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Thermodynamic analysis of amines–lasalocid complex formation

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Abstract

The ionophore lasalocid A selectively transports amines across lipid membranes. Free energy differences of amine–lasalocid complex formation reactions and the activation barriers of the complex transport were calculated by the finite difference thermodynamic integration method. Four catecholamines were treated: serotonin > dopamine > noradrenaline > adrenaline. The concept of transfer selectivity was introduced, combining binding and activation barrier selectivities. The transfer selectivities correlate well with the relative selectivities of the amines under study. © 1997 Elsevier Science B.V.

Keywords: Biogenic amines; Lasalocid A; Amines–lasalocid complexes; Selectivity; Thermodynamic integration; Free energy differences

1. Introduction

The antibiotic lasalocid A (formerly X 537A; Fig. 1) is able to transport metal ions and amines (Fig. 2) [1–6] across lipid membranes.

It is known that the reactions which form the amine–lasalocid complexes occur at the membrane–water interface. Unlike the Ca^{2+} transport, in which the cation is bound by two antibiotic molecules, the transfer of amines is carried out according to a monomeric scheme [2]. Yet, unlike the complexes of inorganic cations [7–14], amines crystallize in an equimolar salt with lasalocid A [15]. According to X-ray data the antibiotic has virtually the same cyclic conformation in all salts.

The ability of lasalocid A to selectively transport catechol-amines across lipid membranes [2] led to this study of the thermodynamics of the amine–lasalocid complex formation reactions. Four typical catecholamines were considered: serotonin > dopamine > noradrenaline > adrenaline (Fig. 2). In the previous paper [16] molecular mechanics technique was used to study the structural fitting between the antibiotic and the four aforementioned amines. Interaction between complexes and water or membrane were ignored. The complex formation reaction was treated as two competing processes: the interaction between amines and lasalocid and the strain arising as a result of the distortion of the conformations of the unbound compounds. The complex stability was evaluated by the 'fitting energy' F_i consisting of the sum of three terms I_i, ΔL_i, and ΔA_i; where I_i is the interaction energy between the antibiotic and the ith amine, ΔL_i is the strain energy of

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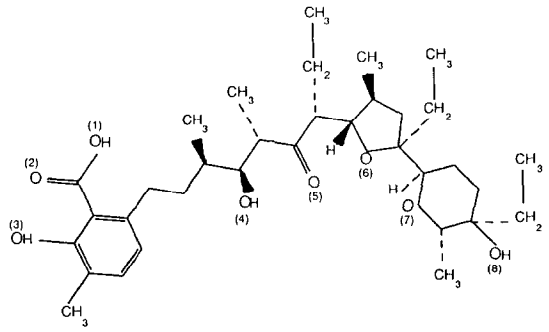


Fig. 1. Structure of lasalocid A.

lasalocid bound to the *i*thamine, ΔA_i is the strain energy of the *i*th amine. The strain energies were calculated as the differences between intramolecular energies of bound and free compounds. All structural and energy parameters were calculated by using Insight and Discover programs of BIOSYM Technologies, San Diego. The energy was represented by the sum of five terms: stretch, bending, torsion, van der Waals and Coulomb. The CVFF potentials of BIOSYM were used.

The cyclic conformation of the antibiotic was taken from Cambridge Data Base [8] and then refined by the energy minimization technique (Fig. 3). The optimal conformations of the amines were calcu-

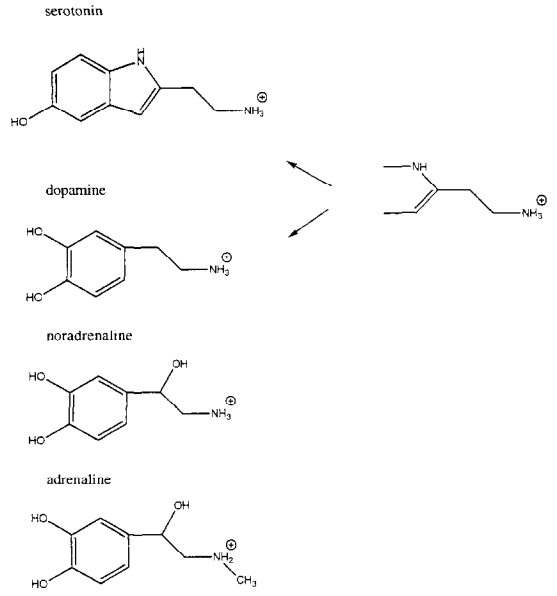


Fig. 2. Structure of amines. The directions of the last three amine mutations are from the top to the bottom of the figure.

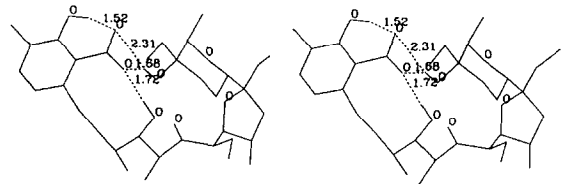


Fig. 3. Stereo drawings of the unbound lasalocid A cyclic conformation. Hydrogen bonds are shown.

lated by varying the torsion angles using the 'rotor' technique of Discover. Then the optimal structure of amines were docked to the cyclic conformation of lasalocid in such a way that the ammonium groups of the amines reached the ligand oxygens of the lasalocid, and that the phenyl rings of the amines overlaid the aromatic ring of the lasalocid. These 'rough' complex structures were refined by energy minimization starting from different torsion angles.

Finally, the favorable structures of the complexes were found. In all the structures the ionophore has cyclic conformation, in which the ligand oxygens are in contact with the ammonium group (Fig. 4). As in the crystal salts [7–15] this structure contains a hydrogen bond between terminal carboxyl and hydroxyl groups (Fig. 3). The molecule has the shape of a cup with polar orifice and interior and hydrophobic exterior. The plane of the aromatic ring is parallel to the entrance plane of the 'cup'. Note that this structure looks as if it had been 'designed' to bind both the ammonium group and the phenyl ring of amines (Fig. 4). For the first three structures the nitrogens of the primary amino group are located in the 'basket' formed by the lasalocid ligand oxygens (Fig. 4a,b). The same pattern was observed in the crystal [15]. In contrast, in the adrenaline complex the methylated amino group is not attached to the ligand oxygens but is located over the carboxyl group of the ionophore (Fig. 4c). The best overlap of the amines and ionophore aromatic rings was observed for the six-membered serotonin ring (Fig. 4a). Obviously this results from the larger size of the serotonin indol ring compared with the phenyl rings of the amines (Fig. 2). It is reasonable to assume that the calculated structures occur during the transfer processes. Apparently the phenyl rings of the amines would be in touch with the aromatic ring of a ionophore that matches the hydrophobic interactions on the membrane–water interface.

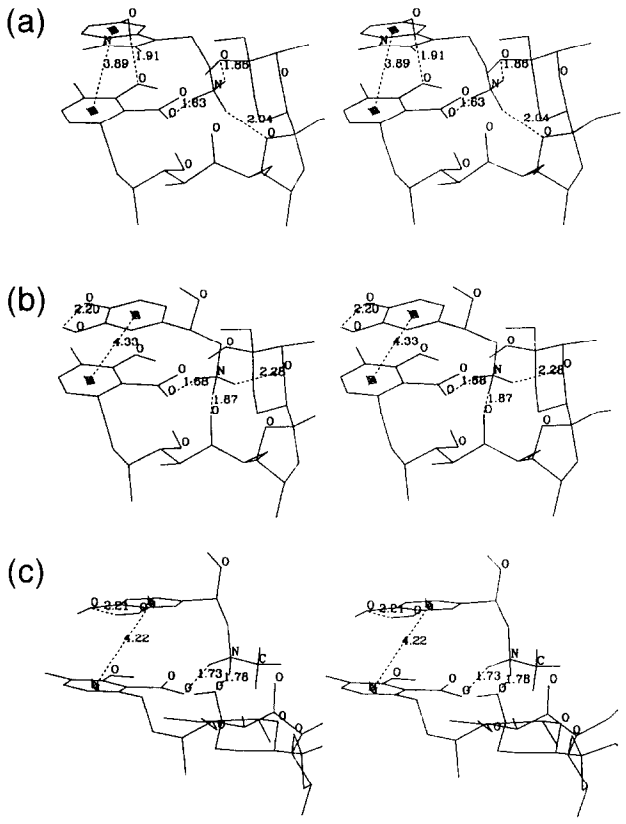


Fig. 4. Typical structures—stereo views—of amine–lasalocid complexes: (a) serotonin; (b) noradrenaline (dopamine is similar); and (c) adrenaline. Distances between ring geometrical centers and hydrogen bonds—except intramolecular lasalocid hydrogen bonds—are drawn.

The F_i values correlate with the permeability coefficients which were measured on lipid membranes doped with lasalocid A [2], but their differences are rather large compared with the relative selectivities within the sequence of the amines [16]. This means that other factors which regulate the antibiotic selectivity, such as the interaction of the complexes with water and membranes, probably exist [17].

In this communication the finite difference thermodynamic integration (FTDI) method [18] was used to study the complex formation reactions. The interactions with water were included in the discussion.

2. Method

FDTI combines the perturbation method (PM) with thermodynamic integration [18]. The main advantage of FTDI is that unlike PM, large changes in free energy can be calculated in fewer steps. The FTDI formalism, implemented in Discover 2.9 package of BIOSYM Technology was used for free energy difference calculations of the reactions of the amines–lasalocid complex formation.

The complex transfer process can be represented in two steps: the complex formation reaction on the membrane–water interface and the complex transfer across an internal zone of the membrane:

$$A^{+}_{w} + L^{-}_{wm} \overset{K}{\Leftrightarrow} \left[(AL)_{wm}\right]_1 \overset{k_m}{\rightarrow} \left[(AL)_{wm}\right]_2 \qquad (1)$$

where A^+ and L^- are amine and lasalocid respectively; w, m, and wm denote the bulk water, internal membrane zone, and first and second interface—1 and 2 in Eq. (1)—respectively; K is the equilibrium constant of the reaction; k_m is the rate constant of the complex transfer across membrane.

The rate of transfer v can be calculated by the following formula:

$$v = [AL]k_m = [A^+][L^-]Kk_m =$$
$$= [A^+][L^-]\exp(-\Delta G/k_B T)\, b \exp$$
$$(-\Delta G^*/k_B T) =$$
$$= [A^+][L^-]b \exp(-(\Delta G + \Delta G^*)/k_B T), \quad (2)$$

where $[AL]$, $[A^+]$, and $[L^-]$ are the respective reagent concentrations; ΔG is the reaction free energy; b is the preexponential factor; and ΔG^* is the activation free energy barrier of the complex transfer across the membrane.

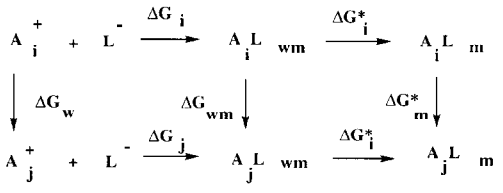
The relative selectivity s_{ij} of the ionophore for amines i and j can be evaluated as the ratio of their rates of transport v_i and v_j at equal concentrations of the amines and ionophore:

$$s_{ij} = v_i/v_j = \exp\Big(\big((\Delta G_j - \Delta G_i) + (\Delta G_j^* - \Delta G_i^*)\big)/k_B T\Big) =$$
$$= \exp((\Delta\Delta G + \Delta\Delta G^*)/k_B T)$$
$$= \exp(\Delta\Delta G_S/k_B T) = \exp(\Delta\Delta G_s/k_B T), \quad (3)$$

where $\Delta\Delta G = \Delta G_j - \Delta G_i$, $\Delta\Delta G^* = \Delta G_j^* - \Delta G_i^*$, and $\Delta\Delta G_S = \Delta\Delta G + \Delta\Delta G^*$.

The ionophore is selective to amine i if $s_{ij} > 1$ ($\Delta\Delta G_S > 0.0$), and vice versa if $s_{ij} < 1$. Let us refer to $\Delta\Delta G_S = \Delta\Delta G + \Delta\Delta G^*$ as the transfer selectivity.

Thus, the ionophore transfer selectivity $\Delta\Delta G_S$ is defined as the sum of the binding selectivity $\Delta\Delta G$ and activation barrier selectivity $\Delta\Delta G^*$. Both of these values can be calculated by the FDTI method so as to satisfy the following thermodynamic cycles [19–21]:



where ΔG_w, ΔG_{wm}, and ΔG_m are the relative free energies of the mutation in water, on the membrane–water interface and inside membrane, respectively.

These cycles require that:

$$\Delta G_j - \Delta G_i = \Delta\Delta G = \Delta G_{wm} - \Delta G_w,$$
$$\Delta G_j^* - \Delta G_i^* = \Delta\Delta G^* = \Delta G_m - \Delta G_{wm}. \quad (4)$$

Thus, the needed $\Delta\Delta G$ and $\Delta\Delta G^*$ values can be obtained from three free energies of the mutation ΔG_w, ΔG_{wm}, and ΔG_m.

The ΔG_w energies can be calculated by the FDTI procedure applied to a water box containing mutated amines. The simulations of the membrane–water interface or internal membrane zone are rather problematic. That is why the complexes were considered to be surrounded by water molecules or in the gas phase, respectively. Some reasoning would justify these approximations. There is no indication that membranes alter the selectivity of lasalocid A. For example, the permeability coefficients measured in lipid bilayer membranes containing lasalocid were forty and ten times higher for dopamine and noradrenaline respectively than for adrenaline [2]. Both two first compounds gave crystalline salts containing equimolar amount of the amine and antibiotic, but all attempts to crystallize the lasalocid salt of the third one were unsuccessful [15]. The shape of the complexes is dominantly defined by the cyclic conformation of the antibiotic which is identical for all structures (Fig. 4; [16]). Most complex polar groups are located on the complex interior. Apparently the hydrophobic chains of lipids are not completely adaptable to the complex shape and contact only partially with the surface of the complexes located inside the membranes. It is believed that non-specific van der Waals interactions of the complexes with internal non-polar membrane chains would not contribute remarkably to the free energy differences, but could restrict the flexibility of the complexes. Probably, the complexes located on the membrane–water interface are completely accessible to water molecules; that is why the ΔG_{wm} energies were calculated like the ΔG_w ones. The amines and their complexes with lasalocid were assumed to be in a cubic water box 21 Å in size, with the periodic boundary conditions. The ΔG_m energies of complexes located inside membrane were obtained by applying the FDTI procedure to the isolated complexes (the gas phase). The tethering forces implemented in Discover package were used

to prevent the dissociation of the complexes. These forces confine the movement of the molecules in MD simulations (see for example [22]) and in this particular case, to some extend, represent the lipid chain influences.

The energies of intra and intermolecular interactions were represented by the sum of five terms: stretch, bending, torsion, van der Waals and Coulomb. The CVFF potentials of BIOSYM were used. The most favorable structures of amines and complexes [16] were taken as the starting points for MD simulations.

Noradrenaline was mutated from dopamine by the conversion of the methylene hydrogen to hydroxyl group and adrenaline from noradrenaline by the conversion of the ammonium hydrogen to the terminal methyl group (Fig. 2). The procedure of serotonin–dopamine mutation was more complicated because of the large structural differences between the indol and phenyl rings of these two amines. Both rings were generated from the 'chimera' resembling the flat aromatic structures (Fig. 2). Fifty intervals of the coupling parameter and six quadrature points in the numerical integration were chosen. The molecular dynamics procedure was run at 300 K with 50 ps of the equilibrium period and 50 ps of the sampling period for each quadrature point.

3. Results and discussions

The free energy differences $\Delta\Delta G_S$, $\Delta\Delta G$, and $\Delta\Delta G^*$ are summarized in Table 1 along with fitting energy differences ΔF calculated earlier [16]. It is possible to compare them with the experimental transfer selectivities, which can be obtained by the Eq. (3)—$\Delta\Delta G_S = k_B T \ln(v_i/v_{i+1})$ ($i = 1, 2$, and 3)—on the basis of the measured transport rates v_i. The permeation of biogenic amines across artificial lipid membranes (bilayer membranes) doped with lasalocid A was investigated by means of electrical conductivity measurements and fluorescence spectroscopy [2]. The v_i/v_{i+1} values obtained from these measurement are as follows: $1.73 < 3.95 < 10.55$. The corresponding $\Delta\Delta G_S$ values are $0.33 < 0.82 < 1.41$ (kcal/mol).

The fitting energy differences ΔF correlate with the experimental transfer selectivities $\Delta\Delta G_S$, but their differences are very large. All binding selectivity values $\Delta\Delta G$ are lower than the ΔF and correlate with them. Thus, FDTI modelling of the amine–lasalocid complex formations in the water phase led to better agreement with the experimental data than just the minimization procedure. Nevertheless, the values $\Delta\Delta G$ for the last two transitions are still rather high, compared with experimental $\Delta\Delta G_S$, especially for the noradrenaline–adrenaline transition. Fortunately, the activation barrier selectivities $\Delta\Delta G^*$ contribute contrary to the transfer selectivity values $\Delta\Delta G_S$, reducing disagreement between the simulated and experimental data. In other words, the less stable complexes quickly traverse the hydrophobic membrane interior. Actually, we can see in Table 1 that the corresponding values $\Delta\Delta G^*$ are negative. At a first glance it could be reasonably explained only for the last noradrenaline–adrenaline transition for which the translocation of the terminal hydrophobic methyl group into the membrane is energetically favorable. The negative value $\Delta\Delta G^*$ for the dopamine–noradrenaline transition could be described on the basis of the strain energy. The dopamine complex is less strained than the noradrenaline one (ΔA term in

Table 1
Energy parameters (kcal/mol) of amine–lasalocid complexes

	$\Delta\Delta G_S^a$	$\Delta\Delta G^b$	$\Delta\Delta G^{*c}$	ΔF^d	$\Delta\Delta L^e$	$\Delta\Delta A^f$	ΔI^g
Serotonin → dopamine	0.45	0.55	−0.10	0.71	−0.70	−4.94	6.35
Dopamine → noradrenaline	1.30	2.31	−1.01	3.32	0.08	2.73	0.51
Noradrenaline → adrenaline	4.37	8.19	−3.82	12.76	−0.82	−0.21	13.28

[a,b,c] Transfer, binding, and barrier selectivities respectively.
[d] Fitness energy differences.
[e,f] Strain energy differences of lasalocid and amines.
[g] Amine–lasalocid interaction energy differences.

Table 1). This strain is remarkably reduced in the gas (membrane) phase, where the complex is more flexible.

Thus, the $\Delta\Delta G_S$ values determining the ionophore selectivity correlate rather well with those calculated from the experimental data [2] and even reproduce the relative selectivities in the row of the amines. Given the simplicity of the reaction scheme, there is no reason to expect better matching between the calculated and experimental values. The ionophore selectivity on the structural level could be described comparing the free energy differences and intramolecular and intermolecular interaction energies (Table 1). The 'massive' serotonin interacts more strongly with lasalocid than other amines (ΔI term in Table 1). The noradrenaline complex is more strained than the equivalent dopamine, which is predominantly determined by the intramolecular interactions of the amines (ΔA term in Table 1). The hydroxyl group attached to the asymmetric carbon of noradrenaline (Fig. 2) restricts its conformational adaptation. This strain is remarkably reduced going from the water to the gas (membrane) phase, where the complex is more flexible (term $\Delta\Delta G^*$ in Table 1). The adrenaline complex differs from the other ones by the high value of the interaction energy (ΔI term in Table 1). This is because the terminal methyl group of adrenaline hinders the interaction of the amino group with the ligand ionophore oxygens (Fig. 4c). The translocation of the methyl group into the membrane essentially compensates these energy losses ($\Delta\Delta G^*$ term in Table 1).

4. Conclusion

The selective ability of lasalocid A (Fig. 1) to transport catecholamines across lipid membranes motivated this work. The thermodynamic of amine–lasalocid complex formation and transport were studied by the FDTI technique. Four cathecholamines were considered: serotonin > dopamine > noradrenaline > adrenaline (Fig. 2).

The transfer process was studied in two steps: the complex formation reaction between the amines and antibiotic at the membrane water interface and the transfer of the complexes across the internal membrane zone. Thermodynamic cycles, which allow to calculate the free energy differences for the complex formation reactions and the transfer activation barriers, were introduced. The transfer selectivity consisting of binding plus activation barrier selectivities was derived from the kinetic equations describing the transfer process. The transfer selectivities correlate well with those calculated from the rates of transport in the sequence of the four amines. The binding affinities correlate with overall selectivities but counteract the transfer activation barriers.

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