Investigation of the Self-Assembly Pathway of Pentanuclear Helicates by Electrospray Mass Spectrometry

Annie Marquis-Rigault, Annick Dupont-Gervais, Alain Van Dorsselaer and Jean-Marie Lehn*

Abstract: The self-assembly of the pentanuclear double helicates **Hh**, **Ha** and **He** from the corresponding oligobipyridine strands **Lh**, **La** and **Le** and Cu^{I} ions has been investigated by NMR and electrospray mass spectrometry (ESMS). Whereas **Hh** is assembled rapidly (in less than 20 min), **He** (about 20 h) and especially **Ha** (about 60 h) form much more slowly. The rate decreases strongly with increasing steric bulk of the substituents in the 4,4′-positions on the bipyridine units; this indicates that the search processes (wrapping, unwrapping) that lead to the final helicate are strongly hindered by the size of the substituents. The ESMS data give information about the species present in solution under different conditions and allow the formulation of possible formation pathways, which may involve, in particular, helicates of hairpin type.

Keywords
bipyridine ligands · helicates · kinetics · mass spectrometry · self-assembly

Introduction

Helicates are a class of inorganic compounds formed by self-assembly of two or three ligand strands in an helicoidal fashion around several metal ions.[1-8] Double helicates containing up to five metal cations (usually copper(I) or silver(I)) surrounded by two oligo-2,2′-bipyridine (oligobpy) ligands have been investigated (Fig. 1).

The formation of the di- to tetranuclear double helicates is usually a fast process, in which the thermodynamic equilibrium is quickly reached (less than 5 min). However, helicates of high nuclearity (e.g. pentahelicates) bearing bulky groups in the 4,4′-positions of the bipyridine moieties only reach thermodynamic equilibrium in hours or days, depending on the nature of the substituents R (Fig. 1). Here we investigate and describe the kinetics of formation of three pentahelicates with R = H, $CONEt_2$ and COOEt (Fig. 1) by NMR and electrospray mass spectrometry (ESMS) in order to gain information on their formation pathway.

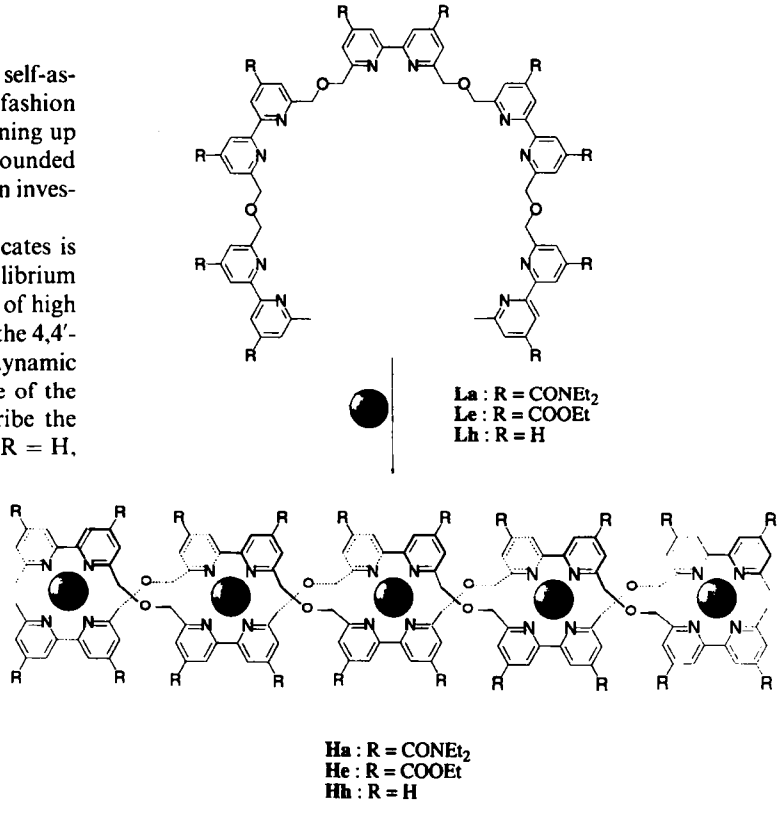

Fig. 1. Formation of a pentahelicate from its components; M represents Cu^{I} or Ag^{I} metal ions (**L** = ligand, **H** = helicate; **a** = amide, **e** = ester, **h** = H).

Results

^{1}H NMR investigations: At a concentration of 10^{-3} M, the pentanuclear copper(I) helicate **Hh** was fully formed 10 minutes after mixing; its NMR spectrum was that of the final complex and remained unchanged with time. In contrast, the evolution of the NMR spectrum for the formation of the complex **Ha** was slow and could be followed as a function of time. This helicate bears diethylamide groups on the bipyridine units

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(ligand **La**), and as many as 40 ethyl groups are brought close together when the double helical complex is formed (Fig. 1). One hour after mixing the ligand **La** and the copper(I) salt at a concentration of about 10^{-3} M, the ^{1}H NMR spectrum exhibited a great number of peaks in the aromatic region, which could not been interpreted (Fig. 2b), apart from the characteristic signals of the pentahelicate **Ha** itself. None of the peaks observed could be attributed to the free ligand **La**, since the signals of the methylene protons of the bridges were located in the region of those of the final complex.[1] Sixty hours later, the ^{1}H NMR signals showed the presence of only the helicate **Ha**, while the unknown peaks had disappeared (Fig. 2a). Under the through the presence of three peaks corresponding to increasingly charged species formed by successive loss of the BF_4^- anions (Fig. 3 and Table 1).

A solution containing the ligand **La** at a concentration of 10^{-3} M was then mixed very quickly with a stoichiometric amount of Cu^I (1 equiv of **La**, 2.5 equiv of $[Cu(CH_3CN)_4]BF_4$), and the resulting mixture was injected into the ES mass spectrometer. Spectra were recorded at regular time intervals;the first measurement was about 1 min after injection. Apart from some minor peaks corresponding to the final helicate **Ha**, already formed after 1 min, the spectra showed the presence of several well defined peaks, which could be attributed

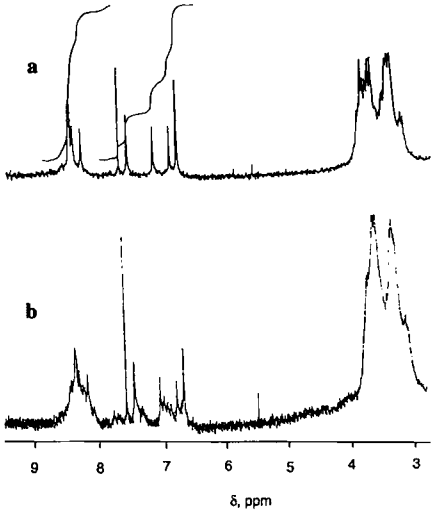

Fig. 2. ^{1}H NMR spectra of the mixture [2 **La** + 5 Cu^+]: a) 3 weeks and b) 10 min after mixing.

Table 1. ES mass spectra: characteristic ions and expected *m/z*. The peaks actually detected in the ES mass spectra are marked by asterisks.

Species	Ions	L = **La** [a] (R = $CONEt_2$)	L = **Le** [b] (R = COOEt)	L = **Lh** [c] (R = H)
LCu_2	$[LCu_2]^{2+}$	1047.8*	912.4*	552.1*
	$[LCu_2 + BF_4]^+$	2182.6	1911.9	1191.2
LCu_3	$[LCu_3]^{3+}$	719.7*	629.5*	445.8* (+ $2CH_3CN$ + HBF_4)
				475.1* (+ $2CH_3CN$ + $2HBF_4$)
				459.5* (+ $3CH_3CN$ + HBF_4)
				488.8* (+ $3CH_3CN$ + $2HBF_4$)
	$[LCu_3 + BF_4]^{2+}$	1123.1*	987.7*	627.4
	$[LCu_3 + 2\,BF_4]^+$	2333.1	2062.4	1341.7
L_2Cu_5 = **H**	$[L_2Cu_5]^{5+}$	850.9*	742.7*	454.4*
	$[L_2Cu_5 + BF_4]^{4+}$	1085.4*	950.1*	589.7*
	$[L_2Cu_5 + 2\,BF_4]^{3+}$	1476.2*	1295.8*	815.3*
	$[L_2Cu_5 + 3\,BF_4]^{2+}$	2257.9	1987.2	1266.5
	$[L_2Cu_5 + 4\,BF_4]^+$	4602.7	4061.3	2619.9

[a] Mol. wt. **La** = 1968.5 Da. [b] Mol. wt. **Le** = 1697.8 Da. [c] Mol. wt. **Lh** = 977.1 Da.

same conditions of solvent and concentration, the ^{1}H NMR spectrum of the ligand **Le**, bearing the less bulky ethyl ester groups on the bipyridine moieties (Fig. 1), showed only the helicate after only two hours.

In order to gain information about the nature of the possible intermediates generated during the self-assembly of the pentahelicates, we followed the formation of some of them by ESMS.

ESMS investigations: ESMS is known to be a soft ionization method which allows the observation of supramolecular complexes in solution in most usual organic or aqueous solvents and over a wide concentration range (10^{-6} to 10^{-2} M). It offers the possibility of controlling fragmentation during the ionization process through the accelerating cone voltage V_c.[9-10] ES mass spectra are usually clear and straightforward to interpret owing to the presence of several pseudomolecular peaks corresponding to the successive loss of counteranions. It has already been used to characterize several metal complexes,[9] and, recently, to determine the nature of intermediate species present in equilibrium in solution.[10] The potential of the method seems enormous, since it appears that ESMS can give a quantitative image of species present in equilibrium in solution, provided some precautions are taken; this applies to synthetic supramolecular entities[10a-11] and protein/inhibitor associations.[12]

Helicate ***Ha***: ESMS on a several month old solution allowed the unambiguous identification of the pentahelicate **Ha** (M = Cu^I), already fully characterized by ^{1}H NMR spectroscopy (Fig. 2a),

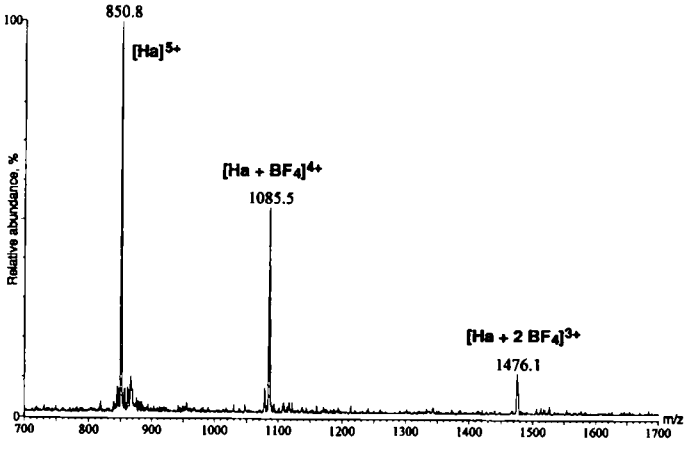

Fig. 3. ES mass spectrum of a solution of **Ha** (M = Cu^I) in CH_3CN/CH_2Cl_2 1/1.

to [(**La**)Cu_2] (major) and to [(**La**)Cu_3] (minor) (Fig. 4 and Table 1).

The stoichiometry of [(**La**)Cu_2] was unambiguously determined by comparison of the observed and calculated isotopic patterns. The simultaneous presence of two states of charge attested the correctness of the proposed composition of [(**La**)Cu_3]. The evolution of the relative intensity of the peaks due to [(**La**)Cu_2], [(**La**)Cu_3] and **Ha** as a function of time is presented in Fig. 4 and Table 2. Assuming that the response factors, independent of time, are the same from spectrum to

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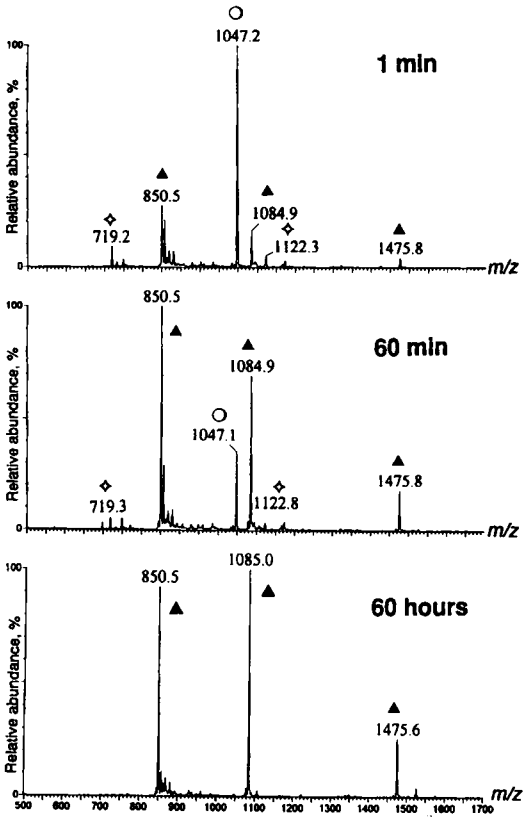

Fig. 4. ES mass spectrum of a solution containing 2 equiv of **La**, 5 equiv of $[Cu(CH_3CN)_4]BF_4$ measured 1 min, 60 min and 60 h after mixing; ▲: **Ha**; ○: $[(La)Cu_2]$; ◇: $[(La)Cu_3]$.

Table 2. Relative amounts of the species $[(La)Cu_2]$, $[(La)Cu_3]$ and **Ha** as a function of time.

t (min)	**Ha** (%)	$(La)Cu_2$ (%)	$(La)Cu_3$ (%)
1	30.2	61.6	8.1
10	44.5	45.1	10.4
15	51.1	40.2	8.7
30	63.4	30.4	6.2
45	71.8	22.7	5.5
60	78.9	16.9	4.3
150	92.6	5.6	1.8
300	96.7	3.3	0
3600	99.6	0.4	0

spectrum in the course of the experiment, this quantitative treatment is permissible, since it involves only the comparison of the relative intensities of the peaks in the same spectrum. The concentrations of the two identified intermediates $[(La)Cu_2]$ and $[(La)Cu_3]$ fell steadily: after 60 minutes they had decreased considerably, and after 60 hours, the ES mass spectrum showed the presence of only the final complex **Ha**. The same study was performed three times with good reproducibility. At 10^{-5} M, the formation process involves the same species $[(La)Cu_2]$, $[(La)Cu_3]$ and **Ha**, but is much slower (several weeks for a complete formation of the pentahelicate!). This dependence on concentration could imply that the limiting step is not an internal rearrangement of a nonhelix to a helix.

*Helicates **He** and **Hh***: The same ESMS experiment was performed with two other oligobpy ligands (**Le** and **Lh**), in order to explore the importance of the substituents of the bpy moieties (ethyl ester and H respectively, see Fig. 1).

With ligand **Le**, where the substituents are less bulky than the diethylamide groups in ligand **La**, the assembly of the helicate appeared to be faster, and the ESMS experiment was performed at a concentration that was ten times lower (10^{-4} M). Under these conditions the pentahelicate was formed in about three hours. The same intermediate species as in the previous case were observed in the ES mass spectra, with $[(Le)Cu_2]$ as the main species at the beginning of the reaction and only traces of $[(Le)Cu_3]$ present. This latter intermediate may either be consumed very quickly or is only weakly detected.

Ligand **Lh** represents the reference compound for characterizing the role of substituents in the self-assembly of the pentahelicate. The major difficulty in this experiment was the insolubility of the free ligand. Fortunately, the complex once formed is very soluble in most solvents,[2] but it was necessary to shake the solution for three minutes before injection. Since the formation of **Hh** was expected to be very fast, the ligand was diluted to a concentration of 10^{-5} M. Under these conditions, besides the final complex **Hh**, again the same intermediate species $[(Lh)Cu_2]$ and $[(Lh)Cu_3]$ were observed. In this case, $[(Lh)Cu_3]$ could be detected with up to three CH_3CN molecules. Due to the lack of ligand, no further kinetic experiments could be performed, and the formation time of **Hh** under these conditions could not be determined.

Discussion

According to previous results,[5] the formation of the pentahelicate could follow the following scheme [Eq. (1–6), L = pentabpy ligand] involving successive binding of the five Cu^I ions (Fig. 5, top).

$$L + Cu \longrightarrow LCu \quad (1)$$

$$LCu + L \longrightarrow L_2Cu \quad (2)$$

$$L_2Cu + Cu \longrightarrow L_2Cu_2 \quad (3)$$

$$L_2Cu_2 + Cu \longrightarrow L_2Cu_3 \quad (4)$$

$$L_2Cu_3 + Cu \longrightarrow L_2Cu_4 \quad (5)$$

$$L_2Cu_4 + Cu \longrightarrow L_2Cu_5 \quad (6)$$

However, the observation by ESMS of the intermediate kinetic species LCu_2 and LCu_3, common to the three systems studied, is not compatible with such a model.

It is attractive to consider a parallel formation pathway [Eq. (7–10)] through a convergent process limiting the number

$$L + Cu \longrightarrow LCu \quad (7)$$

$$LCu + Cu \longrightarrow LCu_2 \quad (8)$$

$$LCu_2 + Cu \longrightarrow LCu_3 \quad (9)$$

$$LCu_2 + LCu_3 \longrightarrow L_2Cu_5 \quad (10)$$

of intermediate structures, such as that represented in Fig. 5 (bottom). The structure of LCu_2 cannot be deduced from ESMS data, but other results[13] as well as CPK models suggest that it could have a hairpin conformation. One could then imagine that LCu_2 opens up to bind a third copper(I) cation and then combines with another LCu_2 unit to give the final structure L_2Cu_5.

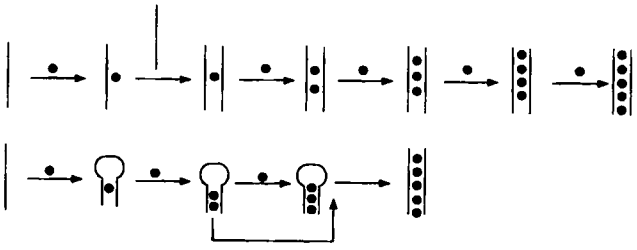

Fig. 5. Two possible formation pathways involving successive binding of Cu^I ions (top) and convergent combination of intermediates species (bottom) (● = Cu^I, — = L). The localization of the ions in the successive pathway is arbitrary. The hairpin structure are hypothetical although in line with other results [13].

Both formation pathways described above may be operating and others can be envisaged. Indeed, in ESMS experiments, the time necessary to mix the compounds (> 30 s) and to inject the resulting mixture into the ES source limits the observable species, the first spectrum being only measured after at least one minute. By this point, the mixture already shows a certain amount of helicate, which may have been formed right after mixing the ligand and Cu^I. One could imagine that the proportion of helicate formed according to one or the other mechanism depends on the initial concentration of the solution, with the successive binding mechanism being all the more important when the solution is more concentrated. Stopped-flow studies are in progress to investigate the simultaneous operation of both mechanisms.

Finally, it should be noted that, in the presence of a large excess of Cu^I salt (10 fold), ^{1}H NMR spectra and ESMS showed that the helicate remains intact, while an excess of ligand regenerates LCu_2 and LCu_3. This suggests that LCu_2 and LCu_3 are both kinetic and thermodynamic species under certain conditions: in presence of an excess of ligand, the ES mass spectrum, showing LCu_2, LCu_3 and L_2Cu_5 remained unchanged after several weeks. Moreover, fragmentation of the helicate by CAD[14] (collisionaly activated dissociation) in the ES source degrades L_2Cu_5 into LCu_2 and LCu_3 (Fig. 6). The intensity of

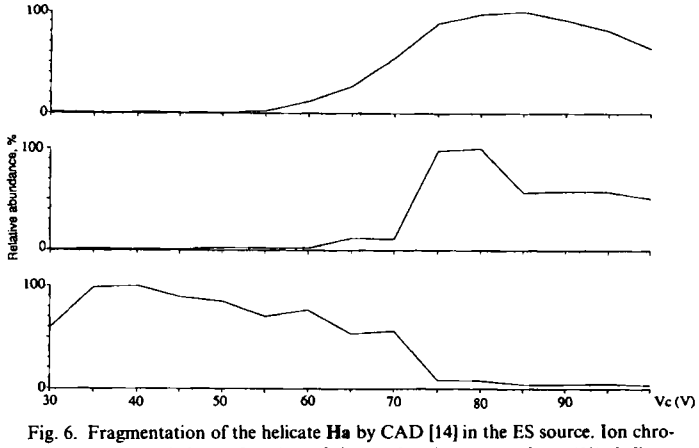

Fig. 6. Fragmentation of the helicate **Ha** by CAD [14] in the ES source. Ion chromatograms recorded as a function of the accelerating cone voltage (V_c): helicate $[\mathbf{Ha}]^{5+}$ (bottom, $m/z = 851$), $[(\mathbf{La})Cu_3]^{3+}$ (middle, $m/z = 720$) and $[(\mathbf{La})Cu_2]^{2+}$ (top, $m/z = 1048$). The helicate **Ha** disappears at about 70 V, while $[(\mathbf{La})Cu_2]$ and $[(\mathbf{La})Cu_3]$ appear at the same voltage.

the cone voltage V_c permits the internal energy of the species to be increased, so that this process may be likened to the melting of a DNA duplex into its components under thermal heating.

Conclusion

The present study has shown that steric factors play a major role in determining the rate of pentahelicate formation and that two discrete species LCu_2 and LCu_3 are common to the three systems studied. These are kinetic as well as thermodynamic intermediates depending on the conditions. The rate is slower for bulkier substituents on the oligobpy ligand; this indicates that steric bulk hinders the search process by which the system proceeds towards the final double helicate. Although the information obtained is mainly qualitative (and not necessarily quantitative nor exhaustive), ESMS is shown to be a precious tool for the investigation of such complicated processes, giving an indication about features such as the composition of intermediates, not readily available from NMR or UV spectroscopic data. ESMS thus revealed the presence of LCu_2 and LCu_3 complexes, probably of hairpin type, a result that provides important information for establishing the mechanism of formation and in particular the role of wrapping and unwrapping processes in the assembly of double helicates.

Experimental Procedure

^{1}H NMR spectra were recorded on a 200 MHz Bruker spectrometer in CD_3CN at a concentration of about 10^{-3} M. ESMS experiments were performed on a VG BioQ triple quadrupole mass spectrometer with an m/z range of 4000 (VGBio Tech, Altrincham, UK). The accelerating cone voltage V_c [9–10] was set at 20 V to minimize fragmentation processes. In a typical experiment, 500 μL of a solution containing the ligand **La**, **Le** or **Lh** in CH_3CN/CH_2Cl_2 (1/1, v/v) was mixed very quickly with 500 μL of a 2.5 times more concentrated solution of $[Cu(CH_3CN)_4](BF_4)$ in the same solvent. Immediately after mixing, this solution was injected into the mass spectrometer in a continuous flow at about 5 μL min^{-1} with a syringe pump. ES mass spectra were recorded in the multi-channel acquisition mode (MCA) at different intervals of time (every 2 min at the beginning of the experiment).

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