Departamentos de Química Inorgánica y Nuclear[1] y Farmacia[2], Universidad Nacional Autónoma de México

Histamine interaction with Zn^{2+} and Cu^{2+} porphyrins

J. J. Recillas-Mota[1], M. J. Bernad-Bernad[2], J. Gracia-Mora[1]

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Dr. Jesus Gracia-Mora, Facultad de Quimica, Universidad Nacional Autónoma de México, México D.F., Ciudad Universitaria 04510, México
jgracia@servidor.unam.mx; jgracia@gmail.com

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Histamine may be present in biological fluids and in pharmaceutical dosage forms such as antiallergenic agents; when in excess, it causes a disorder called histaminosis. Many techniques have been developed to determine the concentration of this compound but the application of such methods is complicated and laborious, requiring expensive equipment and long times. A better alternative is to design chemical sensors. In the work reported here, six metalloporphyrins (Cu^{2+} or Zn^{2+}) with different peripheral groups – benzoate, tosylate and carboxylate – were studied. The stability constants for these compounds were determined with histamine at different temperatures. Histamine is strongly bound to metallic porphyrins containing Cu^{2+} and Zn^{2+}; however, the binding force does not depend exclusively on the metal center. Stabilization of the complex is strongly influenced, in some cases, by the lateral chains of the porphyrin. This possibility implies that this system can be very selective for this biogenic amine.

1. Introduction

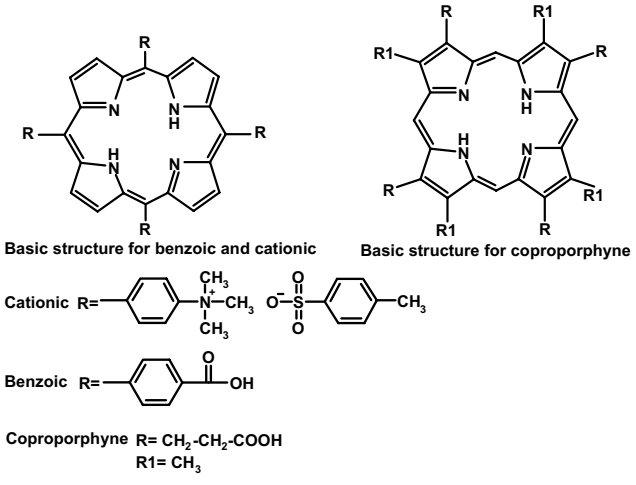
Histamine occurs resides both in the blood of patients with problems such as severe allergic reactions and in foodstuffs such as seafood (Niwa et al. 2000), playing important roles in gastric secretion and the regulation of neuronal functions, such as the sleep-wake cycle and food intake (Beaven 1978; Watanabe et al. 1990). Histamine toxicity leads to a wide variety of symptoms, e.g., urticaria, edema, abdominal cramps, headache, hypotension, palpitation and a burning sensation in the mouth (Taylor 1985).

On the other hand, an immunoglobulin preparation in which a controlled proportion of histamine is bound to the active protein fraction has been used as an anti-allergic treatment (Gushchin et al. 1999). The complex drug was developed on the basis that histamine bound to serum is able to inhibit antigen-induced histamine release from human peripheral blood basophils and rat peritoneal mast cells (Ohnishi et al. 1985). However, the results of excessive administration of histamine are known (Gilbert et al. 1980). Therefore, the histamine content in body fluids such as plasma and urine, and in pharmaceutical dosage forms should be strictly controlled.

Traditionally, this biogenic amine has been quantified using conventional analytical methods (Onal 2007) such as HPLC, gas chromatography, enzymatic techniques, etc. All these methods are very good but they do have disadvantages. For example, they depend on expensive equipment and have long analysis times, both factors that affect their usefulness. As the number of analyses required has increased over time in several complex media, fast, cheap and specific methods are needed.

Among the most novel methods to overcome this problem are chemical sensors (Delmarre and Bied-Charreton 2000; Egashira et al. 1988, 1990; Gupta and Misra 1997; Male et al. 1996; Olafsdottir et al. 1997; Puerro et al. 1999), and some of the most important of these are metalloporphyrin systems (Delmarre and Bied-Charreton 2000; Marques et al. 1999; Mikros et al. 1988; Mizutani et al. 1999; Ogoshi and Mizutani 1999; Puerro et al. 1999; Sirish and Schneider 1999; Zhang et al. 2004). These materials are used as a microelectrode matrix and have the advantage of being highly selective – although they also depend on a complex electronic system.

The high selectivity for the substrate in some supramolecular systems offers an alternative approach to this problem and, in particular, metal porphyrins have appropriate characteristics for this task in that they can coordinate axially to the histamine and thus generate a change in color that is appropriate for a chemical sensor. In addition, spe-



cificity can be introduced into this system through the incorporation of an appropriate lateral group that can also interact with the histamine.

In the work described here the interaction of histamine with three different copper porphyrins and three zinc porphyrins was studied with the aim of finding a molecule able to specifically recognize histamine.

2. Investigations, results and discussion

The complex formation reaction was rationalized in terms of spectral shifts and increased absorption in the porphyrin spectra.

Metalloporphyrin absorption spectra in aqueous solution, pH = 9, at 298 K, with and without histamine, show the same maximum peak at 378 nm, while the peak at around 390 nm shifts to 406 nm when the histamine concentration increases (Fig. 1). These spectral changes suggest interaction between the two species. No significant changes where observed in UV-visible spectra obtained at different temperatures.

To determine the stability constants, non-linear fitting was performed on the models discussed above and the typical curves (binding isotherms) are shown in Fig. 2. These fittings allowed the constant values ΔH and ΔS to be obtained for the different temperatures, and the values are shown in Tables 1 and 2. In the molecular mechanics studies it was found that the lowest energy is obtained with the system consisting of copper coproporphyrin and two histamines, followed by the equivalent zinc complex and then the cationic porphyrin with copper (Figs. 3 and 4).

The copper and zinc complexes of the benzoic porphyrin fitted the 1 : 1 model better. The behavior observed with the other compounds differed from that observed for the benzoic porphyrin, the model taking into account the 1 : 1 and 1 : 2 equilibria being more appropriate for the temperatures studied.

In general, the coproporphyrin generates the most stable complexes. It could be suggested that this stabilization arises due to an interaction through hydrogen bonds between the terminal amine group ($-NH_2$) of the histamine and the carboxylate group of the porphyrin. Additionally, the characteristic groups of the coproporphyrin ($R-CH_2CH_2COO^-$) are more flexible than the benzoate of the benzoic porphyrin and are therefore able to interact more easily with the $R-NH_2$ group.

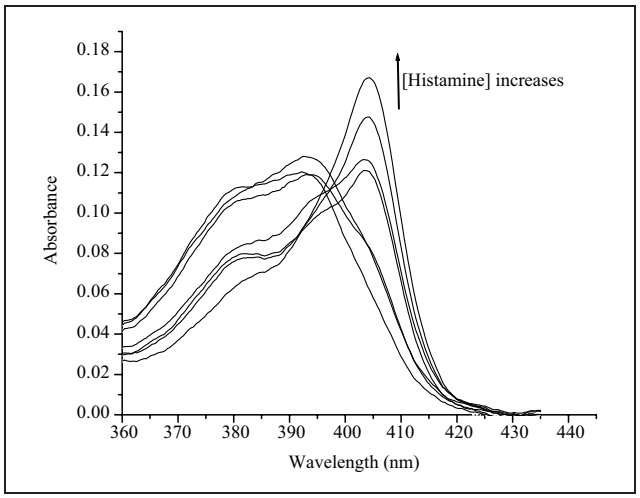


Fig. 1: Typical spectral changes with histamine coordination. Porphyrin benzoic-Cu with histamine

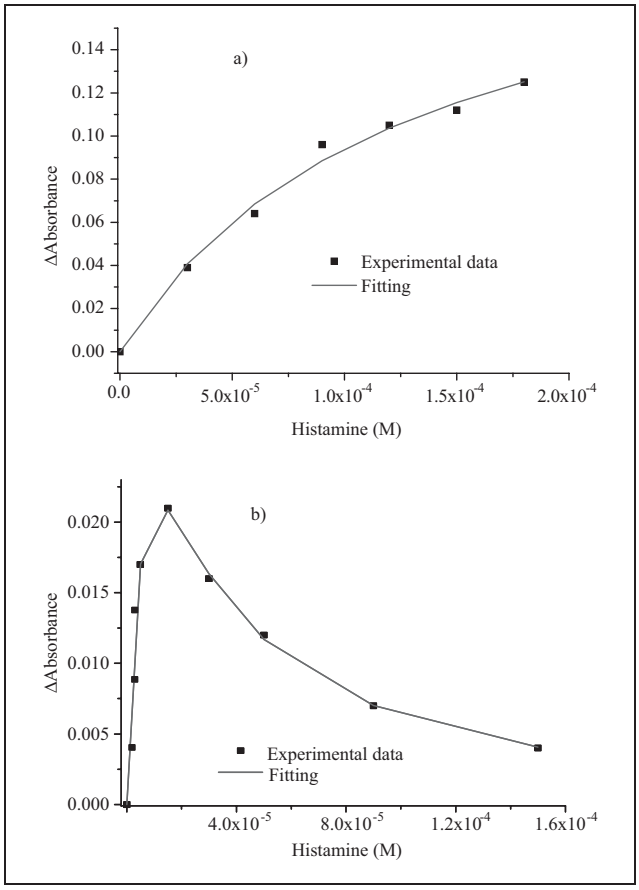


Fig. 2: Typical fitting to (a) 1 : 1 model, Eq. (1) and (b) 1 : 2 model, Eq. (2)

From molecular modeling studies, it is observed (Fig. 3) that the lateral chains of the coproporphyrin ($R-CH_2-CH_2-COO^-$) very easily form a hydrogen bond with the $R-NH_2$ group of the histamine, while the benzoate groups of the benzoic porphyrin are not sufficiently flexible for this process to occur; the approximate distance between the carboxylate and the $R-NH_2$ is 4.5 Å, i.e. far enough that a hydrogen bond is not formed (Fig. 4). Based on this information, it may be supposed that the phenomenon discussed above is a contributing factor to the behavior of

Table 1: Stability constants at different temperatures

T (K)/Benzoic	Copper		Zinc	
	$K_1(M^{-1})$	K_2 (M^{-2})	$K_1(M^{-1})$	K_2 (M^{-2})
293	10728.6		11.21	
295.5	8695.4		10.96	
298	7868.3		10.44	
300.5	6423.0		9.93	
303	5909.3		9.25	
T (K)/Cationic				
293	520.7	11328.2	1734.0	5319.7
295.5	463.6	15000.3	1865.2	5567.1
298	507.0	11603.1	2450.1	3537.8
300.5	432.3	29201.9	3116.1	2116.7
303	434.3	28836.9	2898.4	2267.3
T (K) /Coproporphyrin				
293	0.0359	3.80×10^9	2160	2.72×10^5
295.5	0.0262	3.72×10^9	2670	1.75×10^5
298	0.0389	3.41×10^9	2930	1.50×10^5
300.5	0.0123	3.02×10^9	2670	1.57×10^5
303	0.0387	3.46×10^9	2640	1.49×10^5

Table 2: Thermodynamic parameters for the metalloporphyrins with histamine

Porphyrin	K_1		K_2	
	ΔH_1 (cal/mol)	ΔS_1 (cal/mol K)	ΔH_2 (cal/mol)	ΔS_2 (cal/mol K)
Benzoic-zinc	−3530.6	−7.2		
Benzoic-copper	−10670.7	−18		
Coproporphyrin-zinc	−989.9	+12.4	−3165.4	+13.2
Coproporphyrin-copper	−19813.1	−74	−2246.6	+36
Cationic-zinc	+11025.0	+52.4	−19137.2	−48
Cationic-copper	−1713.7	−6.4	+17939.0	+79.6

the benzoic porphyrin, which fits the 1 : 1 model better than the 1 : 2 system. In contrast, the coproporphyrin, which is much more flexible, can be envisaged as stabilizing two histamine molecules.

When the K_1 values are compared for the series of porphyrins with Zn^{2+}, the compound with the highest association constant is the coproporphyrin, while the complex that has the smallest association constant is that with the benzoic porphyrin. The latter association constant is approximately two orders of magnitude lower than that of the coproporphyrin. The cationic compound, as in the Cu^{2+} series, has an intermediate behavior.

On the other hand, comparing the values of K_2 shows that the complex with the highest stability constant is that of the coproporphyrin and this is approximately 100 and 10^5 times higher than those found for the zinc and copper cationic porphyrins, respectively.

Regarding the complexes with the two cationic porphyrins, although these are structurally similar to the benzoic porphyrins, the former compounds have a differently charged positive functional group. The results obtained for the cationic porphyrins do not fit a 1 : 1 model well but they show a good fit to the 1 : 2 model, which means that this compound coordinates 2 histamine molecules.

The complexes of cationic porphyrins that also contain a benzene ring should, in principle, behave in a similar way to the benzoic metalloporphyrins. However, this is not the case and the stability constant values show intermediate behavior in the series. The values of these constants indicate that they associate to two histamine molecules, but they are less stable systems than those based on coproporphyrin. It is believed that this is due to the lack of flexibility in the cationic group. These findings are consistent with the results from molecular mechanics studies.

Of the six compounds studied, the one with the greatest binding association for histamine is the coproporphyrin derivative with Cu^{2+}, followed by the coproporphyrin with Zn^{2+} (Table 1). These data show that, in general terms, Cu^{2+} forms more stable complexes and this is consistent with some examples found in the literature (Myari et al. 2001; Torok et al. 1998a, 1998b), where it has been reported that histamine has a higher complexation constant with Cu^{2+} than with Zn^{2+} in aqueous solution.

Jencks (1987) pointed out that a favorable ΔH may result from solute-solute interactions or from increased solvent-solvent interactions, which are made possible by the greater solvent order. An unfavorable ΔS suggests that the formation entropy is largely associated with a loss of reagent mobility.

On the other hand, the ΔH and ΔS values (Table 2) are in agreement with the discussion of stability constants. In general, the high and negative ΔH values indicate strong association with histamine and this is characteristic of the formation of a coordination bond. ΔS generally has low values, indicating that the entropy contributions are not very important. This suggests that interactions among the solvent molecules are small compared with the interaction between the reagents. This applies for the benzoic por-

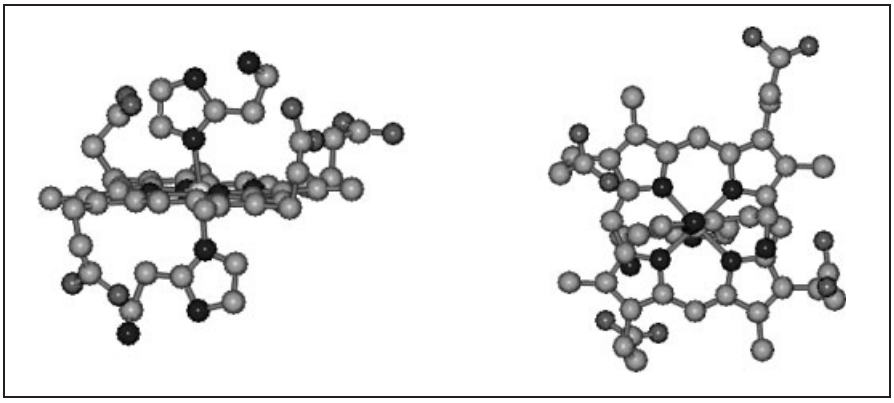


Fig. 3:
Calculated structures for coproporphyrin-histamine

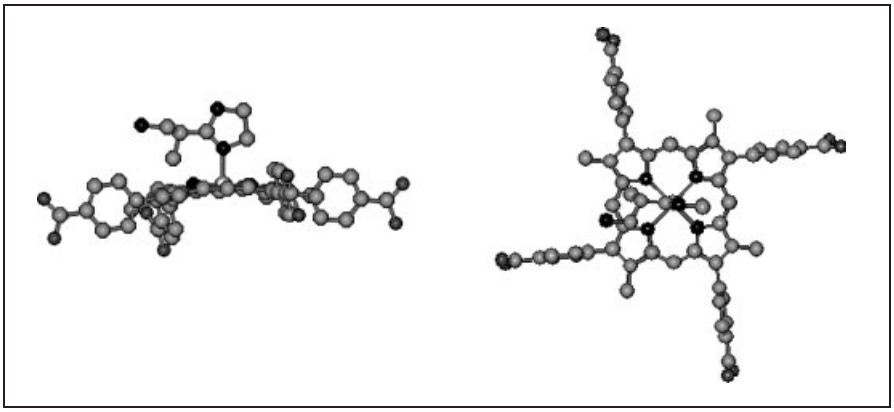


Fig. 4:
Calculated structures for benzoic porphyrin-histamine

phyrin and the coproporphyrin. However, as far as ΔH_1 of the cationic-Zn^{2+} porphyrin and ΔH_2 of its Cu^{2+} complex are concerned, the results show a positive ΔH; this can be attributed to some non-stabilizing interaction due to the positive charge of the ammonium group, since this porphyrin differs from the benzoic system mainly in the nature of the charge. Nevertheless, in these two cases the entropy contribution is favorable, in contrast to the other compounds studied in this work, which are stabilized by the enthalpy factor.

In conclusion, histamine associates strongly with the Cu^{2+} and Zn^{2+} metal porphyrins. However, the force of the association does not depend exclusively on the metal center. The stabilization of the complex is, in some cases, strongly influenced by the lateral chains of the porphyrins. This is the case for the copper coproporphyrin with histamine, since their association constant is much higher than that of the other compounds under investigation, possibly due to the formation of an interaction between the lateral chain of the coproporphyrin and the primary amine of the histamine. In general terms, in the studied systems the entropy effects are not very important and the contributions to the stabilization are provided mainly by the enthalpy factor. It is suggested that this trend is due to the formation of strong interactions. This research suggests that copper coproporphyrin could be a good option for developing a specific chemical sensor to analyze histamine.

3. Experimental

3.1. Reagents

The porphyrins used in this study were 3,8,13,18-tetramethyl-21*H*,23*H*-porphine-2,7,12,17-tetrapropionic acid dihydrochloride (coproporphyrin), 5,10,15,20-tetrakis(4-trimethylammoniophenyl)porphyrin tetra(*p*-toluenesulfonate) (cationic), and 4,4′,4″,4‴-(21*H*,23*H*-porphine-5,10,15,20-tetrayl)tetrakis(benzoic).

These porphyrins and Tris were obtained from Aldrich. The histamine was obtained from Fluka as the free base. The salts $ZnCl_2$ and $CuCl_2$ were obtained from Baker and NaCl from Mallinckrodt. All of these compounds were of AR grade and were used without further purification.

3.2. Determination of stability constants

Porphyrin solutions (8.2×10^{-7} M) were prepared and the first step was metallation in the reaction mixture, an aqueous solution buffered at pH = 9, ionic strength 0.1 M at 298 K. It was found that equilibrium for metallic porphyrin formation is reached in three days. The six complexes that were studied are water soluble at pH = 9 and at a concentration of 8.2×10^{-7} M and self association was not observed.

The direct spectroscopy method was used to determine the stability constants. Porphyrin solutions (8.2×10^{-7} M) were prepared with different histamine concentrations ranging from 0 M to 1.8×10^{-4} M. The solutions were allowed to react for 24 h. The experiments were carried out at the following temperatures: 293 K, 295.5 K, 298 K, 300.5 K and 303 K.

In the determination of the stability constants between the porphyrin complexes and histamine two possible types of behavior were considered: 1 : 1 (K_1) in which one histamine molecule is coordinated axially and the 1 : 2 (K_2) system in which the two axial positions are occupied by two histamine molecules. The calculation of K_1 was carried out by a non-linear fit from the Δabsorbance data for the complex without histamine and with different concentrations of histamine, according to Eq. (1). The value of K_2 was determined with a non-linear fit to Eq. (2).

$$\Delta Abs = \frac{K_1[P]_t\,[H]\,\Delta\varepsilon}{1 + K_1[H]} \quad (1)$$

$$\Delta Abs = K_1[P]_t\,\frac{\Delta\varepsilon_1[H] + K_2\,\Delta\varepsilon_2[H]^2}{1 + k_1[H] + K_2K_1[H]^2} \quad (2)$$

where K_1 and K_2 are, respectively, the equilibrium constants, $[P]_t$ is the total porphyrin concentration, [H] is the total histamine concentration and $\Delta\epsilon$ is the difference in molar absorptivity between the porphyrin with and without histamine. The enthalpy and entropy values were determined according to the Van't Hoff equation.

3.3. Molecular mechanics

The structures of the histamine, each of the six porphyrins and their possible complexes were optimized with Spartan Pro (Wavefunction Inc.), using molecular mechanics with the MMFF force field. The convergence approach was 0.1 kcal/mol Å in energy gradient.

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