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Dopamine: Acid-base properties and membrane penetration capacity

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ABSTRACT

Dopamine and 4 related compounds were studied by ^{1}H NMR-pH titrations and a case-tailored evaluation method. The resulting acid-base properties of dopamine are quantified in terms of 3 macroscopic and 12 microscopic protonation constants and the concomitant 3 interactivity parameters. The species- and site-specific basicities are interpreted by means of inductive and shielding effects through various intra- and intermolecular comparisons. The site-specific basicities determined this way are key parameters for the prediction of pharmacokinetic behavior and receptor-binding at the molecular level.

1. Introduction

Dopamine is one of the few biogenic amines of vital importance in the central nervous system. The lack of this small molecular neurotransmitter in the substantia nigra of the ventral midbrain is generally known to be the cause of Parkinson's disease, one of the most feared ailments afflicting today 10 million people in the world. The transport and reactions of dopamine in the central nervous system are all influenced by the number and location of charges and concomitant acid-base properties. The molecule of dopamine contains one amino and two phenolate sites as basic centers. The gross charge of dopamine therefore varies between -2 and +1, and the molecule exists in 8 different forms when the locations of mobile hydrogens are also considered. Owing to the importance of dopamine, its analytical chemistry and biochemistry have been studied extensively. In fact, its acid-base properties have been reported in as many as 13 papers [1–13], and 4 of those contain site-specific protonation constants [2,5,7,8]. Unfortunately, some of these data sets are certainly dubious or highly conflicting (e.g [5]. vs [8].). An additional, practical shortcoming of most reported studies on dopamine protonation is the lack of unbiased pH calibration, which is a typical source of errors in basic media, where the protonation processes of dopamine take place.

In the present work our aim was to determine the protonation constants of dopamine, for the molecule as a whole (macroscopic level), and also, for its basic sites (microscopic level). Since the two phenolate and the amino sites of dopamine protonate in a highly overlapping fashion, microscopic protonation constants are necessary to untangle the protonation pathways. On the basis of the constants determined we also drew conclusions that refer to the membrane-penetrating properties of dopamine. In order to determine the protonation constants we used NMR-pH titrations with unbiased, referenced pH calibration and appropriate evaluation method, including studies on 3 auxiliary model compounds.

Table 1 summarizes the protonation constants reported for dopamine in the literature [1–13]. It is clear from the data that the most dubious value is logK_1: roughly half of the literature sources put this high value at ca. 13, while some report it to be ca. 12 (log henceforth means the base 10 logarithm). This is not surprising, as it is exteremely difficult to determine such a high protonation constant value in aqueous solution, especially with an ionic strength under 1.0 mol·dm^{-3} (this fact brings a great deal of concern regarding the logK_1 value of reference 8 and 13). With a ^{1}H NMR-pH titration method using in situ pH indicators optimized for strongly basic media [14,15] we can, in principle, determine the first macroscopic protonation constant with greater certainty. NMR spectroscopy in this study also has the added benefit of being insensitive to the possible oxidation of dopamine and the carbonate error of glass electrode at highly basic media, compared to other techniques. As for the possible other methods, in UV spectroscopy a slight change in absorbance due to oxidized dopamine distorts the measurement, in pH-potentiometry the contamination of the titrand with air must be thoroughly sought after at high pH. The microscopic protonation constants available in the literature

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Table 1
The protonation constants of dopamine found in the literature. The method of analysis is given as Pot for potentiometry, UV for UV-VIS spectrophotometry, NMR for ^{1}H NMR spectroscopy; *: reference 2 calculated the microscopic protonation constants from the macroscopic protonation constant values found in reference 1. The macroscopic and microscopic protonation constants in the table are given as defined in this work. Values are given in mean and ± standard deviation, where available.

Ref	Method	I (mol·dm^{-3})	t (°C)	logK_1	logK_2	logK_3	log$k_O{}^O$	log$k_O{}^N$	log$k_{ON}{}^O$	log$k_{OO}{}^N$
[1]	Pot	0.1	20	–	10.63 ± 0.07	8.87 ± 0.03	–	–	–	–
[1]	UV	0.1	20	–	–	8.92 ± 0.06	–	–	–	–
[2]	*	0.1	20	–	–	–	10.60	9.44	8.90	10.06
[3]	Pot	0.1	25	–	10.31	8.86	–	–	–	–
[4]	Pot	0.5	20	12.05 ± 0.01	10.60±0.01	9.06 ± 0.01	–	–	–	–
[5]	NMR (in D_2O)	–	27	–	–	–	11	10.4	9.6	10.2
[6]	Pot	1.0	25	–	10.50	8.96	–	–	–	–
[7]	UV	0.1	25	11.98 ± 0.06	10.52 ± 0.02	9.05 ± 0.04	9.00 ± 0.03	–	–	–
[8]	Pot	0.2	25	13.1 ± 0.2	10.41 ± 0.01	8.89 ± 0.01	–	–	–	–
[8]	UV	0.2	25	–	10.40	8.82	10.36	9.39	8.87	9.95
[9]	Pot	0.5	25	–	10.39 ± 0.13	9.09 ± 0.05	–	–	–	–
[10]	Pot	0.6	25	–	10.49	8.93	–	–	–	–
[11]	Pot/UV	1.0	25	12.81 ± 0.05	10.55 ± 0.01	9.05 ± 0.01	–	–	–	–
[12]	UV	0.5	20	12.04 ± 0.02	9.95 ± 0.03	8.61 ± 0.03	–	–	–	–
[13]	Pot	0.2	25	13.10 ± 0.02	–	8.89 ± 0.02	–	–	–	–

are incomplete. In the present work we elucidate the complete microspeciation scheme of dopamine using three strategically chosen auxiliary compounds and a case-tailored deductive method.

2. Materials and methods

2.1. Materials

All chemicals were purchased from Sigma, and were used without further purification.

2.2. NMR spectroscopy measurements

NMR spectra were recorded on a Varian 600 MHz spectrometer at 25 °C. The solvent was an aqueous solution with H_2O:D_2O, 95:5, V/V (1.0 mol·dm^{-3} ionic strength), using DSS (sodium 3-(trimethylsilyl)-1-propanesulfonate) as the chemical shift reference compound. The sample volume was 600 μL, titrand and pH indicator concentrations were ca. 5 mM. In ^{1}H NMR experiments pH values were determined by internal indicator molecules optimized for NMR [14,15], and the water resonance was diminished by a double pulse field gradient spin echo sequence (number of transients = 16, number of points = 16384, acquisition time = 3.33 s, relaxation delay = 1.5 s). The ^{1}H NMR indicators used at 1.0 mol·dm^{-3} ionic strength were imidazole (pH range 5.5–8.9 and 13-), sarcosine (pH range 8.7–11.8), and acetone oxime (pH range 11.4–13.1).

2.3. Data analysis

For the analysis of NMR titration curves (proton chemical shifts versus pH), the software Origin Pro 8 (OriginLab Corp., Northampton, MA, USA) was used. In all regression analyses the non-linear curve fitting option was used with the following function [16]:

$$\delta_{obs(pH)} = \frac{\delta_L + \sum_{i=1}^{n} \delta_{H_1L} \cdot 10^{\log\beta_1 - i\cdot pH}}{1 + \sum_{i=1}^{n} 10^{\log\beta_1 - 1\cdot pH}} \quad (1)$$

where δ_L is the chemical shift of the unprotonated ligand (L), δ_{HiL} values stand for the chemical shifts of successively protonated ligands, where n is the maximum number of protons that can bind to L, and β is the cumulative protonation macroconstant. The standard deviations of logβ values from the regression analyses were used to calculate the Gaussian propagation of uncertainty to the other equilibrium constants derived in the Results chapter.

3. Results

Fig. 1 depicts the macro- and microscopic protonation schemes of dopamine. Macroequilibria (top line) indicate the stoichiometry of the successively protonated ligand and the stepwise macroscopic protonation constants. In the microspeciation scheme the 8 microspecies with their one-letter symbols (a, b . . . h), and the 12 microscopic protonation constants are depicted (k^{pO}, $k_{pO}{}^N$, $k_{pO}{}^{mO}$ . . .). The superscript of k indicates the protonating group while the subscript (if any) shows the site(s) already protonated. The pO, mO and N symbolize the para-phenolate (at carbon position 1 of the aromatic ring), meta-phenolate (at carbon position 2 of the aromatic ring) and amino sites, respectively. Some examples of macro- and microconstants of dopamine are:

$$K_1 = \frac{[HL^-]}{[L^{2-}][H^+]} \quad K_2 = \frac{[H_2L]}{[HL^-][H^+]} \quad K_1K_2 = \beta_2 = \frac{[H_2L]}{[L^{2-}][H^+]^2} \quad (2)$$

$$k^{pO} = \frac{[b]}{[a][H^+]} \quad k^N_{pO} = \frac{[f]}{[b][H^+]} \quad k^{mO}_{pON} = \frac{[h]}{[f][H^+]} \quad (3)$$

Concentrations of the various macrospecies comprise the sum of the concentration of those microspecies that contain the same number of protons. For example:

$$[HL^-] = [b] + [c] + [d] \quad (4)$$

$$[H_2L] = [e] + [f] + [g] \quad (5)$$

The following equations show the relevant relationships between the micro- and macroconstants to our calculations [17]:

$$K_1 = k^{pO} + k^{mO} + k^N \quad (6)$$

$$K_1K_2K_3 = k^{pO}k^{mO}_{pO}k^N_{pOmO} = k^{mO}k^{pO}_{mO}k^N_{pOmO} = \ldots \quad (7)$$

Equation (7) can be written in 6 different, equivalent ways depending on the path of protonation; this relationship can be referred to as Hessian-relationship of protonation constants because of the resemblance to the Law of Hess (i.e. the sum of protonation constant values in log units is constant between the same start- and end-points regardless of the path of protonation). To characterize all of the microscopic bacisities, the introduction and utilization of auxiliary compounds are necessary.

For the elucidation of the microspeciation scheme, first the protonation constant values of catechol and guaiacol were determined (see Fig. 2) to assess the perturbance of a methyl group on the dihydroxyphenyl moiety. This was important since the