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Research Article

The Role of L-arginine in Inclusion Complexes of Omeprazole with Cyclodextrins

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Abstract. In this study, we investigate how the effect of L-arginine (ARG) and cyclodextrins upon omeprazole (OME) stability and solubility. The effect of the presence of ARG on the apparent stability constants ($K_{1:1}$) of the inclusion complexes formed between OME and each cyclodextrin, β-cyclodextrin (βCD), and methyl-β-cyclodextrin (MβCD) is studied by phase solubility diagrams and nuclear magnetic resonance (NMR) spectroscopy. The interaction of OME with those cyclodextrins, in the presence of ARG, is characterized using NMR spectroscopy and molecular dynamics simulations. ARG significantly increases the drug solubility and complex stability, in comparison to inclusion complexes formed in its absence. The effect is more pronounced for the OME:βCD complex. ARG also contributes to a larger stability of OME when free in aqueous solution. The combination of ARG with cyclodextrins can represent an important tool to develop stable drug formulations.

KEY WORDS: cyclodextrins; L-arginine; molecular dynamics simulation; NMR spectroscopy; omeprazole.

INTRODUCTION

Omeprazole (OME; Fig. 1a) is a proton pump inhibitor in gastric parietal cells. This drug has been widely used in the treatment of peptic ulcer, efflux esophagitis, and the Zollinger–Ellison syndrome (1,2). However, OME is poorly soluble in water and shows low physicochemical stability at neutral and acidic conditions (3,4). All these drawbacks give rise to difficulties in obtaining an oral pharmaceutical formulation with an acceptable bioavailability due to its rapid degradation in the stomach (5–7).

Cyclodextrins are cyclic oligosaccharides that contain a somewhat lipophilic central cavity and a hydrophilic outer surface (8). The internal hydrophobic cavity facilitates the inclusion of a number of guest molecules (9–12), and cyclodextrins have successfully been used as drug carriers to improve drug solubility, chemical stability, dissolution, and bioavailability or to decrease unfavorable side effects (13–20).

Natural cyclodextrins have low aqueous solubility (21), and to surpass this limitation, chemically modified cyclodextrins have been synthesized with improved water solubility and greater solubilizing and complexing power than the natural cyclodextrins (22). An example is methyl-β-cyclodextrin (MβCD with r=CH_3, Fig. 1b), a chemically modified βCD (23,24). Moreover, it has been observed that the addition of suitable auxiliary substances can significantly increase the cyclodextrin complexing ability by multicomponent complex formation (25). Arginine (ARG, Fig. 1c) has been studied as an auxiliary component mainly in systems involving hydroxypropyl βCD (HPβCD) as complexation agent (26–29). The use of the OME:βCD:ARG system has been reported by other authors (30,31). The process by which the ARG acts at a molecular level in these systems has not, however, been previously addressed.

On previous studies, we have reported on the use of cyclodextrins in order to increase OME solubility (11,32). In the present study, we incorporate ARG as a third component in the formation of the inclusion complexes. The goal of the present work is to describe and explain the effects over the drug stability and solubility created by the introduction of ARG in systems where cyclodextrin inclusion complexes are formed. We also address the direct interaction between the drug and ARG. Nuclear magnetic resonance (NMR) spectroscopy is used to determine the stoichiometry and arrangement of the multicomponent inclusion complexes formed. Molecular dynamics simulations are performed with the intent of, in conjunction with the former technique, obtaining some insight on the arrangements and preferential interactions involving the components of the system.

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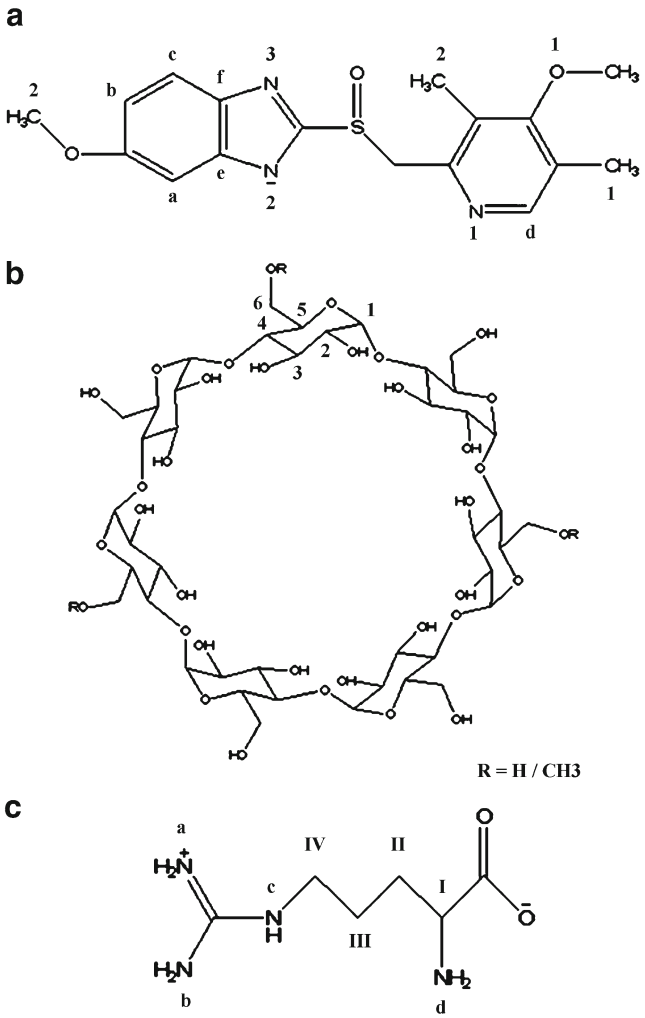

Fig. 1. Molecular structures of OME (**a**), βCD/MβCD (**b**), and ARG (**c**)

MATERIALS AND METHODS

Materials

βCD, M_W=1,135 g/mol and MβCD, M_W=1,190 g/mol and an average degree of substitution, D.S.=0.5, were kindly donated by Roquette (Lestrem, France) and OME, M_W= 345.42 g/mol, by Belmac Laboratory, S.A. (Madrid, Spain). Deuterium oxide (D_2O; 99.97%) was purchased from Eurisotop (Peypin, France), and triethanolamine, tris(hydroxymethyl)aminomethane, L-arginine, L-lysine, and triethylamine were supplied from Panreac (Santiago de Compostela, Spain). All other reagents were of the highest purity available from commercial sources. All products were used as received.

Phase Solubility Studies

Phase solubility diagrams were performed using the method reported by Higuchi and Connors (33). Increasing concentrations of cyclodextrins, βCD (0–13.2 mM), and MβCD (0–42 mM) were added to excess amounts of OME, containing a fixed concentration of ARG (57.9 mM). The concentration of the alkali agent was selected in order to maintain the pH=10 in the final solutions, so as to avoid drug degradation. Suspensions were stirred at room temperature until reaching equilibrium (96 h). All suspensions were filtered through a 0.45 μm membrane filter (Millipore), suitably diluted, and analyzed spectrophotometrically (UV-1603, Shimadzu, Japan) at 306 nm. The $K_{1:1}$ values for the complexation were calculated using Eq. 1:

$$Ks = \text{slope}/S_0(1 - \text{slope}) \quad (1)$$

Preliminary solubility studies between OME alone and other different alkali agents (triethanolamine, tris(hydroxymethyl)aminomethane, L-lysine, and triethylamine) were performed. A direct correlation between the improvement in drug solubility and the pH of the final solutions was observed. ARG, L-lysine, and triethylamine increased the OME solubility with higher efficacy comparatively to triethanolamine and tris(hydroxymethyl)aminomethane. These alkali agents were, thus, selected to investigate the influence in drug solubility in the presence of a constant concentration of βCD (10 mM). When βCD was present, only ARG showed the ability to increase the drug solubility, with the other alkali agents OME solubility maintained or decreased. It is also noteworthy that, when βCD is present, the improvement in drug solubility was found not to be pH dependent suggesting that the other alkali agents compete with OME for the entrance in the cavity. For these reasons, ARG was the third component selected in this study.

NMR Spectroscopy

^{1}H-NMR spectra were recorded at 25°C on a Varian 500 MHz spectrometer using a 5-mm NMR probe and a simple pulse-acquire sequence with solvent presaturation. Acquisition parameters consisted of 24 k points covering a sweep width of 8 kHz, a pulse width of 18 μs, and a total repetition time of 15 s. Digital zero filling to 64 k and a 0.5 Hz exponential were applied before Fourier transformation.

$K_{1:1}$ values were determined by NMR spectroscopy. Sample solutions were prepared by dissolving pure materials in 600 μL of D_2O in order to maintain constant concentrations of OME (3 mM) and ARG (10 mM) and changing cyclodextrin concentration in a molar ratio relatively to drug concentration from 1:0.1 until 1:7 (OME:cyclodextrin). Chemical shifts variations (Δδ) caused by complexation were measured. A nonlinear least squares procedure resorting to the Levenberg–Maquardt algorithm (34) on the differences observed in the chemical shifts due to the presence of cyclodextrins was used in order to estimate and compare the values of $K_{1:1}$ of the inclusion complexes obtained by different methodologies.

To determine the arrangement of the multicomponent inclusion complexes, prepared by freeze-dried method, samples were dissolved to obtain an OME final concentration of 6 mM. In the preparation of the inclusion complexes, cyclodextrins and ARG were added in a proportion 1:1 and 6:1, respectively, according to phase solubility studies that indicate an increase in OME solubility with the amount of ARG. This

proportion produces a pH~10, which was imposed as an upper limit. Reference solutions were prepared by separately dissolving an appropriate amount of OME, ARG, and cyclodextrins directly in 600 μL of D_2O. The values of Δδ caused upon complexation were measured.

Computational Methods

Molecular dynamics (MD) simulations were performed using the GROMACS package (35) and employing the GROMACS ffgmx force field (36).

The βCD structure was obtained from the Hetero-compound Information Centre - Uppsala (HIC-Up) online database (37), and that of MβCD was built by editing the original βCD structure and adding the substitution groups. The initial structures of the OME and ARG molecules were supplied as pdb files created in-house. The structures were then converted to GROMACS input files (conformation and topology) using PRODRG (38). The deprotonated form of OME, present in solutions with pH=10 (39,40), was addressed by extracting the benzimidazole proton when running PRODRG. A pm3 level optimization of the partial charges distribution for the deprotonated molecule OME and ARG molecules was established using GAMESS (41) and introduced into the respective topology input files.

In summary, pdb files for βCD, MβCD, OME, and ARG were converted into GROMACS format, with charges established, respectively, resorting to PRODRG (cyclodextrins) and pm3 calculations.

Four systems were used in order to rationalize different aspects observed in the experimental results. In all systems, only one molecule of OME is present. One of the systems is composed by the OME molecule and a single molecule of ARG, so as to assess the specific interactions between the two molecules. In a second model system, 1:6 excess of ARG is present, establishing a closer correspondence to the experimental setup. The interactions between ARG and the inclusion complexes were studied in two separate systems where OME was included in the cavity of βCD and MβCD, respectively, with a 6:1 ratio in ARG molecules to the inclusion complex.

In order to maintain electroneutrality, one sodium ion was introduced in every system. The long range electrostatic interactions were handled by the particle mesh Ewald method (42). The solvent was considered explicitly using the simple point charge water model and an algorithm for rigid water molecules (43). The system was enclosed in a cell with periodic boundary conditions. Smaller cell sizes, not lower than $30 \times 30 \times 25$ Å^3, were used for the systems where only OME and ARG were present, including at least 750 water molecules. In systems where cyclodextrins were present, the volume was at least $33 \times 33 \times 33$ Å^3, including more than 1,000 water molecules. The simulation was conducted in the NPT ensemble at a constant temperature of 300 K with coupling to an external bath (44).

An equilibration run of at least 3 ns was done previous to the production trajectory runs, without constraints. The formation of the inclusion complex is a result of a pre-equilibration process in the absence of ARG, and equilibration was only considered to be completed upon inclusion. The production runs were carried for 15 ns in the case of 1:1 OME:ARG and 6 ns for every other system. A time step of 1.5 fs was used throughout.

RESULTS AND DISCUSSION

Phase solubility diagrams of OME in solution containing ARG and each cyclodextrin are shown in Fig. 2a. Solubility profiles of OME with βCD (ai) and MβCD (aii) within the concentration range studied displayed a typical AL-type diagram, indicating the formation of 1:1 stoichiometry OME:cyclodextrin inclusion complexes in ARG solution. The OME solubility in ARG aqueous solution was 3.4 mM. In presence of the maximum concentration of βCD, the solubility of OME presented a 1.9-fold increase (to 6.4 mM) and a 3.7-fold increase when MβCD was used (to 12.7 mM).

The $K_{1:1}$ values for the inclusion complexes formed by OME and the cyclodextrins, in the absence and presence of ARG (Table I), were calculated (1) according to Higuchi and

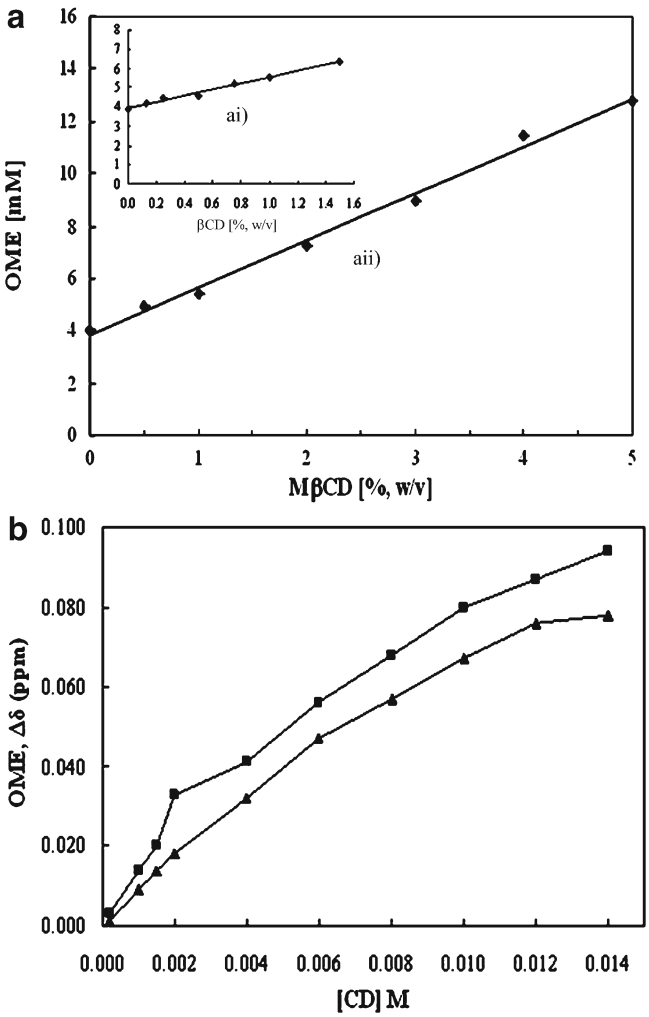

Fig. 2. **a** Phase solubility diagrams of OME:βCD (*ai*) and OME:MβCD (*aii*) in ARG aqueous solution. **b** ^{1}H-NMR chemical shifts increments of OME (CH_3 2 group) *versus* βCD (triangles) and MβCD (squares) increased molar concentrations in ARG aqueous solution

Table I. OME Solubility in Presence of CDs with and without ARG (S_2), Slope (D_2), and $K_{1:1}$ Values Calculated by Two Different Methods

Inclusion complex	S_2 (M, 10^{-3})[a]	D_2 (10^{-2})[b]	$K_{1:1}$ (M^{-1})	$K_{1:1}$ (ppm)
OME:βCD	5.8±0.093[c]	16.9	56.9±2.335[c]	60.0[c]
OME:ARG: βCD	6.4±0.008	18.1	65.0±1.495	74.0
OME:MβCD	11.3±0.118[c]	19.4	77.4±1.388[c]	90.0[c]
OME:ARG:MβCD	12.7±0.005	21.4	80.1±1.236	101.6

Each value represents the mean of three determinations ± standard deviation (SD)
[a] OME solubility in CD solutions (13.2×10^{-3} M βCD and 42×10^{-3} M MβCD) with and without ARG
[b] Slopes of the phase solubility diagrams achieved in inclusion complexes
[c] Results from (11) and used for comparison purposes

Connors (33) and (2) using a nonlinear least squares procedure. For this purpose, we selected the protons of OME that showed the largest Δδ (Fig. 2b) in the presence of increased concentrations of βCD or MβCD in ARG aqueous solution. The inclusion of OME in the cyclodextrin cavity has been observed to increase the solubility of the former (11). However, the presence of the alkali agent, ARG, increases even more the solubility and $K_{1:1}$ values for both inclusion complexes.

The increase in $K_{1:1}$ for the complex OME:βCD was larger than that for OME:MβCD in the presence of ARG (Table I). Note, however, that the complexes formed with MβCD were already more stable before the introduction of the basic amino acid. It should also be noted that these values, calculated by the two independent methods, are similar.

The ^{1}H-NMR spectra of inclusion complexes in the ARG aqueous solution are presented in Fig. 3, and the discussion follows the labeling presented in the panels of Fig. 1. The Δδ values for the inclusion complexes are presented in Table II.

In the system OME:ARG (data not shown), it was possible to observe that all protons in both rings of the drug presented larger Δδ due the presence of ARG, indicating possible interactions between both compounds. At the same time, ARG presented more accentuated Δδ in the protons near the amino group, suggesting the involvement of this group.

In general, the changes in Δδ due to the introduction of ARG in the system are larger for the protons on the OME:βCD complex (Table II) than the changes observed for Δδ values for the OME:MβCD complex (Table II). This is clearly visible in the ones pertaining to protons located in the included part of OME (Ha, Hb, Hc, and methoxy 2) and in the βCD protons inside the cavity (H3 and H5). The overall behavior is compatible with an increased stabilization of the OME:cyclodextrin complexes due to ARG.

In Δδ values for the OME protons when complexed with βCD in the absence and the presence of ARG (Table II), significant changes are observed essentially for the portion of the molecule that is inserted in the cyclodextrin cavity (Hb, Hc, and methoxy 2). The Δδ for methoxy 1 also presents a significant alteration in the presence of ARG. Changes in the protons of the βCD are also observed when ARG is present, specially for protons H5 (larger in absolute value) and H6 (smaller in absolute value). The changes observed for the Δδ in the protons of the OME in the complex with MβCD are

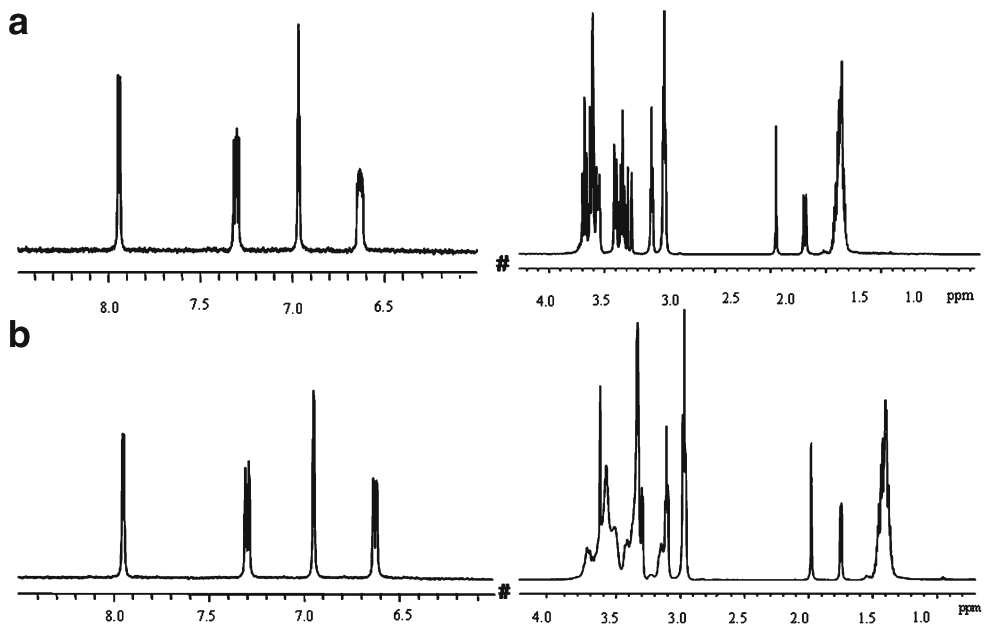

Fig. 3. ^{1}H-NMR spectrum of inclusion complex OME:βCD (**a**) and inclusion complex OME:MβCD (**b**) in ARG aqueous solution

Table II. Δδ for the Inclusion Complexes Formed between OME and βCD/MβCD in Absence and Presence of ARG Aqueous Solution

Assignment	Δδ (δcomplexed – δfree)			
OME	OME:βCD[a]	OME:βCD:ARG	OME:MβCD[a]	OME:MβCD:ARG
Ha	−0.001	−0.003	−0.025	−0.020
Hb	−0.015	−0.042	−0.036	−0.045
Hc	−0.005	−0.030	−0.024	−0.033
Hd	0.001	0.010	0.010	0.028
Methoxy 1	−0.023	0.013	−0.025	0.005
Methoxy 2	−0.011	−0.032	−0.026	−0.018
Methyl 1	0.000	0.000	−0.001	0.012
Methyl 2	0.041	0.038	0.060	0.041
βCD/MβCD				
H1	−0.019	−0.025	−0.049	−0.051
H2	−0.019	−0.026	−0.044	−0.042
H3	−0.054	−0.061	−0.054	−0.072
H4	−0.018	−0.024	−0.020	−0.019
H5	−0.026	−0.050	−0.086	−0.083
H6	−0.041	−0.033	−0.037	−0.035
Methyl-6′	–	–	−0.055	−0.033

[a] Results from (11)

similar for the included and the external part of the molecule (Table II). However, the most significant changes are observed in Hd, methoxy 1, and methyl 2 which are located in the exposed part of OME. The presence of ARG only changes slightly the Δδ of the protons of MβCD except for H3 (larger in absolute value) and methyl 6′ (smaller in absolute value). Δδ of ARG protons show significant alteration in the chemical shift of H_I, which is more important for the system where MβCD is present.

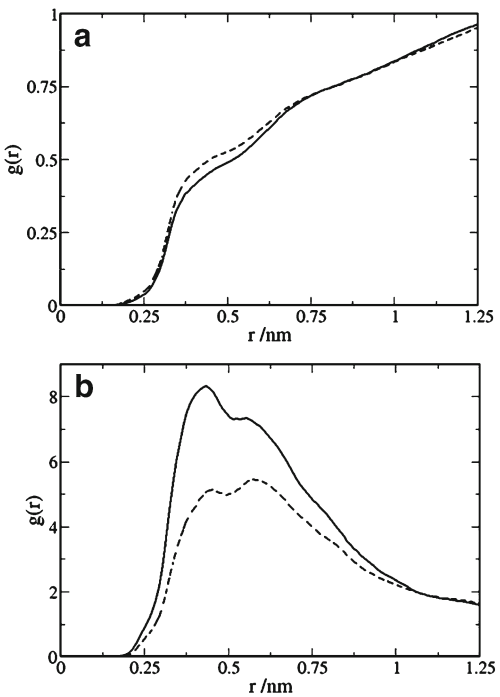

Fig. 4. Radial distribution function of water (**a**) and ARG (**b**) around the exposed part of OME in the inclusion complex with βCD (*full line*) and MβCD (*dashed line*)

Regarding the radial distribution function (RDF) of water and ARG around the 6-methoxy-2-((4-methoxy-3,5-dimethylpyridin-2-yl) methylsulfinyl) part of OME, the portion of the molecule not included in the cavity (Fig. 4), it is possible to observe that the RDF for water in the proximity of OME (Fig. 4a) presents smaller values in the OME:βCD complex, which are originated by a larger probability density of ARG in the proximity of OME (Fig. 4b).

The structure of the multicomponent inclusion complex formed with βCD is illustrated in the snapshot of Fig. 5. ARG molecules located at the wider side of the cavity minimize the interaction of OME with the solvent, thus increasing the stability of the complex. This effect, although being more significant in the OME:βCD complex, is not sufficient to make this complex more stable than the one formed by OME and MβCD.

The main reason suggested for the larger stability of the OME:MβCD complex in the absence of ARG was the larger protection given to OME by a deeper inclusion in the cavity

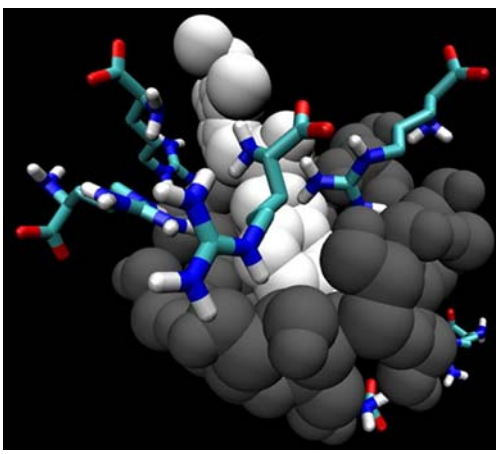

Fig. 5. Snapshot from the molecular dynamics simulation of the multicomponent inclusion complex formed between OME (*light gray*) and βCD (*dark gray*) in the presence of ARG (*colored*)

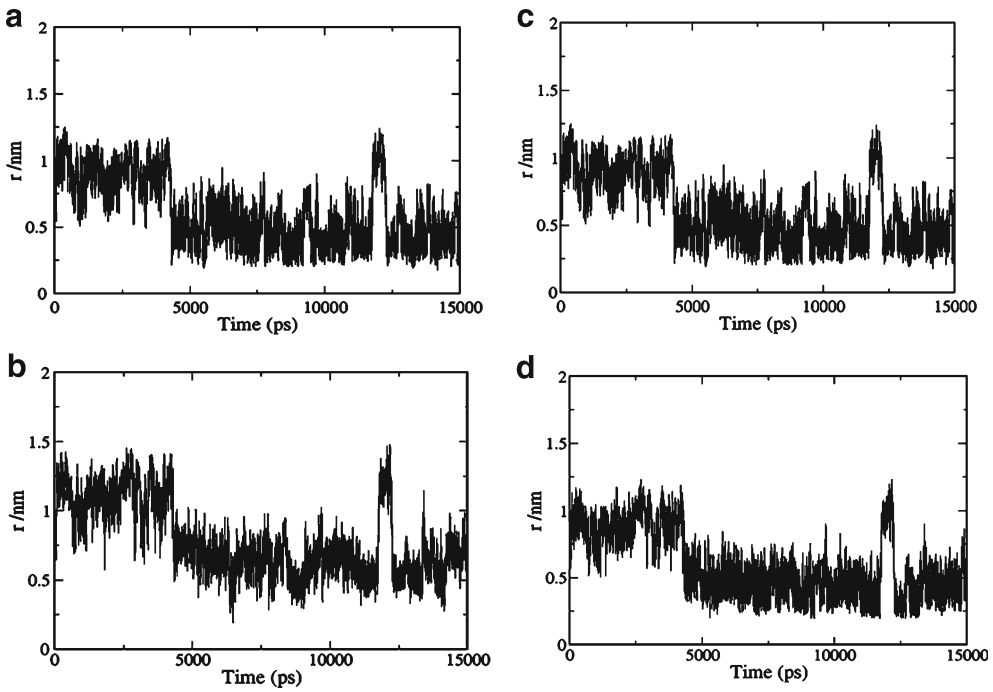

Fig. 6. Hydrogen H(c,ARG) (**a**) and hydrogen H(a,ARG) (**b**) distances to the N(2,OME) nitrogen and time evolution of the distances H(c,ARG)–N(2,OME) (**c**) and H(c,ARG)-N(3,OME) (**d**) along the trajectory

(11). When ARG is present in the system, the difference between the average distance from the e and f atoms of the OME to the center of mass of the O4 atoms of the cyclodextrin that can be considered as a reference to the center of the cyclodextrin inner cavity of the two complexes is not significant. This can explain to a large extent the large difference observed for the $\Delta\delta$ in the OME:βCD and the small difference in the $\Delta\delta$ for OME:MβCD upon the addition

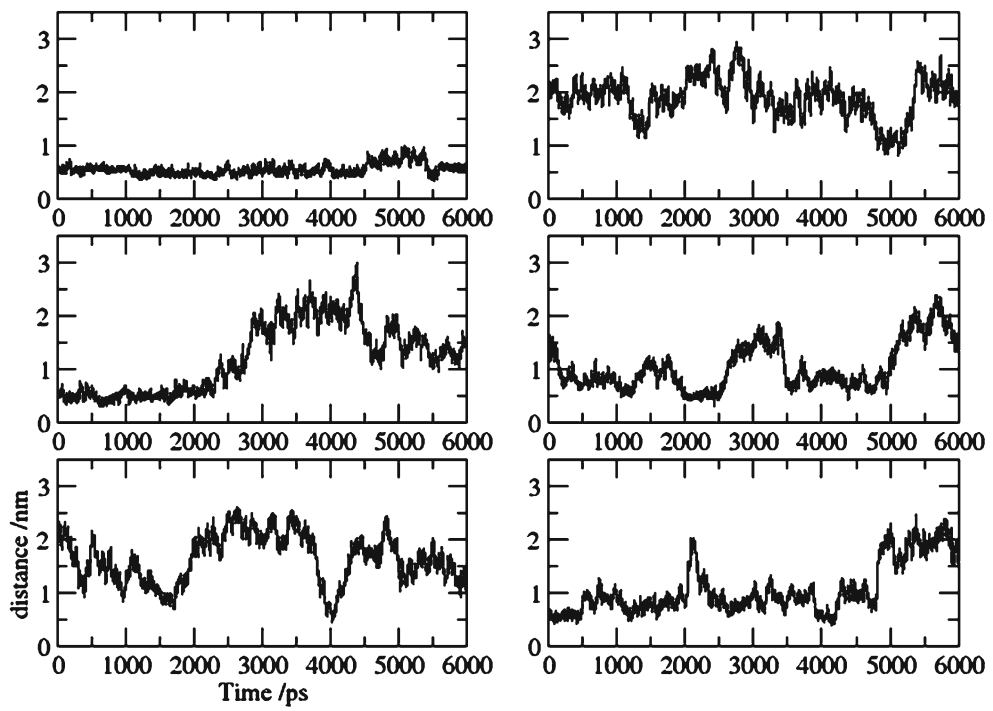

Fig. 7. Distance from the center of mass of OME to the center of mass of each ARG molecules along the trajectory in the system where an excess of ARG is present. Each panel corresponds to a different ARG molecule

of ARG, leading to overall similar Δδ results when the ARG is present. Upon the OME:ARG interaction in aqueous solution, to highlight this interaction, the system is studied in the absence of cyclodextrin. In a first approach, a 1:1 system is inspected to understand how the two molecules interact. In a second approach, a 1:6 excess of ARG is present, to establish a closer correspondence to the experimental setup.

In the system where OME and ARG are in a proportion of 1:1, H(c,ARG) was consistently closer than H(a,ARG) to the nitrogen atom N(2,OME) (Fig. 6). These H(c,ARG)–N(2, OME) distances are very small (approximately 3 Å), and by the residence time in close proximity, we have a clear indication of hydrogen bond formation. Also, H(c,ARG) interacts in a similar fashion with N(2,OME) and N(3,OME) atoms (Fig. 6), leading us to the conclusion that the probability of hydrogen bonding in the two cases is similar. These results are indicative of a direct electrostatic effect in the interaction between ARG and OME in the considered protonation states, and the formation of a hydrogen bond between the region of the largest opposite charge density in each molecule.

Figure 7 shows the distances between the center of mass of OME and the center of mass of each ARG molecule, when a large excess of ARG is present in solution. Superimposing the different panels shows that most of the time, ca. three molecules of ARG located in the proximity of an OME molecule. This large local concentration implies a significant desolvation of the OME molecule, as suggested before, which may be a factor contributing to the larger stability and solubility of OME in the presence of ARG (11).

The combined analysis of the results obtained from NMR and MD studies show that ARG plays an active role in the multicomponent complex formation by having a tendency to be located near the inner ring surface of the cyclodextrin.

CONCLUSION

NMR experiments and MD simulations were performed for studying the effect of the presence of ARG on the stability of inclusion complexes of OME:cyclodextrin and on the solubility and stability of OME.

Higher values of $K_{1:1}$ for the OME:cyclodextrin complexes were obtained with the introduction of ARG in the system. Also, an increase in drug solubility in the presence of ARG was recorded. Both these observations have been correlated with a minimization of the contact between the drug and the solvent. Although the increase in solubility is more pronounced in the OME:βCD:ARG system, overall, the OME:MβCD:ARG still leads to better results.

ARG was observed to play an important role in the increase of drug solubility and stability of free OME molecules. This is mainly attributed to the amino acid, by establishing hydrogen bonds with drug molecules, forcing a significant desolvation of the OME molecule.

Taking into account that improvements in OME solubility and stability are two important parameters to address in drug formulation with better bioavailability, the combination of cyclodextrins and alkali agents, namely ARG, seems suitable to attain this final purpose.

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