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Influence of amino acid sequence on the interaction of short peptides containing 3-[2-(9-anthryl)benzoxazol-5-yl]-alanine with β-cyclodextrin

Research Article

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Abstract: The influence of peptide sequence and Leu chirality in linear and cyclic peptides containing 3-[2-(9-anthryl)benzoxazol-5-yl]alanine on interaction with β-cyclodextrin were studied using fluorescence and NMR spectroscopy. The analysis of enthalpy-entropy compensation effect ($\alpha=1.05\pm0.02$ and $T\Delta S_0^0=15.1\pm0.5$ kJ mol^{-1}) indicates that the entropic contribution connected with the solvent reorganization is the major factor governing the peptides-β-cyclodextrin complexation. Moreover, spatial orientation of guest-host molecule depends more than association constant on Leu residue configuration. However, the cyclization of the peptide chain substantially decrease the association constant with β-CD. An analysis of 2D NMR spectra reveals that inclusion complex is formed by penetration of cyclodextrin cavity from wider and narrow rims by anthryl group in the case of Box(Ant)-SPKL or anthryl and Leu residues for Box(Ant)-SPK(D)L analogue.

Keywords: *Cyclodextrin • Peptides • Fluorescence spectroscopy • NMR spectroscopy • Inclusion complex*

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1. Introduction

The α-, β-, and γ-cyclodextrins (CD) are toroidally shaped polysaccharides consisting of six to eight D-glucopyranose residues, respectively, linked by α-1,4 glycosidic bonds into a macromolecule. The cavities of CDs are relatively hydrophobic and have internal diameter of 4.7-8.3 Å [1,2]. Each cyclodextrin has its own ability to form inclusion complexes with various guest molecules with suitable polarity and dimension because of their special molecular structure [1,2]. Therefore, CDs are ideal molecules for the study of small molecule binding. Peptides and proteins cannot penetrate entirely into CD cavity because of its bulky and hydrophobicity. Moreover, the peptides backbone may influence on the formation of inclusion complexes [3-10]. Imperfect fit of the guest to the CD cavity leads to association constant decrease and formation of inclusion complex as well as association complexes in which peptide side chain can interact with the cavity and the wall of CD [9,10]. Moreover, CDs can recognize not only the size and shape but also the chirality of amino acids and their derivatives [9-12]. To further explain the influence of the side chain on interactions of CD with peptides, in this work we present the results of our studies on the binding process of the linear and cyclic peptides with β-cyclodextrin in water solution. It is well know that the presence of Pro-Lys in the peptide sequence is β-turn maker [13]. However, the presence of this dipeptide is not sufficient to make a full β-turn of the peptide with sequence Ser-Pro-Lys-Leu [14]. The addition to the N-terminus of Ser-Pro-Lys-Leu peptide, a hydrophobic amino acid, which can interact with C-terminal Leu-residue by hydrophobic interaction should fortify the tendency to form a β-turn. As a N-terminal hydrophobic amino acid 3-[2-(9-anthryl) benzoxazol-5-yl]-alanine (Box(Ant)) was chosen. This non-proteinogenic amino acid, whose photophysical properties are sensitive to any small changes in their environment, forms a stable inclusion complex (K=780 M^{-1}) with β-CD [15]. Thus, the peptides Box(Ant)-SPKL and Box(Ant)-SPK(D)L were synthesized to study the influence of Leu chirality on binding constant with

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β-CD as well as the structure of the inclusion complex. Additionally, a group of shorter peptides, devoid of some amino acids (Box(Ant)PKL, Box(Ant)KL and Box(Ant)L as well as Box(Ant)EL), were studied too. As a model compound Box(Ant)NHMe was used.

It has been know that high resolution 2D ^{1}H NMR (NEOSY or REOSY) as well as the fluorescence spectroscopy give reliable information about the structure, stoichiometry, binding constant and thermodynamic informations on the complexes of CDs and these methods were applied in our studies.

2. Experimental procedure

β-CD was purchased from Roth. BoxAnt was synthesized according to the procedure published in [15]. Peptides were synthesized using F-moc strategy according to the procedure published in [16]. Compounds were purified using semipreparative HPLC (Kromasil column, C-8, 5 µm, 250 mm long, ID=10 mm). The mobile phase was a gradient running from 20% to 80% of aqueous solution of acetonitrile with addition of 0.1% of TFA. The identification of products was based on ^{1}H NMR spectra (COSY, TOCSY, NOESY in D_2O) recorded on Varian, Mercury 400BB spectrometer (400 MHz) and mass spectra (Bruker Biflex III, MALDI).

2.1. Fluorescence measurements

Absorption spectra of all peptides studied in water were recorded using a Perkin-Elmer Lambda 40P spectrophotometer. Fluorescence spectra were recorded using a Perkin-Elmer LS-50B spectrofluorimeter with 3.0 nm band-width for excitation and emission. The steady–state emission spectra were measured at 15, 20, 30, 40, 50 and 60^{0}C. Temperature was maintained using a Julabo F26-MP refrigerated circulator. To the aqueous solution of β-CD 200 µl of stock solution of appropriate peptide was added (c=3×10^{-5} M). The optical density of the sample at the excitation wavelength (λ= 384 nm) did not exceed 0.1. Because of low solubility, Box(Ant)L and Box(Ant)EL were dissolved in water solution containing 1% v/v of MeOH and 10% v/v of MeOH, respectively. All measurements were made triplicate.

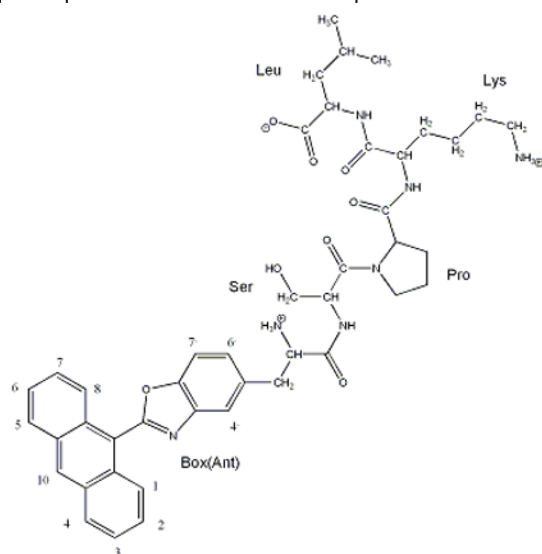

Figure 1. Structure of Box(Ant)-SPKL peptide with hydrogen atoms numbering

2.2. NMR measurements

Proton 2D NMR spectra of equimolar mixture of Box(Ant)SPKL or Box(Ant)SPK(D)L peptides (11 mM) with β-CD (11 mM) were recorded on a 499.89 MHz Varian spectrometer at the Nuclear Magnetic Resonance Laboratory at the Technical University of Gdańsk. The experiments were carried out in D_2O. Two-dimensional ^{1}H-^{1}H nuclear Overhauser enhanced spectroscopy (NOESY) spectra were recorded at 30°C, the mixing time (t_{mix}) was 0.35 s. The NMR spectra were processed using MestReNova software (Mestrelab Research S.L.). The structure of Box(Ant)SPKL and hydrogen atom numbering is shown in Fig. 1.

2.3. Determination of equilibrium constants

In the fluorimetric titration method the equilibrium constants of complex with 1:1 stoichiometry are usually calculated using the non-linear least-square methods applying the following equation [9,10]:

$$I_{CH} = \frac{I^0_{CH} + I^0_{CH\beta CD} K[CD]_0}{1 + K[CD]_0} \qquad (1)$$

or in linear form:

$$\frac{1}{I_{CH} - I^0_{CH}} = \frac{1}{K(I^0_{CH\beta CD} - I^0_{CH})[CD]_0} + \frac{1}{I^0_{CH\beta CD} - I^0_{CH}} \qquad (2)$$

where: I_{CH} - the fluorescence intensity of the chromophore in the presence of various, [CD] concentration; - the fluorescence intensity of the chromophore in water; $I^0_{CH\beta CD}$ - the fluorescence intensity of 'pure' 1:1 (CH:βCD) complex, whereas K - denote the association constant for 1:1 complex.

Eqs. 1 and 2 are valid for a large excess of CD over dyes and with the assumption that during the excited state lifetime the conversion of the uncomplexed dye to the complexed one and *vice versa* can be excluded since the corresponding guest exchange rate constants are small [17-20].

3. Results and discussion

3.1. Steady-state fluorescence spectra

Addition of the β-CD to the aqueous solution of studied peptides caused an increase of Box(Ant) fluorescence intensity, as it was observed for 3-[2-(9-anthryl)benzoxazol-5-yl]-alanine [15], and simultaneous hypsochromic shift of its emission band (Fig. 2).

Such changes of fluorescence spectra indicate the formation of an inclusion complex between fluorophore and β-cyclodextrin [15]. Application of Eq. 2 reveals no deviation from a straight line for higher CD concentration. Thus, only a simple complex with 1:1 stoichiometry is formed and our fluorescence data were analyzed according to the Eq. 1 assuming formation of one type of complex with 1:1 stoichiometry. An example of nonlinear fitting of integral fluorescence intensity changes of Box(Ant)SPK(D)L versus β-CD concentration is presented in Fig. 3.

The obtained binding constants at different temperatures as well as the thermodynamic parameters calculated from the van`t Hoff equation for all studied peptides are collected in Table 1. Data collected in Table 1 show a substantial influence of the peptide chain on the binding constant of inclusion complexes with β-CD (because of 10% v/v of MeOH content in the solvent the value of Box(Ant)EL is not taken into account in the discussion). For the model compound Box(Ant)NHMe in 20°C the binding constant is about 420 dm^3 mol^{-1} and is lower than that of Box(Ant) with unblocked amino acid moiety (K=590 dm^3 mol^{-1}) [15]. Substitution of the methylamide group by Leu residue substantially decreases the binding constant (K=300 dm^3 mol^{-1}). It suggests that the ionized carboxylic group of Box(Ant) is essential to form stable inclusion complex because of a hydrogen-bond network formation between the hydroxyl groups of cyclodextrin and unblocked amino acid moiety. This is not consistent with the literature data showing that the ionized guest molecules form weaker inclusion complexes with cyclodextrins than neutral ones [2,12,21-23] because of formation a strong hydration sphere around charged amino and carboxyl groups not allowing for deep penetration of the β-CD cavity [12,21]. However, in our case, the fluorophore is large and separated from the amino acid moiety by benzoxazole ring, thus the above-mentioned hydrogen bond network may be formed on the opposite to the anthryl penetration rim of the cyclodextrin.

The transformation of carboxyl group into N-methyl amide group or substitution by Leu residue probably destroys the hydrogen bond network decreasing the binding constant. Separating Box(Ant) and Leu by a

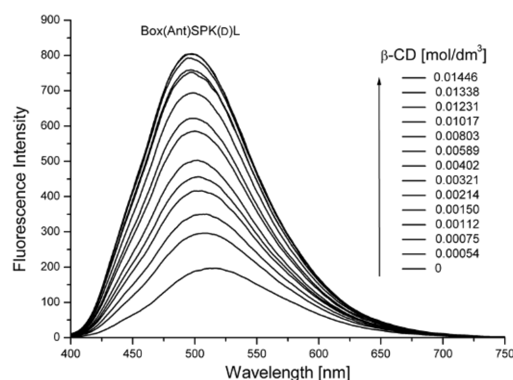

Figure 2. The changes of fluorescence spectra of Box(Ant)-SPK(D)L upon β-CD addition.

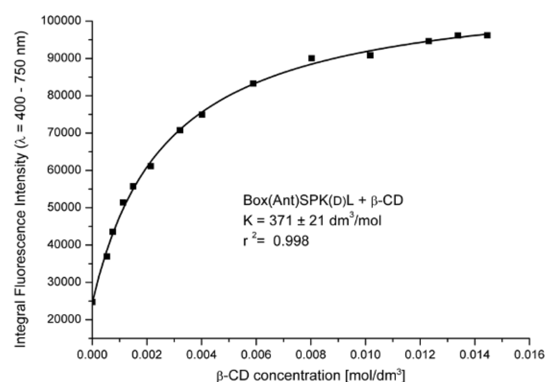

Figure 3. Nonlinear fit of integral fluorescence intensity changes of Box(Ant)-SPK(D)L fluorescence versus β-CD concentration according to Eq. 1.

Lys residue substantially increases the binding constant possibly due to restoring the hydrogen bond network and anchoring the peptide to CD by polar amino group of Lys. Introducing additional amino acid residues, Pro and Ser (Box(Ant)PKL and Box(Ant)SPKL), regularly decreases the binding constant value. Positioning close to the fluorophore rather polar serine residue (Box(Ant)SPKL), which should enable to form a hydrogen bond-network with a cyclodextrin hydroxylic rim, decreases the binding constant as compared to that of Box(Ant)PKL peptide indicating that the interactions with the surrounding water are stronger than those with hydroxylic groups of CD. Thus, for different peptide sequences, an interaction of the peptide chain with the surrounding solvent or cyclodextrin hydroxyl rim plays an essential role in the stabilization of inclusion complexes. The peptide cyclization stiffens the peptide chain and thereby changes its conformation. Such changes can hinder the penetration of the cyclodextrin cavity by a chromophore. For cyclic peptides studied, c-[(Box(Ant)SPK(D)L] and c-[Box(Ant)SPKL], the binding constants are much lower than for linear ones with the same sequence. Moreover, the inclusion complex stability depends on the configuration of Leu residue (nearly twice higher for

peptide containing L-Leu residue than with D-Leu) as it was observed for cyclic Leu-enkephalins [9]. However, the configuration of C-terminal Leu residue in the linear peptides does not have influence on the complex stability (in the range of experimental error). Thus, the differences between binding constants among peptides studied seem to depend on sequence, amino acid polarity and chirality as well as conformation of peptide chain.

3.2. The enthalpy-entropy compensation

For all studied peptides the enthalpy of complexation process is negative and in the range from -68.3±3.0 kJ mol^{-1} for c-[(Box(Ant)SPK(D)L] to -11.3± 0.8 kJ mol^{-1} for Box(Ant)L. A high value of ΔH observed for c-[Box(Ant)SPK(D)L], which is a twice of that observed for c-[Box(Ant)SPKL], is worth mentioning. It is compensated by a highly unfavorable change of entropy of complexation (ΔS=-193±9 J mol^{-1} K^{-1}). For the linear analogues of aforementioned peptides (Box(Ant)SPKL and Box(Ant)SPK(D)L), the configuration of Leu residue influences on the entropy and enthalpy of complexation while not changing the equilibrium constant is because of the compensation effect.

The diverse chemical and biochemical supramolecular systems, including cyclodextrins, can be analysed consistently using the following equations [2,10,24-30]:

$$T\Delta\Delta S^0 = \alpha\,\Delta\Delta H^0 \tag{3}$$

$$T\Delta S^0 = \alpha\,\Delta H^0 + T\Delta S_0^0 \tag{4}$$

$$\Delta\Delta G^0 = (1-\alpha)\Delta\Delta H^0 \tag{5}$$

The slope (α) obtained from Eq. 3 indicates to what extent the enthalpic gain ($\Delta\Delta H^0$) is cancelled by the accompanying entropic loss ($\Delta\Delta S^0$) and only a fraction (1-α) of the enthalpic gain can contribute to the enhancement of complex stability. Moreover, the intercept ($T\Delta S_0^0$) represents the inherent complex stability (ΔG^0) obtained at ΔH^0=0 (as far as the $T\Delta S_0^0$ term is positive). It has been suggested that the slope (α) and the intercept ($T\Delta S_0^0$) of the regression line are related to the degree conformational changes and solvent reorganization round of both host and guest upon complexation, respectively [25,28,29]. The thermodynamic parameters obtained from the fluorimetric titration of peptides studied with β-cyclodextrin are presented in Fig. 4 whereas the correlation between ΔH and TΔS is presented in Fig. 5.

The linear correlation for 8 data points gives the following parameters: α=1.05±0.02 and $T\Delta S_0^0$=15.1±0.5 kJ mol^{-1} with correlation coefficient r^2=0.996. These data are a little higher than the literature values presented by Rekharsky and Inoue [2] for β-cyclodextrin inclusion complexes obtained for more rigid guest molecules, however, they are comparable with data obtained for [Leu]-enkephalins (1.01±0.13 and $T\Delta S_0^0$=18.7±3.7 kJ mol^{-1}) [10]. The value of the slope obtained in this studies indicates that the enthalpic gain is not reflected in the net increase of complex stability. The rearrangement of the peripheral hydrogen-bond network and the conformation changes of peptides studied should be considered as responsible for relatively large slope obtained for peptides-cyclodextrin complexes studied [2,24]. Moreover, the high $T\Delta S_0^0$=15.1 kJ mol^{-1} value, higher than observed for N-AcTyr amides [30], indicates that the entropic contribution of the solvent reorganization is the major factor in the peptides-β-cyclodextrin complexation.

3.2.1. ^{1}H NMR studies

As was stated in the Introduction section, 2D NMR spectroscopy is an essential method for the conformational studies of CDs complexes because intensity of a NOE correlation signal detected between the relevant protons at the NOESY spectrum gives information that two protons are closely located in the space [31]. Therefore, it is possible to estimate the orientation of guest molecule in the CD cavity using the assigned NOE correlation.

Among peptides studied, two of them, Box(Ant) SPKL and Box(Ant)SPK(D)L, were selected for NMR study because of possibility of β-turn formation and different Leu configuration. The presence of β-turn in the aforementioned peptides confirms the 2D NMR spectra (Figs. 6 and 7) on which crosspeaks between Box(Ant) protons and Leu residue protons as well as Lys or Pro $C^{\alpha}H$ proton are seen. The weighted volumes of observed pairs of protons belonging to Box(Ant) and Leu indicate that the D-Leu residue is closer to the Box(Ant) residue than L-Leu residue. The lack of additional crosspeaks of remaining amino acid residues (except that for Lys or Pro $C^{\alpha}H$ protons) indicate the existence of a β-turn stabilized by hydrophobic interactions between Box(Ant) and Leu residues.

3.2.2. ^{1}H NMR studies of Box(Ant)SPKL and Box(Ant) SPK(D)L with β-CD

2D NOESY spectra presenting crosspeaks indicating on dipole-dipole interactions between the protons of β-CD and Box(Ant)SPKL or Box(Ant)SPK(D)L are presented in Figs. 8 and 9. The strength of the dipole-dipole interactions, as a thickness of line (determined basing on a volume of the crosspeak) between the appropriate pairs of protons, are presented in Figs. 10 and 11.

Table 1. Binding constants and thermodynamic parameters obtained for Box(Ant)NHMe and peptides containing Box(Ant) with **β**-cyclodextrin (r^2 denotes the quality of the fit).

	K [dm³ mol⁻¹]							ΔH [kJ mol⁻¹]	ΔS [J mol⁻¹K⁻¹]
Temperature [°C]	**10**	**15**	**20**	**30**	**40**	**50**	**60**		
Box(Ant)NHMe	479 ± 41 r^2 = 0.999	-	415 ± 36 r^2 = 0.998	287 ± 40 r^2 = 0.998	253 ± 32 r^2 = 0.997	213 ± 29 r^2 = 0.997	-	- 16.2 ± 01.7 r^2 = 0.996	- 5.6 ± 2.0
Box(Ant)L (1% v/v MeOH)	340 ± 22 r^2 = 0.999	-	300 ± 29 r^2 = 0.999	239 ± 21 r^2 = 0.998	221 ± 24 r^2 = 0.998	188 ± 23 r^2 = 0.996	-	- 11.3 ± 0.8 r^2 = 0.998	- 8.5 ± 2.8
Box(Ant)EL (10% v/v MeOH)	503 ± 36 r^2 = 0.997	362 ± 36 r^2 = 0.995	315 ± 31 r^2 = 0.995	223 ± 24 r^2 = 0.995	179 ± 23 r^2 = 0.994	150 ± 22 r^2 = 0.993	134 ± 16 r^2 = 0.996	- 23.1 ± 2.3 r^2 = 0.961	- 30.6 ± 7.6
Box(Ant)KL	-	658 ± 38 r^2 = 0.995	568 ± 27 r^2 = 0.995	463 ± 37 r^2 = 0.995	360 ± 39 r^2 = 0.994	257 ± 34 r^2 = 0.993	-	- 19.0 ± 1.6 r^2 = 0.980	- 11.9 ± 5.2
Box(Ant)PKL	738 ± 45 r^2 = 0.998	501 ± 36 r^2 = 0.996	422 ± 32 r^2 = 0.996	260 ± 25 r^2 = 0.996	178 ± 12 r^2 = 0.999	148 ± 13 r^2 = 0.998	109 ± 14 r^2 = 0.996	- 33.7 ± 2.8 r^2 = 0.983	- 64.7 ± 9.3
Box(Ant)SPKL	384 ± 27 r^2 = 0.998	-	356 ±18 r^2 = 0.999	290 ±24 r^2 = 0.997	242 ± 36 r^2 = 0.993	200 ± 29 r^2 = 0.994	-	-12.2 ± 1.4 r^2 = 0.969	6.6 ± 4.5
c-[Box(Ant) SPKL]	272 ± 33 r^2 = 0.995	-	196 ± 15 r^2 = 0.998	126 ± 26 r^2 = 0.989	80 ± 18 r^2 = 0.992	-	-	-28.1 ± 2.5 r^2 = 0.989	-52.5 ± 8.3
Box(Ant)SPK(D)L	463 ± 23 r^2 = 0.999	-	371 ± 21 r^2 = 0.998	281 ± 17 r^2 = 0.998	235 ± 23 r^2 = 0.996	174 ± 27 r^2 = 0.992	-	-17.8 ± 0.8 r^2 = 0.995	-11.7 ± 2.6
c-[Box(Ant) SPK(D)L]	349 ± 108 r^2 = 0.964	-	126 ± 37 r^2 = 0.977	59 ± 18 r^2 = 0.992	21 ± 14 r^2 = 0.984	3 ± 7 r^2 = 0.995	-	-68.3 ± 3 r^2 = 0.999	-192.6 ± 9.1

The addition of β-CD to the solution of peptides causes the lowfield shift of the chemical shift of the protons of Pro, Leu and Box(Ant) residues while the highfield shift of H^3 and H^5 protons of β-CD. The values of chemical shift of remaining protons of β-CD practically do not change. For Box(Ant)SPKL, crosspeaks between anthryl substituent of benzoxazole and H^3 and H^5 protons of β-CD are recorded (Fig. 8). It indicates that only the anthryl group interacts with cyclodextrin forming an inclusion complex whereas the changes of proton position observed for Pro and Leu residue are caused by conformational changes. For the peptide containing D-Leu residue, apart from the crosspeaks observed for Box(Ant)SPKL, additional crosspeaks between $C^{\delta}H_3$ protons of Leu and C^3H, C^5H and C^6H protons of β-CD occur (Fig. 9). The weighted volumes of observed signals of pairs of protons belonging to both peptides studied and β-CD can be directly compared because of comparable binding constant (from fluorescence measurements) and the same condition of NMR measurement (the same concentration of both peptides and cyclodextrin). Analyzing the weighted volumes of observed signals of pairs of protons of Box(Ant)SPKL (Fig. 10), one can see that the strength of the interaction of the anthryl substituent with both C^3H and C^5H protons of β-CD is similar, indicating the penetration of cyclodextrin cavity by an anthryl group from both wider and narrower sides with a little preference of the wider side. The formation of inclusion complex by penetrating the cavity from both sides of β-CD torus was already described in the literature [17,19,28]. Additionally, apart from the symmetry of the anthryl substituent, the weighted volumes of crosspeaks observed for $C^{4,5}H$ and $C^{1,8}H$ aromatic protons are not identical contrary to the crosspeaks for the $C^{2,7}H$ and $C^{3,6}H$ protons. Thereby, anthryl group does not penetrate the cyclodextrin cavity along its symmetry axis but possibly the longer symmetry axis of anthryl group forms an angle with the symmetry axis of β-CD. The lower values of weighted volumes observed for $C^{1,8}H$ than $C^{10}H$ aromatic proton support this assumption. Contrary to the Box(Ant)SPKL, the interactions of Box(Ant)SPK(D)L with β-CD are more diversified indicating a different depth of the penetration of the anthryl group inside the β-CD cavity and its different spatial orientation. As in the previous case, the interactions of the anthryl group with both C^3H and C^5H protons of β-CD are present. Additionally, the weighted volume of crosspeak observed for $C^{10}H$ aromatic proton and C^3H proton of β-CD is also bigger than that of C^5H proton. These results suggest that in this case, the anthryl group also prefers to penetrate β-CD cavity from the wider side. Moreover, diversified volumes of crosspeaks between β-CD protons and $C^{4,5}H$ and $C^{1,8}H$ proton indicate that for Box(Ant)SPK(D)L, the anthryl group also forms an angle with the symmetry axis of β-CD. However, the lower differences in the crosspeaks volumes between $C^{4,5}H$ and $C^{1,8}H$ aromatic protons and cyclodextrin protons than that observed for L-Leu analogue suggests that this angle is smaller. Apart from crosspeaks of the anthryl group and β-CD protons, for

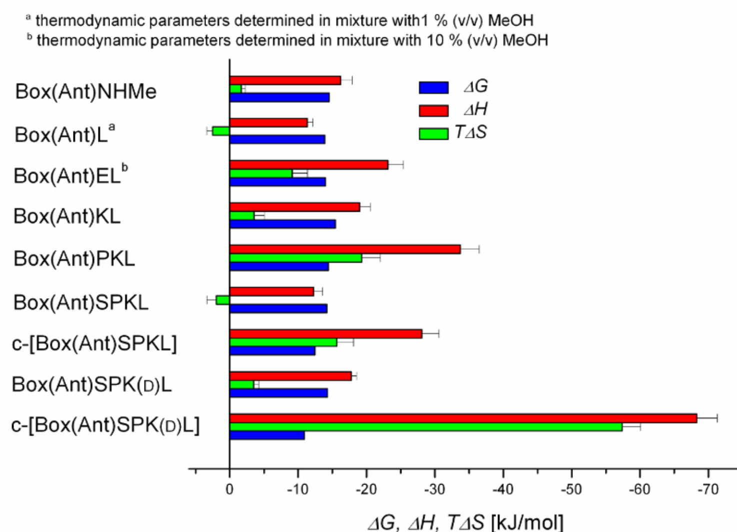

Figure 4. Plot of thermodynamic parameters values of (ΔG, ΔH, ΔS) of complexation process of studied compounds with β-CD.

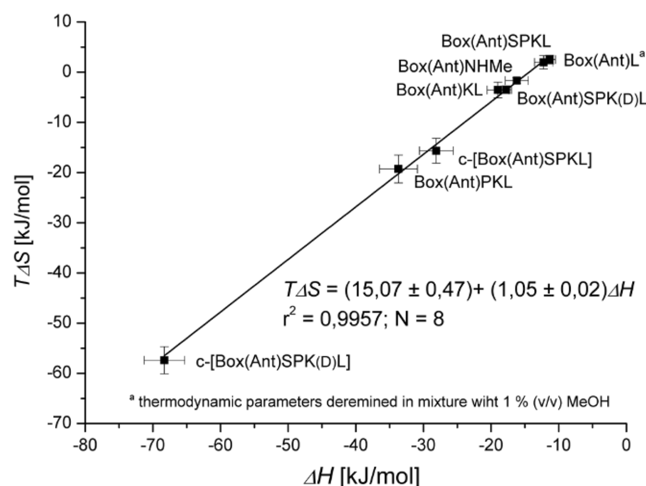

Figure 5. Enthalpy-entropy compensation plot for inclusion complexes of compounds studied with β-CD obtained from fluorescence titration.

Box(Ant)SPK(D)L relatively strong interactions between δ protons of Leu and C^3H, C^5H and C^6H protons of β-CD are observed. Taking into account the structure of peptide studied, two types of interactions of peptide hydrophobic groups are possible: independent penetration of β-CD cavity by anthryl group and Leu residue or simultaneous penetration by both residues. The interaction of δ protons of Leu and C^3H protons of β-CD is weaker than that with C^5H protons contrary to the *eg.* $C^{10}H$ anthryl proton. This seems to be a result of simultaneous complex formation in which δ protons of Leu shallowly penetrate the β-CD cavity from the one side whereas the anthryl group penetrates the β-CD cavity from the other side. In the case of independent interaction of the anthryl group and D-Leu residue with β-CD cavity, the equal crosspeak's volumes of $C^{\delta}H_3$ and anthryl aromatic protons with C^3H and C^5H cyclodextrin protons should be produced. Additionally, the same crosspeaks should be observed for the analogue containing L-Leu residue. Thus, the different Leu configuration changes the peptide conformation allowing the D-Leu analogue for simultaneous interactions of both its hydrophobic groups with cyclodextrin cavity.

According to the literature data, if only inclusion complex is formed, there is no interaction between external H^2 and H^4 cyclodextrin protons and guest molecules encapsulated inside β-CD cavity because the interproton distance is higher than 5 Å [32]. Thus, the absence of crosspeaks between peptides studied protons and H^2 and H^4 β-CD ones indicates that contrary to the cyclic enkephalins [9,10] only inclusion complexes are present.

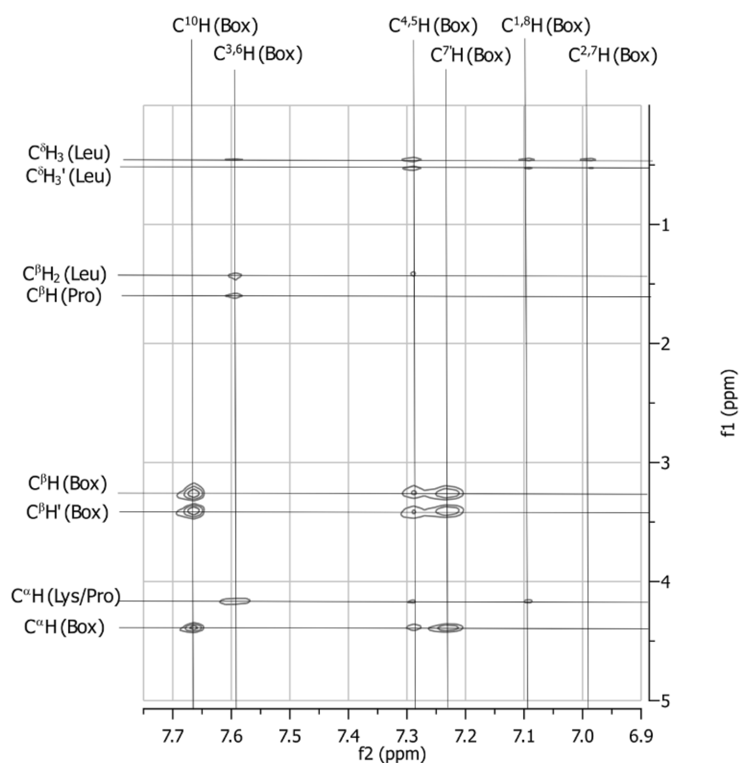

Figure 6. Fragment of 2D NOESY ^{1}H NMR spectrum of Box(Ant)SPKL. Crosspeaks indicating the interactions of anthryl group with Leu, Pro/Lys are presented.

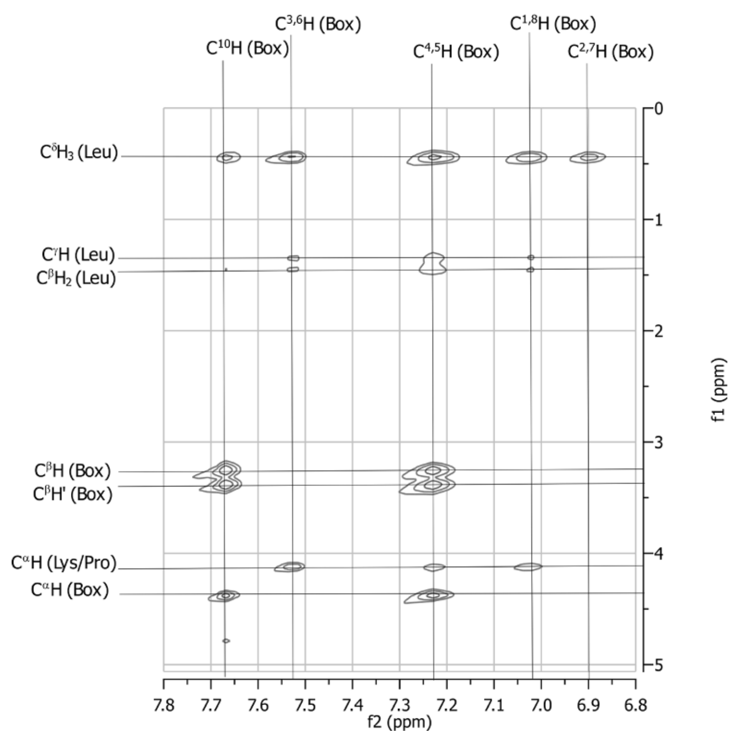

Figure 7. Fragment of 2D NOESY ^{1}H NMR spectrum of Box(Ant)SPK(D)L. Crosspeaks indicating the interactions of anthryl group with Leu, Pro/Lys are presented

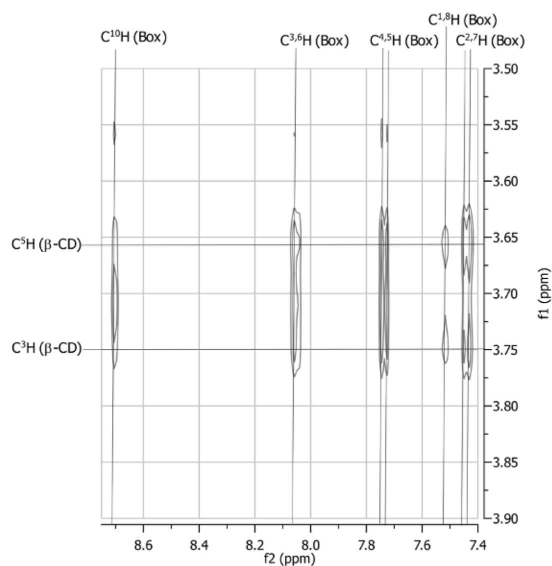

Figure 8. Fragment of 2D NOESY ^{1}H NMR spectrum of Box(Ant)SPKL. Crosspeaks indicating the interactions of anthryl group and β-CD are presented.

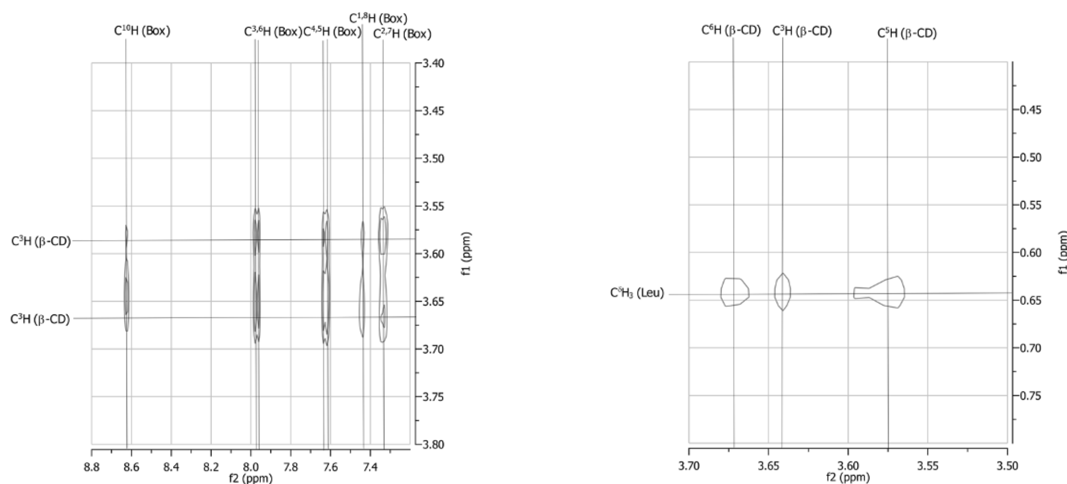

Figure 9. Fragment of 2D NOESY ^{1}H NMR spectrum of Box(Ant)SPK(D)L. Crosspesks indicating the interactions of anthryl group as well as $C^{\delta}H_3$ group of Leu and β-CD are presented.

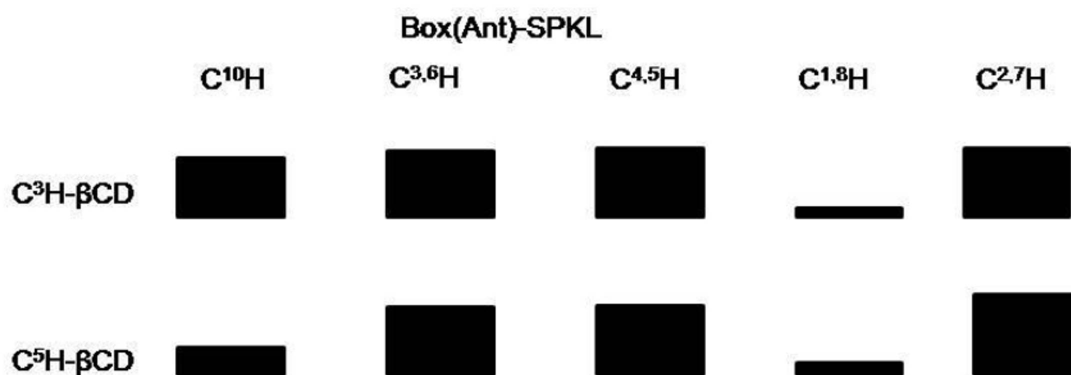

Figure 10. Weighted volumes of observed signals of pairs of protons belonging to β-CD and Box(Ant)SKPL complex in 2D ^{1}H NMR spectrum.

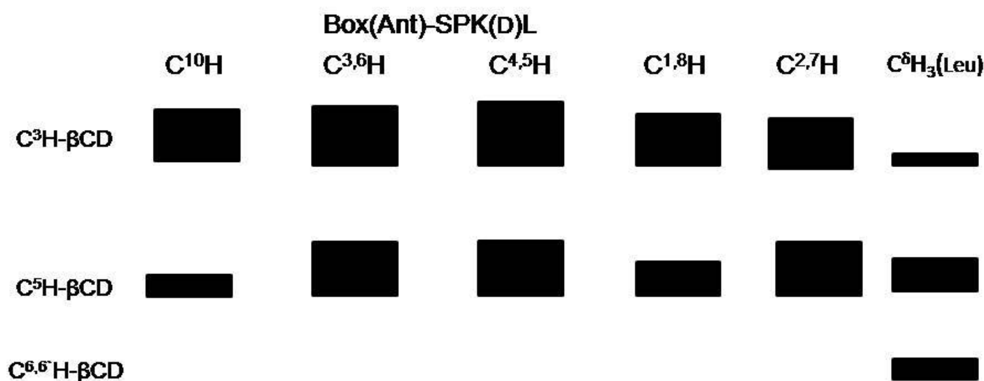

Figure 11. Weighted volumes of observed signals of pairs of protons belonging to β-CD and Box(Ant)SKP(D)L complex in 2D ^{1}H NMR spectrum.

4. Conclusions

For the short linear peptides, changes in peptide sequences cause different interactions of the peptide chain with the surrounding solvent or cyclodextrin hydroxyl rim which play essential role in the stabilization of inclusion complexes. The cyclization of a peptide has substantial influence on the binding constant by causing a lowering of its value compared to the linear peptide. The analysis of 2D NMR spectra of complexes of peptides studied with β-CD allows the determination of the spatial and mutual differences between host and guest molecules as well as the spatial orientation of the anthryl group depending on Leu chirality. Chirality of a Leu residue changes the spatial conformation of the peptide itself as well as the mutual orientation of host-guest molecule. However, it does not influence the binding constant indicating that the additional interactions of Leu residue with cyclodextrin are weak because of the anthryl group entirely governing the whole peptide's interactions.

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