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COMMUNICATION

Structural diversity in native cyclodextrins/folic acid complexes – from [2]-rotaxane to exclusion compound†

Magdalena Ceborska,*[a] Magdalena Zimnicka,[b] Mariusz Pietrzak,[a] Anna Troć,[b] Małgorzata Koźbiał[a] and Janusz Lipkowski[a]

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The formation of different complexes of folic acid depending on the size of the host cyclodextrin resulting in either an exclusion compound (with the smallest α-cyclodextrin) or 2-rotaxane, where cyclodextrin is threaded over folic acid (with β- and γ-cyclodextrins), is presented. The formation is carried out in water which allows both possible application in pharmaceutical sciences and usage of environmentally friendly "green chemistry". The obtained compounds are thoroughly characterized using one and two dimensional NMR, mass spectrometry, differential scanning calorimetry and thermogravimetric analysis.

Folic acid (FA) is one of the forms of vitamin B_9. Deficiency of FA is a major cause of the neural tube effects (NTDs) in newborns; it is also responsible, among others, for disorders such as megaloblastic anemia.[1] Apart from its obvious use as a dietary supplement, it is now used in the drug development process. It is being incorporated in various prodrugs, mainly targeted at cancer treatment. Folic acid in the human body is bound by the folate receptor, which is overexpressed in tumors of the brain, kidney, lungs and breast.[2] Prodrugs are synthesized as combinations of easy to cleave linker conjugates of folic acid with various drugs, such as cytotoxic drugs (*e.g.* camptothecin,[3] taxol[4] and folate-tethered protein toxins (momordin)).[5] Recently folic acid has also been incorporated into γ-cyclodextrin-derived ZnSe and CdSe quantum dots.[6] The wide range of effects that folic acid, both alone and as conjugates with various active compounds, can exert on the human body still causes growing interest in this molecule, in terms of functionalization, synthesis of prodrugs and the drug delivery process.

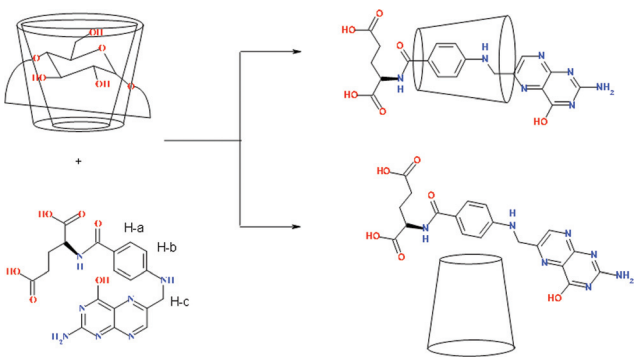
One of the possibilities of increasing the bioavailability of folic acid is formation of an inclusion compound with the host molecule acting as a carrier for an active compound. Native cyclodextrins (CDs) – α, β and γ – seem to be an obvious choice for such host compounds. They are cyclic oligosaccharides built from 6, 7 or 8 glucopyranoside units, respectively; well known for their complexing properties, an ability that is thoroughly acknowledged by the food[7] and pharmaceutical industries.[8]

NMR spectroscopy is widely used for studies of inclusion complexes of cyclodextrins with various guest molecules in solution.[9,10] The formation of the inclusion compound causes the shift changes of the protons belonging to the guest compound and appropriate protons of cyclodextrin.

In the solution of β-CD/FA salt (β-CD/FA) in water, significant changes of inner-surface protons H-3 and H-5 of β-CD (0.12 and 0.09, respectively) suggest that the guest compound should be included inside the CD cavity (Scheme 1).

The changes of chemical shifts for protons of folic acid sodium salt occur for three pairs of protons: H-a (0.05), H-b (0.19) and H-c (0.05). This indicates that these protons are more affected by the proximity of the CD molecule. Moreover, T_1 values of the FA proton signals change dramatically after addition of β-CD to the solution (*i.e.* H-a from 1.49 to 0.73 s, H-b from 0.90 to 0.51 s), indicating a great change of dynamics of the FA molecule, which confirms complex formation.

Furthermore, a 2D ROESY experiment confirms previously obtained data from ^{1}H NMR measurements, at the same time giving more detailed information about the guest conformation (Fig. 1). The 2D ROESY spectrum of the complex displays clear cross-peaks between H-3 and H-5 protons of CD (protons



Scheme 1 Formation of an exclusion compound (α-CD) and 2-rotaxane (β- and γ-CDs) with folic acid.

[a]*Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, PL-01-224 Warsaw, Poland. E-mail: mceborska@ichf.edu.pl; Tel: +0048223433414*
[b]*Institute of Organic Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, PL-01-224 Warsaw, Poland*

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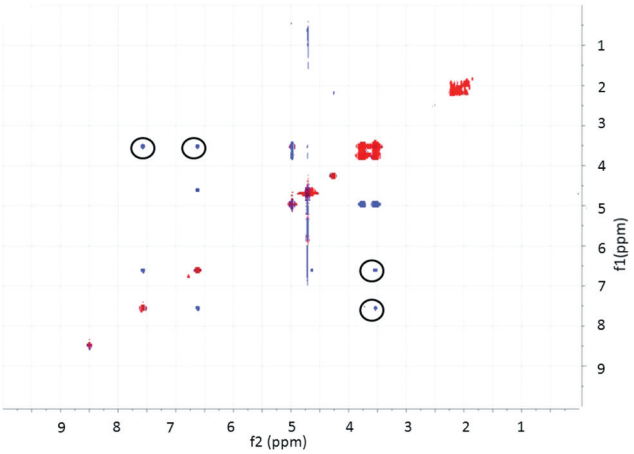

Fig. 1 ^{1}H NMR-ROESY spectrum of the β-CD/FA complex in D_2O at 298 K after the mixing time of 200 ms. Important cross-peaks are marked.

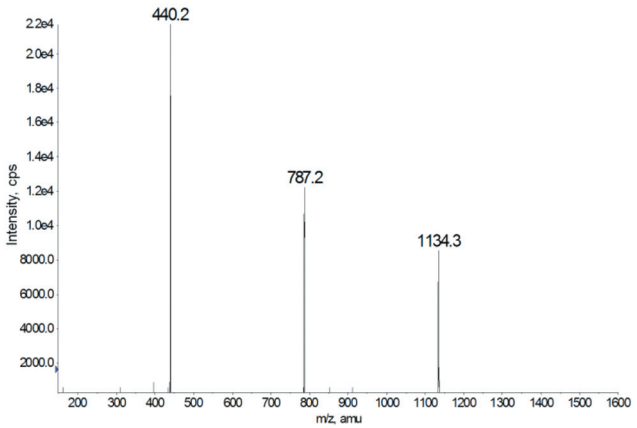

Fig. 2 CID mass spectrum of $[\beta\text{-CD/FA-2H}]^{2-}$ when collided with nitrogen. Collision energy (laboratory frame) was set to 31 eV.

located inside the cavity of β-CD) and H-a and H-b protons of FA salt (protons belonging to the phenol ring in the guest compound).

Surprisingly, the obtained β-CD/FA complex does not form an inclusion compound by incorporating the bicyclic part of FA, but by threading the β-cyclodextrin ring over the FA molecule forming [2]-rotaxane, in which FA serves as a dumbbell. Rotaxanes are very stable if they are not subjected to dethreading (deslipping), thus being perfect compounds for active molecule carriers. In our case the threading is carried out in water, which allows both possible application in pharmaceutical sciences and usage of environmentally friendly "green chemistry".

Similar results were obtained for complexation of FA salt with γ-CD. ^{1}H NMR spectra showed changes for inner-surface protons H-3 and H-5 of γ-CD (0.04 and 0.05, respectively), suggesting the inclusion of a guest compound inside the CD cavity. T_1 values of the FA proton signals also change after addition of γ-CD to the solution (*i.e.* H-a from 1.49 to 1.15 s, H-b from 0.90 to 0.72 s), confirming complex formation. The 2D ROESY spectrum displays cross-peaks between protons pointing towards the cavity (H-3 and H-5) and protons of the phenolic group of FA salt (H-a, H-b). To conclude, the reaction of FA salt with γ-CD results in the formation of [2]-rotaxane.

When the complexation reaction of FA salt was repeated for the smallest α-CD, the results significantly varied from those obtained for β- and γ-CDs. ^{1}H NMR spectra showed shift changes only for H-6-protons situated at the primary side of CD (0.06 ppm), suggesting that the environment for the protons located inside the CD cavity does not change and, although chemical shifts for protons of FA salt (the same as previous experiments) do change, the inclusion complex is not formed. Also other NMR observations show clearly that interaction between FA and α-CD has completely different characteristics than in the case of β-CD/FA and γ-CD/FA. Practically no changes of T_1 values (after addition of α-CD to the FA solution) and the absence of cross peaks between α-CD and FA proton lines in the 2D ROESY spectrum do not confirm any stable complex formation. However, based on the ^{1}H NMR spectra and MS analysis described below, we postulate formation of the exclusion complex that is created by weak interactions of phenolic ring protons of FA with H-6 protons of the primary side of α-cyclodextrin.

The complexes formed between FA salt and α-, β- and γ-CDs were subjected to MS analysis. The primary goal of these experiments was to establish the relative gas-phase stability of the examined complexes. Previous studies on the stability of the complexes of CDs with different "guests" such as oleanolic acid[11] and α,ω-dicarboxylic acids of different chain lengths[12] indicated a direct relationship between the stability of the complexes in solution and in the gas phase. The negative-ESI mass spectrum (Q1) recorded for the solution (methanol–water, 1 : 1) of β-CD/FA complex is dominated by the presence of the peak at *m/z* 787.2 corresponding to the formation of the expected doubly charged inclusion complex. The β-CD/FA complex may suffice as a representative example for the other two complexes, for which analogous observations have been made. The collision-induced decay of the representative β-CD/FA complex is shown in Fig. 2. The complex dissociates into the anions of FA (*m/z* 440.2) and β-CD (*m/z* 1134.3). α-CD/FA and γ-CD/FA follow the same dissociation pathway as observed for β-CD/FA.

The relative stability of α-CD/FA, β-CD/FA and γ-CD/FA was established based on the dissociation efficiency curves (plots of normalized intensity of complexes *vs.* center-of-mass collision energy under single collision conditions) shown in Fig. 3. Collision energy for half-dissociation of the complex was used as a measure of complex stability.

It is apparent from the plots that α-CD/FA is much less stable than the other two complexes, of which γ-CD/FA is more stable. Taking into account the results from the NMR experiments, the significant differences in the gas-phase stability between α-CD/FA and the other two complexes are due to their different structures.

The studied CDs, FA salt and their inclusion complexes in solid state were analyzed in TG and DSC experiments (in the range 25–300 °C, with heating rate 5° min^{-1}). The TG curves for the studied CD complexes and pure FA salt are shown in Fig. 4. The obtained TG and DSC plots of these complexes are different than obtained for parent compounds and indicate that thermal properties of complexes differ markedly from properties

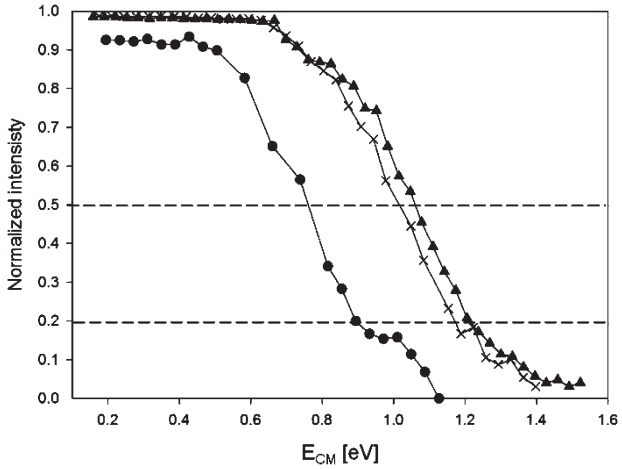

Fig. 3 Dissociation efficiency curves of three CD/FA complexes: α-CD/FA (circle), β-CD/FA (cross) and γ-CD/FA (triangle). The ions were collided with nitrogen (pressure *ca.* 2×10^{-5} Torr) at various collision energies. The *y* axis represents the normalized intensity of the complex in proportion to the sum of the intensities of the fragment ions, *i.e.* anions of folic acid and anions of cyclodextrin. The line at 0.2 of normalized intensity points at the threshold, below which the consecutive fragmentations of the two main dissociation products, *i.e.* anions of FA and anions of CDs, occur.

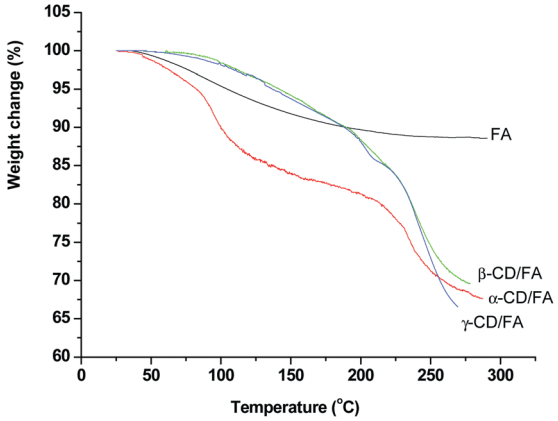

Fig. 4 TG curves of FA salt and complexes with α-, β-, γ-CDs.

of pure α, β, γ-CDs and FA salt. The shapes of TG curves obtained for β-CD/FA and γ-CD/FA salt inclusion complexes are similar, but different than for the α-CD/FA non-inclusion complex. The weight loss is caused by dehydration (under 150 °C) and decomposition of complexes (in the range 150–300 °C).

The DSC thermograms of cyclodextrin complexes showed two peaks in the same temperature range as TG, one endothermic corresponding to loss of water, and the second exothermic connected with decomposition of complexes.

In the present study, preparation of the complexes of FA salt with native CDs is presented. Depending on the size of the host, either strongly bound 2-rotaxane or weakly bound exclusion complex are formed. The obtained complexes are thoroughly characterized by means of NMR, MS and thermal analysis. The synthesis is performed in water, thus meeting the requirements of "green chemistry". Reports on such complexes are rare, and the methodology described here (aqueous solutions, no organic solvents) offers a unique possibility to mimic the natural environment.

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Notes and references

1 R. H. Fung Cheung, J. G. Hughes, P. J. Marriott and D. M. Small, *Food Chem.*, 2009, **112**, 507.
2 S. Santra, C. Kaittanis, O. J. Santiesteban and J. M. Perez, *JACS*, 2011, **133**, 16680.
3 W. A. Henne, D. D. Doorneweerd, A. R. Hilgenbrink, S. A. Kularatne and P. S. Low, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 5350.
4 J. W. Lee, J. Y. Lu, P. S. Low and P. L. Fuchs, *Bioorg. Med. Chem.*, 2002, **10**, 2397.
5 C. P. Lemon and P. S. Low, *J. Biol. Chem.*, 1992, **267**, 24966.
6 M. X. Zhao, H.-F. Huang, Q. Xia, L.-N. Ji and Z.-W. Mao, *J. Mater. Chem.*, 2011, **12**, 10290.
7 G. Astray, C. Gonzalez-Barreiro, C. Mejuto, R. Rial-Otero and J. Simal-Gandara, *Food Hydrocolloids*, 2009, **23**, 1631.
8 R. Arun, K. C. K. Ashkok and V. V. N. S. S. Sravanthi, *Sci. Pharm.*, 2008, **76**, 567.
9 S. Sagiraju and B. S. Jursic, *Carbohydr. Res.*, 2008, **343**, 1180.
10 Z. H. Yang, Y. Chen and Y. Liu, *Carbohydr. Res.*, 2008, **343**, 2439.
11 M. Guo, S. Zhang, F. Song, D. Wang, Z. Liu and S. Liu, *J. Mass Spectrom.*, 2003, **38**, 723.
12 V. Gabelica, N. Galic and E. de Pauw, *J. Am. Soc. Mass Spectrom.*, 2002, **13**, 946.

