 297 nm to 300 nm and the half-width of the peak is growing. For other tested sugars the decrease of fluorescence intensity during titration was much smaller and no changes of emission spectra shape were observed.

Quenching of 3AAPBA fluorescence by sugars does not obey Stern-Volmer equation as can be seen on Fig. 4B. Similar deviations from Stern-Volmer equation were observed for 3-aminophenyl boronic acid bound on gold surface covered by cysteine [7] or naphathalene boronic acid derivative [6]. At lower saccharide concentration portion, the quenching follows Stern-Volmer equation

for fructose and the quenching constant can be estimated as 67.9 M^{-1} [7]. For other tested sugars even such estimation cannot be done.

The reason of deviation of 3AAPBA fluorescence quenching by sugars from Stern-Volmer equation is unknown. It could be caused by absorbance changes during titration of 3AAPBA by sugars. Fig. 5 shows the changes of absorbance spectra of 3AAPBA caused by addition of fructose. After fructose addition the absorbance of 3AAPBA is decreasing and the shape of the spectrum is changing. Similar but much less expressed changes were observed for galactose. Addition of other tested sugars had no influence on absorbance spectrum of 3AAPBA at pH 7. Comparison of fluorimetric and absorbance titration results indicates that at pH 7 3AAPBA forms esters only with fructose and galactose in noticeable extend.

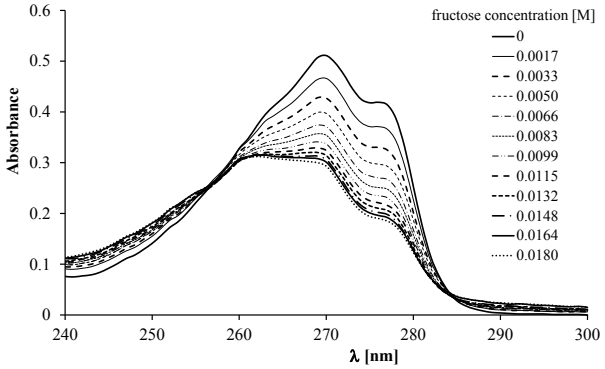

Figure 5. Changes of absorbance spectrum of 10^{-3} M 3AAPBA during titration by fructose at pH 7

The results of time-resolved measurements confirm the observations for steady-state fluorescence measurements. No influence on 3AAPBA fluorescence lifetime was found for glucose, maltose, lactose and sucrose (Tab. 1) up to 0.02 M. The decays were monoexponential with lifetime 2.05 ns.

Table 1. 3AAPBA fluorescence lifetime as the function of sugar concentration at pH 7

glucose			fructose						
c_{glu} [M]	τ_1 [ns]	χ^2	c_{fru} [M]	τ_1 [ns]	f_1	τ_2 [ns]	f_2	$\langle\tau\rangle$ [ns]	χ^2
0.000	2.05 ± 0.01	1.47	**0.000**	2.06 ± 0.01	1.00	-	-	2.06 ± 0.01	1.54
0.002	2.06 ± 0.01	1.50	**0.002**	2.05 ± 0.01	1.00	-	-	2.05 ± 0.01	1.50
0.007	2.05 ± 0.01	1.50	**0.007**	2.03 ± 0.01	1.00	-	-	2.03 ± 0.01	1.58
0.013	2.05 ± 0.01	1.46	**0.013**	2.01 ± 0.02	1.00	-	-	2.01 ± 0.01	1.90
0.016	2.04 ± 0.01	1.51	**0.016**	2.21 ± 0.02	0.81	1.02 ± 0.07	0.19	1.98 ± 0.07	1.41
0.020	2.05 ± 0.01	1.32	**0.020**	2.25 ± 0.02	0.78	1.03 ± 0.06	0.22	1.98 ± 0.06	1.26

For fructose and galactose the decays become biexponential for high sugar concentration (0.016 M for fructose and 0.019 M for galactose) and the second

shorter lifetime about 1.03 ns appeared with slight increase of the longer component to about 2.20 ns. In our previous studies for 3-aminophenylboronic acid it was found that addition of sugar had no influence on fluorescence decays [10] at pH 7 but the range of studied sugar concentrations was less (up to 0.01 M). Esterification of PBA with sugars derivatives is leading to increase of their acidity (decrease of pK_a). The PBA derivatives or their esters in anionic form, when boron atom is in tetrahedral sp^3-hybridization, have different spectral properties than neutral form. For 3-aminophenylboronic acid we assumed that the shorter component of fluorescence decays which appeared when pH was greater than pK_a could be ascribed to its anionic form [10]. We can assume that at very high fructose or galactose concentration the amount of formed ester causing the shift of acidity is high enough that part of 3AAPBA is in anionic form with shorter lifetime.

The observed binding affinity of PBA derivatives with monosaccharides follows the order : fructose > galactose > glucose [6,21]. The strength of PBA derivatives binding to saccharides is determined by the orientation and position of hydroxyl groups in sugar molecule. Only furanose anomers have syn-periplanar pair of hydroxyl groups which can form ester with boronic group due to the steric fit. For fructose the amount furanose form in aqueous solution is about 25%, for galactose 2.5% and for glucose only 0.14% [21]. That is in line with observed affinity of PBA derivatives to monosaccharides.

Generally the observed binding affinity of 3AAPBA with saccharides is less than for 3-aminophenylboronic acid [10]. It could be caused by the presence of a quite big substituent in meta position in 3AAPBA molecule (Fig. 1) which could act a steric hindrance.

Acid-base equilibria of 3AAPBA and its esters

Acid-base equilibrium constants for boronic acid derivatives and their esters with diols can be determined by pH-metric titration or absorbance measurements [16]. The fluorescence of PBA derivatives depends on pH and from this dependence the acid-base equilibrium constant can be also calculated [8]. For 3-aminophenylboronic acid and its esters with sugars the pK values calculated from absorbance and fluorescence measurement were nearly the same [10].

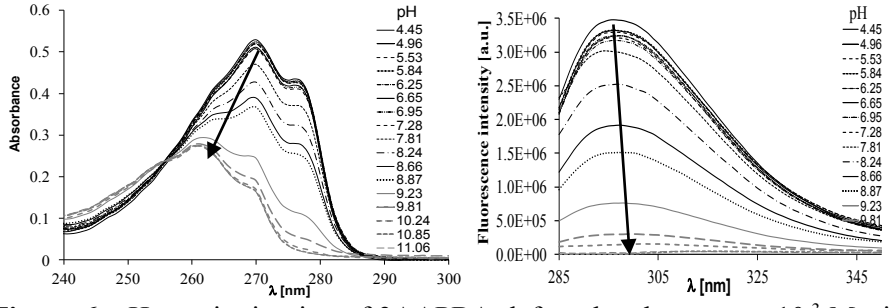

Figure 6. pH-metric titration of 3AAPBA; left – absorbance, c = 10^{-3} M; right – fluorescence, c = 10^{-4} M

Fig. 6 presents the results of pH-metric titration of 3AAPBA recorded by absorbance (left) and fluorescence (right) measurements in pH range from 4.5 to 11.00. With increasing pH absorbance of 3AAPBA is decreasing and hipsochromic shift of the maximum can be observed from 270 nm at pH 4.5 to 260 nm at pH 11. The fluorescence intensity of 3AAPBA is decreasing dramatically with increasing pH and the maximum of fluoresce is shifted from 295 nm to 320 nm. Similar results were obtained for 3AAPBA at the presence of six selected sugars of concentration 0.05 M. Such high concentration of sugars was chosen to shift the equilibrium of esterification to the right side (ester formation). For all sugars the changes of absorbance and fluorescence spectra with increasing pH were similar to that of free 3AAPBA.

Such significant changes of 3AAPBA absorbance and fluorescence spectra with increase of pH indicate that both forms of 3AAPBA neutral with boron in sp^2-hybridisation and anionic with boron in sp^3-hybridisation have different optical properties and spectra. In this case it is impossible to measure independently spectra of neutral and anionic forms of 3AAPBA. To analyse obtained data specific approach must be used.

The obtained pH profile of absorbance was analysed by the method described by Tomsho et al. [22] to find acid-base dissociation constant (pK) for free 3AAPBA and apparent one (pK_a) in the presence of sugars. Briefly, difference spectra were obtained by subtracting from each absorbance spectrum the one for the lowest pH, and from them the total absorbance difference was calculated as a sum of absolute absorbance differences at minimum and maximum. The calculated total absorbance difference (ΔA) was plotted against pH. The plots of ΔA against pH have a sigmoid shape and the value of pH at the point of inflection is equal to pK_a. The values of pK_a can be found by fitting the results to equation (3):

$$\Delta A = \frac{\Delta A_{acid} - \Delta A_{base} \cdot 10^{(pH - pK_a)}}{1 + 10^{(pH - pK_a)}} \tag{3}$$

where: ΔA_{acid} – total absorbance difference of acidic form of APBA; ΔA_{base} – total absorbance difference of basic form of APBA

ΔA_{base} was found as the limiting value of ΔA in the alkaline range and ΔA_{acid} was set as zero. The results are collected in Table 2.

pK_a can be also calculated from fluorescence pH profile using method described by DiCesare et al. [4] by fitting data to equation (4):

$$I = \frac{I_{acid} + I_{base} \cdot 10^{(pH - pK_a)}}{1 + 10^{(pH - pK_a)}} \tag{4}$$

where: I_{acid} – fluorescence intensity of acidic form of APBA; I_{base} – fluorescence intensity of basic form of APBA.

Because the maximum of fluorescence is shifted to longer wavelength with increasing pH, integrated fluorescence intensities in wavelength range 285-340 nm were used in calculations. I_{acid} and I_{base} were found from the plots of total

fluorescence intensity I on pH as the limiting values at low and high pH respectively. The results of calculations of pK for 3AAPBA and pK_a for its esters with sugars from absorbance and fluorescence data are collected in Table 2.

Table 2. Acid-base dissociation constants of 3AAPBA and apparent acid-base dissociation constants of 3AAPBA esters with sugars – comparison of values obtained by different methods

	pK/pK$_a$			
	absorbance		fluorescence	
	from equation (3)	DATAN	from equation (4)	DATAN
3AAPBA	8.80 ± 0.04	8.87	8.76 ± 0.02	8.76
3AAPBA + fructose	6.29 ± 0.02	6.24	6.24 ± 0.03	6.24
3AAPBA + galactose	7.47 ± 0.06	7.48	7.46 ± 0.04	7.45
3AAPBA + glucose	7.94 ± 0.08	8.00	7.92 ± 0.04	7.92
3AAPBA + maltose	8.39 ± 0.06	8.44	8.32 ± 0.06	8.30
3AAPBA + lactose	8.45 ± 0.11	8.50	8.48 ± 0.07	8.48
3AAPBA + sucrose	8.64 ± 0.05	8.87	8.81 ± 0.06	8.80

Spectroscopic methods are generally useful to analyze chemical equilibria. If the components can be obtained in pure form to measure their spectra, or if their spectra do not overlap, analysis of spectroscopic data is generally trivial. To analyse spectroscopic data for systems with overlapping spectra or when components cannot be separated, the chemometric methods can be applied [23-25] basing on principal component analysis. Such approach was used to study multistep acid-base dissociation [24] or DNA-protein interactions from fluorescence measurements [25]. As in case of this study we have the problem of overlapping spectra, we applied DATAN 3.1 program (MultiD Analyses AB, Sweden) [23], which uses the raw experimental data as input for analysis by chemometric methods, to find acid-base dissociation constants. We applied the model of one step dissociation. The results of calculations are shown in Table 2. The values obtained from absorbance and fluorescence data by former methods from equations (3) or (4) respectively and DATAN program are the same. The only exception can be observed for the system 3AAPBA and sucrose for absorbance data.

As it can be seen in Table 2 the pK for 3AAPBA is about 8.8. This value is the same as for phenylboronic acid [18], and a bit lower than for 3-aminophenylboronic acid [12]. The values of apparent pK_a for esters are lower than for free acid and the lowest value is observed for the ester with fructose (about 6.2), which indicates that fructose has the highest affinity towards 3AAPBA. It is consistent with the data for other monoboronic derivatives reported in literature [10, 14]. Esters of phenylboronic acid derivatives with sugars are more acidic than free boronic acid [14]. The shift of pK_a to lower values indicates the affinity of particular sugar to phenylboronic acid derivative. In case of 3AAPBA the studied sugars can be ordered by the increasing acidity of esters as follows: fructose > galactose > glucose > maltose > lactose > sucrose which is the same as for 3-amino phenylboronic acid [10].

Time-resolved fluorescence measurements were carried out for $5 \cdot 10^{-4}$ M 3AAPBA without and with added 0.05 M sugars in the same pH range as in case of absorbance and fluorescence emission measurements. Examples of obtained fluorescence decays parameter are presented in Table 3.

Table 3. Lifetimes of free 3AAPBA and at the presence fructose and glucose in pH range from 4 to 11

pH	τ_1 [ns]	f_1	τ_2 [ns]	f_2	$\langle\tau\rangle$ [ns]	χ^2
			3AAPBA			
3.97	2.04 ± 0.01	1.00	-	-	2.04 ± 0.01	1.82
6.05	2.05 ± 0.01	1.00	-	-	2.05 ± 0.01	1.63
7.98	2.10 ± 0.01	1.00	-	-	2.10 ± 0.01	1.83
9.96	2.22 ± 0.01	0.61	0.31 ± 0.01	0.39	0.74 ± 0.01	1.36
11.00	2.57 ± 0.03	0.19	0.21 ± 0.01	0.81	0.32 ± 0.03	1.18
			3AAPBA + fructose			
3.97	2.23 ± 0.01	1.00	-	-	2.23 ± 0.01	2.23
6.05	2.17 ± 0.01	1.00	-	-	2.17 ± 0.01	2.45
7.98	2.39 ± 0.01	0.38	0.29 ± 0.01	0.62	0.55 ± 0.01	1.53
9.96	2.79 ± 0.04	0.14	0.24 ± 0.01	0.86	0.30 ± 0.04	1.39
11.00	3.25 ± 0.06	0.10	0.26 ± 0.01	0.90	0.27 ± 0.06	1.46

At acidic pH the fluorescence decays are monoexponential for free acid and at the presence of sugars. At pH 4 the lifetime for free 3AAPBA is 2.05 ns and is practically independent on pH up to 8. At pHs greater than pK of 3AAPBA (about 8.8) the fluorescence decays become biexponential and the second shorter lifetime appeared. With growing pH after crossing pK the longer component of decay is increasing and the shorter one is decreasing and in parallel the fractional contribution of the second component is growing. As the result the mean fluorescence lifetime is decreasing to 0.32 ns at pH 11. The shorter component of fluorescence lifetime can be attributed 3AAPBA anion in which the boron atom is in tetrahedral sp^3-hybridization [1].

At the presence of sugars the influence of pH on fluorescence decays is very similar to that of free 3AAPBA. For pHs lower than apparent acid-base dissociation constant pK_a the decays are monoexponential and the lifetime is constant although its value is a bit greater than for free 3AAPBA. For pHs greater than pK_a the decays become biexponential with shorter component which fractional contribution is growing with increasing pH. As the result the mean fluorescence lifetime is decreasing. Table 3 presents results only for fructose but the results for other tested sugars are very similar. We assume that also for 3AAPBA esters with sugars the longer fluorescence lifetime is characteristic for neutral form with boron atom in trigonal planar sp^2-hybridization and the shorter one for anionic one with boron atom in tetrahedral sp^3-hybridization. The influence of pH on fluorescence decays of 3AAPBA and its esters with sugars are consistent with results obtained for 3-amino phenylboronic acid [10].

Conclusions

Optical properties of (3-(acetamidomethyl)phenyl)boronic acid (3AAPBA) are similar to unsubstituted phenylboronic acid. At physiological pH level (pH 7) it is characterized by weak fluorescence with quantum yield 0.062 ± 0.001, low Stokes's shift and lifetime 2.05 ± 0.01 ns. Quenching experiments at pH 7 indicate that 3AAPBA shows weak affinity to form esters with sugars. This is probably caused by the presence of substituent in meta position which could act as a steric hindrance in ester formation. The presence of sugar influenced not only fluorescence of 3AAPBA but also its absorbance spectrum. The results indicated that 3AAPBA is not suitable for sugar sensing.

It is well known that esterification of phenylboronic acid and its derivatives leads to increased acidity of them. The stronger is sugar binding by phenylboronic acid, the greater is the increase of ester acidity. The acid base-base dissociation constant can be evaluated from pH profile of absorbance spectra or fluorescence spectra. The obtained values of pK of 3AAPBA and apparent pK_a for its esters with sugars from absorbance and fluorescence measurements are practically the same showing that the fluorescence measurements are a good tool to study acid-base equilibria of phenylboronic acid derivatives.

With increasing pH the fluorescence and absorbance of 3AAPBA are decreasing. Simultaneously the absorbance maximum is shifting to lower wavelengths and fluorescence maximum to higher ones. This indicates that overall changes of the spectra are caused by overlapping different ones of anionic form of 3AAPBA with boron atom is in sp^3-hybridization and neutral 3AAPBA with boron atom is in sp^2-hybridization. The analysis of fluorescence and absorbance pH profiles using chemometric approach supports this hypothesis. It also allows to find dissociation constants by using raw data without any previous preparation. The chemometric analysis can be a good tool to analyze spectroscopic data while studying acid-base equilibria of PBA derivatives.

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