Chiral Auto-resolution of L and D-Arginine Mixture using Their Cu(II) diastereomeres Complexes

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Starting from plan-tetragonal structure of α-amino acids (AA) complexes with Cu(II), by interpretation of UV-Vis, IR spectra and X-ray structure, we studied the influence of some stabilization factors to the homo and heterochiral diastereomers configuration and found a possibility of chiral separation (auto-separation) of L and D-Arginine mixture by selective crystallization.

Key words: arginine copper complex, chiral resolution, homochiral and heterochiral diastereomers

As it is known [1], all natural α-amino acids (AA) form with Cu^{2+} very stable chelat complexes of the type [(AA)$_2$Cu]$^{2+}$, involving as donor carboxylic oxygen and α-amino nitrogen atoms, in penta-atomic rings.

The copper hexa-coordination is fulfilled following the geometry of a distorted octahedron: 4 shorter bonds (≈1.9 Å) generated by N and O donor atoms belonging to the AA ligands, and two longer bonds (≈ 2.5Å) generated by water molecule, anions present in solution, or, when it is possible, donors from the laterally catena of the AA (the second coordination sphere).

This coordination system induces important consequences:

-the possibility to form *cis-trans* isomers by the reciprocal position of N and O donor atoms, and *syn-anti* isomers, considering the position of lateral catena against the chelatization plan;

-important influence of the solvent proprieties (polarity, dielectric constant, hydrogen bonding), *p*H, dilution, salinity and the nature of the anion on the solubility of these complexes;

-intra or/and intermolecular interactions between laterally catena brought in proximity by complexation, or between functional groups grafted on these and the complex generator. These interactions can be:

- steric (repulsion or fitting);
- electronic (π-interactions);
- hydrogen bonds;
- electrostatic (attraction or repulsion).

In watery solutions, in a large *p*H range (≈ 6–11) [2], copper complex of arginine presents with guanidine group protonated. In these conditions, the complex formation must arrive at the most stable configuration, determined, specially by:

-greater stability of *trans* isomer compared with *cis* [3,-6];

-the nature of anion [7-9];

-the accommodation of positive charge from the laterally catena, brought in proximity by complexation;

-hydration, hydrogen bonds, dielectric properties of the solution.

Tetragonal structure in the first coordination sphere and ligands' chirality lead at the following configurative variants:

-Homochiral complexes (L,L or D,D) – configuration *trans-syn* or *cis-anti,* that is, structure *trans* in the first coordination sphere requires the setting of the both laterally catena at the same part of the coordination plane (*syn*).The structure *cis*, requires the setting on one and another side (*anti*), figure 1.

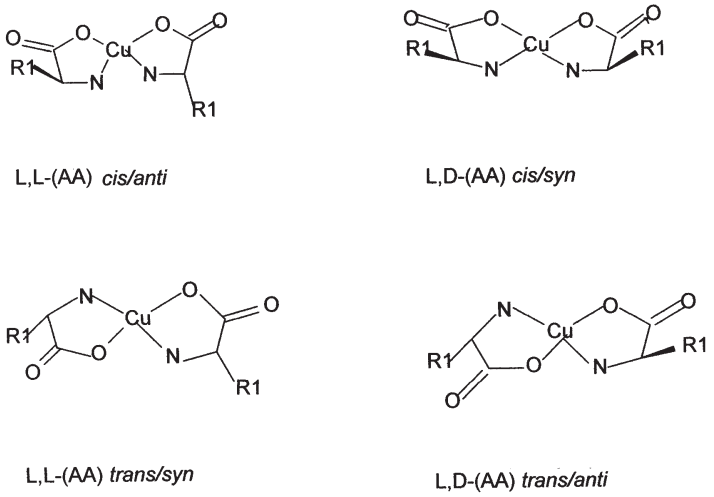
L,L-(AA) *cis/anti*
L,D-(AA) *cis/syn*
L,L-(AA) *trans/syn*
L,D-(AA) *trans/anti*

Fig. 1.Homo and heterochiral diastereomeres of arginine Cu(II) complexes

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-Heterochiral complexes (L,D) – configurations *trans-anti* or *cis-syn.*

The configuration of the formed diastereomers is the consequence of the importance of the contribution of the form *trans* compared with *cis* and *anti* against *syn* at thermodynamic stabilization. The effective reaction conditions, especially, the nature of contra-anion are, also, very important.

If the electrostatic repulsion interactions between laterally positive charged catena are stronger than *trans* stabilization, will form diastereomers which ensure the maximum distance between the charges – *cis-anti* for the homochiral diastereomers and *trans-anti* for heterochiral ones. It is interesting that, in the literature, many papers are talking about the chiral selectivity of copper complexes with AA having ionized groups in laterally catena, but all of them refer to electrostatic attraction between opposite charges [10,11].

The contra-anion can selectively stabilize, intra or intermolecular, a certain configuration (for example, a suitable dianion may stabilize intramolecular *syn* structure).

Of course, these influences can overlap with other having smaller importance.

The configurative differences induce different solubilities and chromatographic behaviors. We can expect, for example, that *trans-anti* diastereomer, having a higher symmetry and a bigger tendency to intermolecular interactions, to be more insoluble, but *cis-syn*, having a smaller symmetry and bigger polarization to adsorb stronger on chromatographic supports.

The aim of our work is to study the possibility of chiral separation of a mixture of L and D arginine following the transformation in diastereomers complexes with copper (II) ion and selective crystallization, and also, to suggest one explanation concerning experimental comportment, through interpretation of UV-VIS, IR spectra and X-ray structure determination.

Experimental part

[L-ArgCuL-Arg]$^{2+}$

20 mmoll L-Arg (3.48 g) were dissolved in 15 mL water. The *p*H is brought at ≈ 7 with HCl 18%. 10 mmoll $CuCl_2$ (1.36 g) was added. The *p*H is corrected at ≈ 7 with Na_2CO_3. The solution was stirred 10 min at 50°C. By evaporation of the solvent at a temperature under 35°C the complex crystallizes and is filtrated and dried in vacuum.

[L-ArgCuD-Arg]$^{2+}$

10 mmoll D-Arg (1.74 g) and 10 mmoll L-Arg are dizolved in 15 mL water. We followed the procedure described above.

[L-ArgCuD-Arg]$^{2+}$/ [L-ArgCuL-Arg]$^{2+}$ 1/1 (25 % D-Arg in a mixture with L-Arg)

15 mmoll L-Arg (2.61 g) and 5 mmoll D-Arg (0.87 g) were dissolved in 15 mL water. It followed on like at the first experiment.

The resulting solution was left to crystallize over night, then it was filtered . The filtered solution was evaporated at half volume and left over night to crystallize. The crystals were filtered. The collected filtrate is diluted with water at 10 mL. In 2 mL from these solution we added 2 mmoll L-Glu (0.294 g) and 1 mmoll of $CuCl_2$ (0.136 g) and the *p*H was corrected at ≈ 7 with Na_2CO_3. It was left over night to crystallize. In another 2 mL filtrate, diluted at 10 mL, we added 2 mmoll D-Glu (0.294 g) and 1 mmoll $CuCl_2$ (0.135 g). The *p*H was corrected at ≈ 7 with Na_2CO_3 and the solution was left over night to crystallize.

.[L-ArgCuL-Arg]$^{2+}$/[L-ArgCuD-Arg]$^{2+}$: 3/1 (12.5 % D-Arg in mixture with L-Arg)

7 mmoll L-Arg (1.218 g) and 1 mmoll D-Arg (0.174 g) were dissolved in 6 mL water. The *p*H was adjusted at ≈ 7 with HCl 18%. We added 4 mmoll $CuCl_2$ (0.544 g) and then stirred 10 min at 50°C, then the solution was left over night at room temperature.

The formed precipitate was filtered . The filtrate was concentrated at half volume and left over night to crystallize, and then, filtered again. In 1 mL filtrate was added 1 mmoll L-Glu and 1 mmoll $CuCl_2$. The *p*H was corrected at ≈ 7 with Na_2CO_3 ,the solution was stirred 10 minutes at 50°C and left over night at room temperature.

We used *pro analyisi* chemicals, the UV-VIS spectra was carried out in watery solution 10^{-3} M. FT-IR spectra was carried out in dry powder. X- ray structure was possible to be determined only for the L,L complex which have enough solubility to obtain good monocrystals. Temperature rising or increasing solubility agents can change the structure and were not used.

Results and discutions

Homochiral complex

The preparation of [L-ArgCuL-Arg]$^{2+}$ produced a bright blue solution. UV-Vis specter presents an absorption maximum at 624 nm. FT-IR spectrum made for dried powder complex, in the range over 600 cm^{-1} is not representative to clarify the *cis* or *trans* structure. Because the large mass of Cu, the characteristic stretch vibrations bands are situated in the range 350–500 cm^{-1} [12].

By slow evaporation of the solvent, it was possible to obtain crystals for the X-ray structure determination. In this respect, the found structure, in conformity with literature data [9], (fig. 2) clarify, in part, the assumptions presented before. The structure in the first coordination sphere is *trans.* This means that the *trans* energetic stabilization is more important than *anti* one, so the electrostatic intramolecular repulsion is not decisive, or there is another mechanism. When the structure in the first coordination sphere is *trans,* the laterally catena for the L,L diastereomers are *syn* situated, which favors the intramolecular interaction with dianion carbonate (used at complex preparation) and grater solubility, but in the L,D diastereomers situation, the catena are in *anti* position, which favors intermolecular interactions and smaller solubility.

Heterochiral complex

A fine blue suspension was obtained, having a very low solubility in water. UV-Vis spectrum presents a characteristic absorption maximum at 618 nm, different from that of homochiral complex. FT-IR spectrum , being in the range over 600 cm^{-1}, gave not information about *cis* or *trans* structure in the first coordination sphere.

Because of the very low solubility, it was not possible to obtain monocrystal suitable for X-ray structure determination, but, taking in consideration the chirality of the both involved legends, the configuration of the complex must be *trans/anti* (double stabilization). The higher symmetry of this complex, the reduced tendency (may be the steric impossibility) to intramolecular interaction with the carbonate dianon favors the intermolecular interaction, aggregation and precipitation of the complex.

D-Arg separation (25 % in mixture with L-Arg)

From the solution left over night at room temperature, crystallized a blue-mauve precipitate, like [L-ArgCuD-Arg]$^{2+}$, having the absorption maximum in UV-Vis spectrum at the same position (618 nm). UV-Vis spectrum

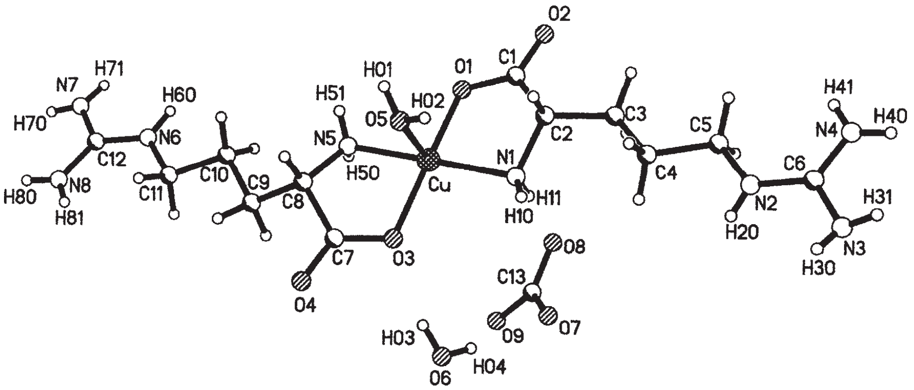

Fig. 2. X-ray structure of the complex{[Cu(arginine)$_2$H$_2$O]CO$_3$. H$_2$O}

Table 1
CRYSTAL DATA AND STRUCTURE REFINEMENT

Identification code	ineda	
Empirical formula	$C_{13} H_{32} Cu N_8 O_9$	
Formula weight	508.01	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P 2_1	
Unit cell dimensions	a = 8.3467(2) Å	α= 90°
	b = 12.0931(2) Å	β= 94.887(2)°
	c = 10.1911(2) Å	γ = 90°
Volume	1024.92(4) Å^3	
Z	2	
Density (calculated)	1.646 Mg/m^3	
Absorption coefficient	1.132 mm^{-1}	
F(000)	534	
Crystal size	0.27 x 0.12 x 0.07 mm^3	
Theta range for data collection	2.62 to 31.00°	
Index ranges	-12<=h<=12, -17<=k<=17, -14<=l<=14	
Reflections collected	27674	
Independent reflections	6467 [R(int) = 0.0371]	
Completeness to theta = 31.00°	99.8 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	1.00000 and 0.91464	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	6467 / 98 / 352	
Goodness-of-fit on F^2	1.012	
Final R indices [I>2sigma(I)]	R1 = 0.0236, wR2 = 0.0535	
R indices (all data)	R1 = 0.0268, wR2 = 0.0541	
Absolute structure parameter	0.002(6)	
Largest diff. peak and hole	0.327 and -0.345 e.Å^{-3}	

of the filtrate have the absorption maximum at the same wave length like [L-ArgCuL-Arg]$^{2+}$. IR spectrum of the precipitate is the same with that of L-ArgCuD-Arg]$^{2+}$. Polarimetric measurements of AA separated from the precipitate, indicate no optic activity, that is, there is a racemic mixture of L and D-Arg.

Based on the results of a Japanese team [10,11], if D-Arg was present in the final filtrate, when treating this with L-Glu, [D-ArgCuL-Glu], with very low solubility, should precipitate. Since this thing did not happen, we concluded that D-Arg was not present. On the other way, adding D-Glu in same conditions, [L-ArgCuD-Glu] make precipitate.

Chiral purification (12.5% D-Arg in a mixture with L-Arg)

The sample which contained only 12.5 % D-Arg conceived like chiral purification one, led at the separation of a precipitate [L-ArgCuD-Arg]$^{2+}$ having the same UV-Vis spectrum as the complex prepared at the second experiment. The test with L-Glu on the filtrate showed that it did not contain important quantity of D-Arg.

X-ray structural characterization of {[Cu(arginine)$_2$ H$_2$O]CO$_3$•H$_2$O}

Homochiral structure (L,L) of the complex {[Cu(arginine)$_2$H$_2$O]CO$_3$•H$_2$O} was confirmed by X-ray measurements. This compound presents a tetragonal-pyramidal structure at central Cu^{2+} ion (fig. 2). The structure is similar with that of the complex [L-ArgCuL-Arg]CO$_3$•H$_2$O [9].

In the literature there are, also, other copper complexes with arginine, structurally characterized which present a tetragonal-pyramidal structure of Cu^{2+} ion, having instead of carbonate different contraanions, like, for example, izophtalate [7], 2,6-pyridindicarboxilat [7], or 1,2,4,5-benzentetracarboxilat [9].

In the first coordination sphere, Cu^{2+} is *trans* coordinated by two arginine molecules which function like bidentat ligands through α-amino nitrogen (Cu-N1: 1.992 Å, and Cu-N2: 1.972 Å) and carboxylic oxygen (Cu-O1: 1.947 Å and Cu-O3: 1.972 Å). These bonds are approximately identical with that from from [9] (Cu-N: 1.977-1.991Å and Cu-O: 1,947-1.966 Å).

The coordination sphere of copper is completed with one water molecule in the pyramidal position, which is bonded through one electron pairs belonging to oxygen atom (Cu-O$_{water}$: 2.289 Å). This bond Cu-O$_{water}$ from the second coppers coordination sphere is the shortest known in the literature connected with this complex type containing arginine. In the similar complex [9], the bond

Cu-O$_{water}$ is 2.319 Å , while in homochiral complexes of the type L,L or D,D with Cu^{2+} *trans* or *cis* coordinated, this bond Cu-O$_{water}$ is situated in the range 2.382 – 2.526 [7]. The AA Arg (arginine) has a terminal guanidinium group protonated, the positive charge being delocalized .

The bonds carbon –nitrogen (C6-N2: 1.326 Å, C6-N3: 1.352 Å, and C6-N4: 1.325 Å) are approximately equal and have values included between that of simple bond C-NH$_2$ and that of double bond C=NH.

The positive charge is equilibrated by the negative charge of the carbonate anion CO_3^{2-}. The complex is stabilized by formation of a great number of hydrogen bonds between the α-amine, ω-amine groups and amidic groups NH with carboxylic oxygen, carbonyl group and water (N-H···O: 2.852-2.871Å), but also through formation of hydrogen bonds between OH groups from water and the oxygen from carbonyl or carboxyl groups (O$_{water}$-H···O: 2.676-2.871Å).

Conclusion

The results consideration corroborated with literature data proves that the electrostatic repulsion effect between guanidinium groups from laterally catena do not conduct to achieve *anti* structures (*cis/anti* for L,L and *trans/anti* for L,D), so it do not prevail over *trans* stabilization effect . Very probable, carbonate dianion favors the achievement of *syn* configuration because of the electrostatic attraction and hydrogen bonds. This effect, coupled with the greater stability of *trans* isomers, leads to the preferred formation of diastereomeres *trans/syn,* for the homochiral complexes (L,L) and *trans/anti* for the heterochiral ones (L,D).

The L,D isomer (*trans/anti*) has a much lower water solubility than L,L (*trans/syn*) being possible the chiral separation by selective crystallization of D-Arg from the mixture with L-Arg like diastereomers complexes with Cu^{2+} cation.

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