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Spectral manifestations of nitrogen-containing amino acids intermolecular interaction with maleimide

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Nitrogen-containing amino acids complexation when one of them is enriched with maleimide has been studied using quantum chemical modeling methods based on density functional theory. Nitrogen-containing amino acids that are part of protein capsules for drug delivery are considered as the studied objects: tryptophan, arginine, lysine and histidine, as well as the maleimide molecule. Based on molecular complexes structures and their infrared spectra calculation, followed by an analysis of the parameters of the hydrogen bonds formed for pairs of interacting amino acids modified with maleimide, it was found that maleimide enhances the intermolecular interaction of nitrogen-containing amino acids. This allows us to conclude that the mechanism of increasing the therapeutic activity of protein delivery capsule enriched with maleimide is due to increased intermolecular interaction of delivery capsule proteins and target proteins in the presence of maleimide.

Keywords: maleimide, nitrogen-containing amino acids, hydrogen bonds, density functional theory, molecular modeling, IR spectra.

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Introduction

Amino acids are organic compounds that are found in cells and are involved in protein synthesis. They supply energy to tissues and muscles and are involved in salt and water metabolism, hormone synthesis, and functioning of the nervous system. Some of them are synthesized in the body, while others are essential (i.e. can only be obtained from external sources). The interactions of amino acids play a key role in targeted therapy based on biodegradable carriers.

In the present study, we consider the intermolecular interaction of two amino acids: one belonging to the protein of the target substance in the body and another one belonging to the protein container for targeted delivery. To evaluate the quality of complexation in the case of application of capsules, nanogels, and other protein drug carriers, we examine the interaction of these two amino acids. The main goal is to enhance this interaction, since both delivery and retention of a capsule or a vesicle (for more complete drug release) play an important part here. One way to enhance the interaction of amino acids is to enrich one of them with maleimide.

Maleimide (maleic acid imide) is an important „building block“ in organic synthesis [1]. Since the thiol–maleimide reaction may proceed under physiological conditions, capsules modified with maleimide functional groups are highly adhesive to mucous tissues [2,3].

The aim of this study is to evaluate the effect of maleimide on intermolecular interaction and complexation of amino acid pairs by calculating infrared (IR) spectra and molecular structures and performing subsequent analysis of the parameters of formed hydrogen bonds. We have already demonstrated [4] that maleimide has the capacity to increase significantly the degree of intermolecular interaction of substances in polyelectrolyte capsules for targeted delivery (polyarginine and dextran sulfate) with amino acids of the protein of the target substance. The present study is a continuation of this research and is focused on the complexation of some of the most important amino acids, which are involved in protein binding, and their interaction with maleimide.

Objects under study and methods of modeling

Three of the four amino acids examined here are essential for the human body. These amino acids are histidine, lysine, and tryptophan. Histidine sets the hemoglobin level and is involved in blood formation. It is also needed for tissue regeneration. Lysine promotes tissue regeneration and normalization of the musculoskeletal system. Tryptophan is a part of RNA proteins and a precursor to serotonin, melatonin, and growth hormone. Tryptophan deficiency leads to a decrease in serotonin, which has a number of adverse consequences: depression, anxiety, increased irritability, and insomnia. Arginine belongs to the group of conditionally essential amino acids. It is produced naturally in the body in limited quantities. Arginine is essential to normal functioning of the body. Its most significant contribution is the capacity to increase the production

of nitrogen oxide, which exerts a beneficial influence on blood flow by dilating blood vessels and making them elastic. Arginine is needed for such purposes as neogenesis, stimulation of the immune system, and support of normal kidney function.

Maleimide is produced as a result of dehydration of a non-cyclic amide formed in the reaction of maleic anhydride with amines [5]; it is used widely to modify biological objects [6]. For example, the synthesis of nanogels functionalized with maleimide was reported in [2]. It was found that, compared to the known mucoadhesive chitosan, maleimide-modified nanogels acquire high mucoadhesiveness on conjunctival tissue *ex vivo*. It was reported in [7] that liposomes with maleimide groups demonstrate better retention *in vitro* on the bladder tissue, which is attributable to their capacity to form bonds with thiols present in the mucosal tissue. These results confirm that nanogels containing maleic acid imide hold much promise as a new platform for sustainable drug delivery.

Maleimide is used widely in chemical bioconjugation of drugs due to its high activity in the formation of molecular complexes. It may react with amino or sulfhydryl groups on proteins, antibodies, enzymes, and other molecules in the body to form stable conjugates. These conjugates may be used to deliver drugs to specific sites in the body or to increase their stability and duration of action.

Molecular modeling of amino acid complexation, which included the calculation of structures and IR spectra of molecules and their complexes, was carried out on the basis of density functional theory (DFT) [8] using the B3LYP functional and the 6-31G(d) [9] basis set in the Gaussian [10] software package. Avogadro and GaussView were used to visualize molecular structures.

Frequency scaling was performed to minimize the discrepancies between the calculated and measured frequencies. This provides an opportunity to bring the calculated data closer to the experimental ones. The following scaling factors used below were derived by comparing the calculated IR spectra with experimental ones: 0.987 (within the $0-1000\,\mathrm{cm}^{-1}$ range), 0.961 ($1000-2000\,\mathrm{cm}^{-1}$), and 0.948 (above $2000\,\mathrm{cm}^{-1}$). These ranges were chosen on the premise that the influence of anharmonicity in the low-frequency region of the spectrum is minimal, since this is the center of a molecule and bonds vibrate uniformly. The $1000-2000\,\mathrm{cm}^{-1}$ range corresponds to the middle of a molecule where the deviation increases. The maximum deviation is observed at the periphery of a molecule (above $2000\,\mathrm{cm}^{-1}$), which is seen in the spectra.

The degree of complexation was studied by analyzing the parameters of hydrogen bonds. The energy of hydrogen bonds was calculated using the empirical Iogansen formula [11,12]:

$$-\Delta H = 0.3\sqrt{\Delta\nu - 40},$$

where $\Delta\nu$ is the frequency shift for valence vibrations of O–H bonds.

Results and discussion

The structures and IR spectra of maleimide, histidine, arginine, lysine, and tryptophan molecules were calculated. In addition, the structures and IR spectra of the resulting pairs of amino acids and molecular complexes obtained by adding a maleimide molecule to a pair of amino acids were calculated and analyzed.

Let us consider the potential for hydrogen bonding of histidine, arginine, lysine, tryptophan, and maleimide molecules. Figures 1 and 2 show the calculated structures and IR spectra of these molecules and their experimental IR spectra taken from the Spectrabase [13–17]. It can be seen that all amino acid molecules have potential for hydrogen binding. The maleimide molecule has only one option to form a hydrogen bond through the N–H amino group (Fig. 2, *b*).

Since the second amino acid in the examined complexes binds to the first one through the hydrogen atom of a carboxyl group, we evaluate the degree of hydrogen bonding exactly through this group for each amino acid. Its vibrations for histidine, arginine, lysine, and tryptophan are manifested at a frequency of $3498\,\mathrm{cm}^{-1}$ (Fig. 1, *a* (4)), $3540\,\mathrm{cm}^{-1}$ (Fig. 1, *b* (6)), $3497\,\mathrm{cm}^{-1}$ (Fig. 1, *c* (5)), and $3498\,\mathrm{cm}^{-1}$ (Fig. 2, *a* (4)), respectively. Vibrations of the amino group of maleimide manifest themselves at a frequency of $3449\,\mathrm{cm}^{-1}$ (Fig. 2, *b* (1)).

Let us consider the intermolecular interaction of pairs of amino acids and the effect of the maleimide molecule on their complexation. The strength of formed hydrogen bonds was evaluated in accordance with the classification given in [18], where hydrogen bonds are considered strong if their energy is $14.34-28.65$ kkal/mol and the hydrogen bridge length is $2.2-2.5$ Å; bonds of a moderate strength have an energy of $3.82-14.43$ kkal/mol and a hydrogen bridge length of $2.5-3.2$ Å; and weak bonds have an energy lower than 2.87 kkal/mol and a hydrogen bridge length of $3.2-4.0$ Å.

It follows from Fig. 3, *a* that a hydrogen bond forms at a frequency of $2672\,\mathrm{cm}^{-1}$ when the carboxyl group of histidine binds to the amino group of lysine. The parameters of this bond, which may be classified as a strong one, are presented in the table: the frequency shift is $826\,\mathrm{cm}^{-1}$, the spectral peak intensity is 2527 km/mol, and the bond energy is 8.4 kkal/mol.

With the addition of maleimide (Fig. 3, *b*), peak (**I**) in the IR spectrum, which corresponds to the O–H vibration of the histidine group, shifts noticeably to the left (the frequency shift was $1135\,\mathrm{cm}^{-1}$, and the spectral peak frequency was $2363\,\mathrm{cm}^{-1}$), implying a significant increase in bond energy. It can be seen from the table that the bond energy increased from 8.4 to 9.9 kkal/mol, and the intensity of the spectral peak rose from 2527 to 2761 km/mol.

When arginine interacts with lysine, a peak at a frequency of $3034\,\mathrm{cm}^{-1}$ is formed in the IR spectrum. This is indicative of the formation of a hydrogen bond between the

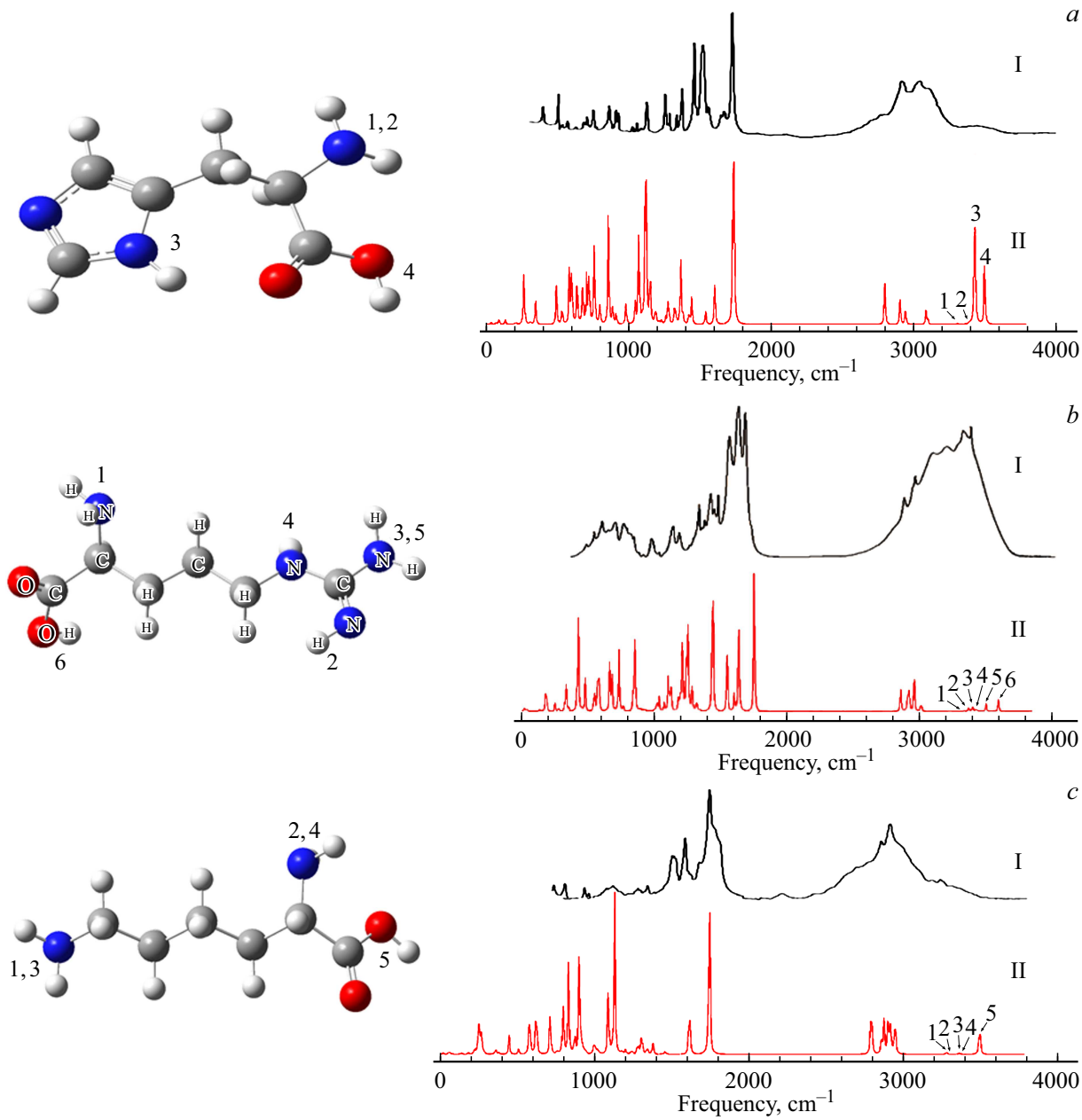

Figure 1. Experimental (**I**) and calculated (**II**) IR spectra and calculated structures of histidine (*a*), arginine (*b*), and lysine (*c*) molecules. Numbers denote the vibrations of molecular groups (and the corresponding spectral peaks) allowing for the formation of a complex based on hydrogen bonds.

carboxyl group of arginine and the amino group of lysine (Fig. 4, *a*).

Peak (**I**), which corresponds to the vibration of the carboxyl group of arginine, shifted to the left by 90 cm^{-1} and is detected at a frequency of 2957 cm^{-1}. The bond energy increased from 6.4 to 7.0 kkal/mol, and the intensity rose from 1080 to 2090 km/mol. All parameters of hydrogen bonds are listed in the table.

The strongest interaction of lysine is observed when lysine binds with tryptophan (Fig. 5, *a*). The vibration of the carboxyl group of lysine manifests itself at a frequency of 2771 cm^{-1} in this case. The frequency shift relative to a single lysine molecule is 726 cm^{-1}, suggesting that this bond is moderate in strength (close to a strong one). The effect of maleimide is revealed by changes in the structure and IR spectrum of the resulting lysine–tryptophan–maleimide molecular complex (Fig. 5, *b*).

If can be seen from the table that the vibration frequency of the carboxyl group of lysine in this complex is 2673 cm^{-1}. Thus, the addition of maleimide did also increase the frequency shift and the bond energy (from 7.9 to 8.4 kkal/mol) in this case. The intensity rose from 1988 to 2221 km/mol as well.

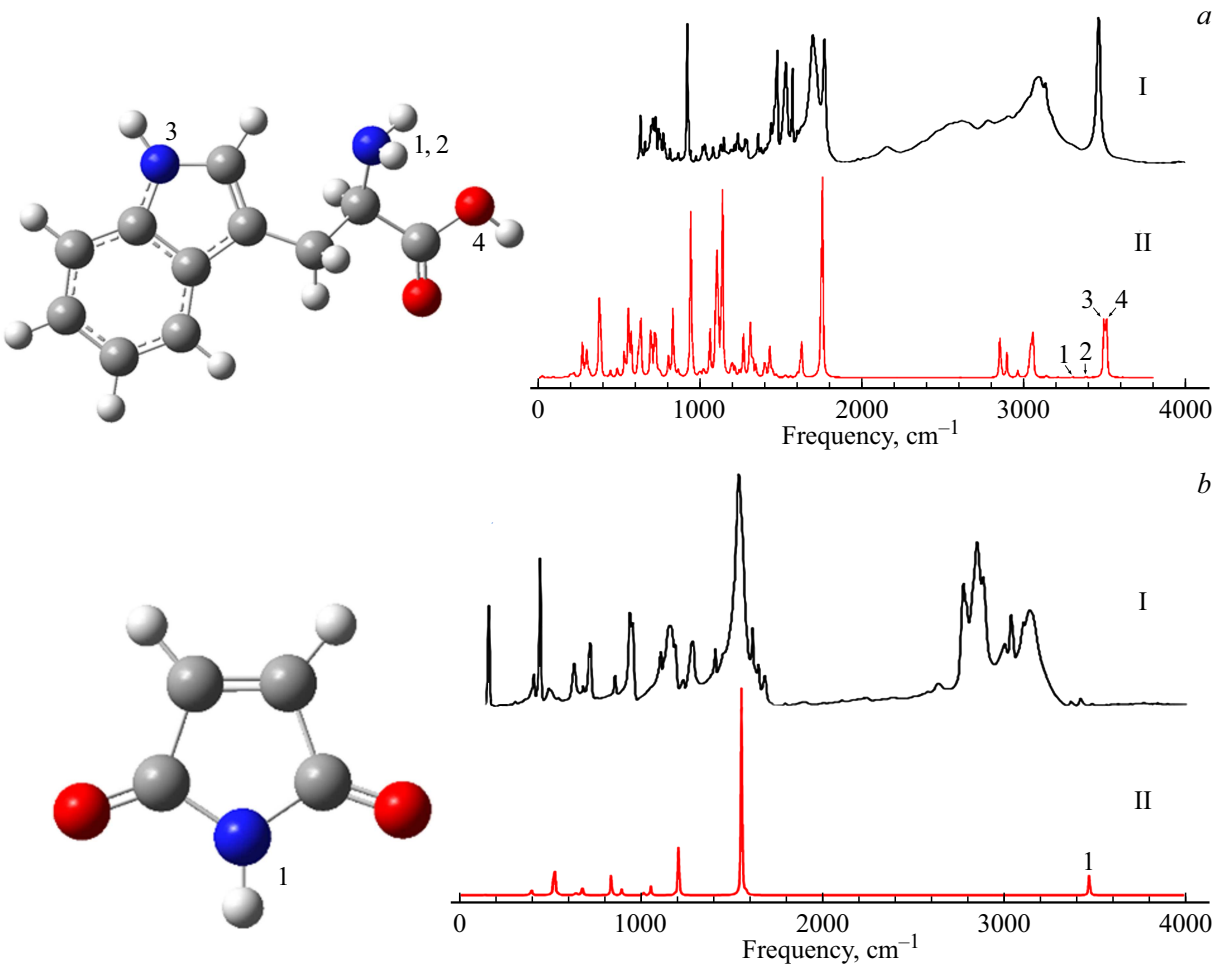

Figure 2. Experimental (**I**) and calculated (**II**) IR spectra and calculated structures of tryptophan (*a*) and maleimide (*b*) molecules. Numbers denote the vibrations of molecular groups (and the corresponding spectral peaks) allowing for the formation of a complex based on hydrogen bonds.

In the complexes discussed above, an increase in strength of moderate-to-strong hydrogen bonds forming between nitrogen-containing amino acids was observed. Depending on the complex type, the increase in bond energy induced by the addition of maleimide varied in magnitude from 0.5 to 1.5 kkal/mol. The pattern is the same in all cases: the addition of maleimide enhances the interaction significantly.

However, there is one exception: when two molecules of arginine interact (Fig. 6, *a*), the interaction of amino acids is much weaker. A hydrogen bond between nitrogen N and the O–H group is formed at a frequency of 3415 cm^{-1}.

The table demonstrates that the interaction of arginine as an amino acid belonging to the carrier protein with other amino acids and maleimide is weaker than that of the other examined molecules. In a complex of two arginine molecules, the frequency shift was 148 cm^{-1}.

The shift in interaction with two maleimide molecules (Fig. 6, *b*) increased just by 6 cm^{-1}. This suggests that the interaction between arginine and maleimide is weak. As it turned out, this is the only one of the examined pairs of amino acids that is characterized by a fairly weakly pronounced intermolecular interaction.

In other cases, the addition of maleimide induces a noticeable increase in the energy and strength of the hydrogen bond in question, indicating enhanced complexation of nitrogen-containing amino acids in the presence of maleimide. The strongest hydrogen bonds correspond to tryptophan and lysine.

Conclusion

The obtained results demonstrated that the addition of maleimide makes it possible to enhance the interaction between nitrogen-containing amino acids. The most profound changes occurred in histidine–lysine, arginine–histidine, and lysine–tryptophan complexes, while the weakest change was observed in the case of two arginine molecules.

Enrichment of a carrier capsule with maleimide allows for the formation of stronger hydrogen bonds with the

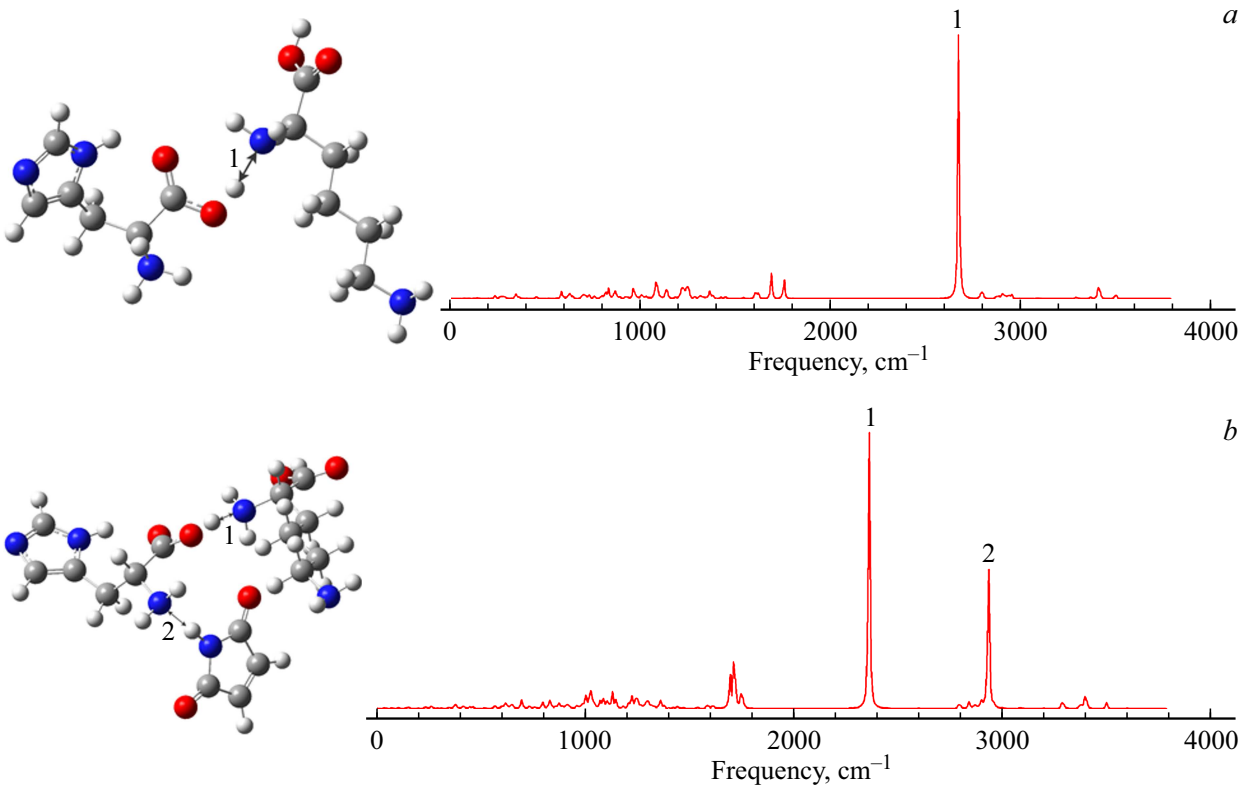

Figure 3. Calculated structures and IR spectra of molecular complexes of histidine and lysine (a) and histidine, lysine, and maleimide (b).

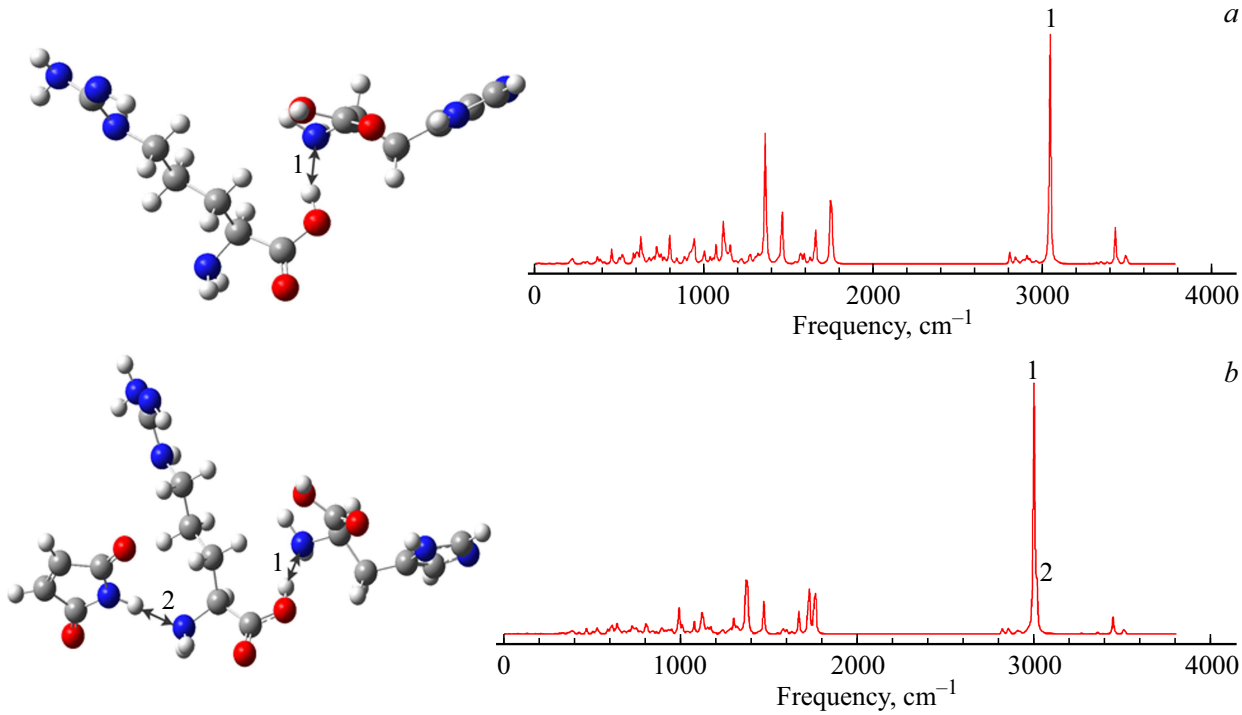

Figure 4. Calculated structures and IR spectra of molecular complexes of arginine and histidine (a) and maleimide, arginine, and histidine (b).

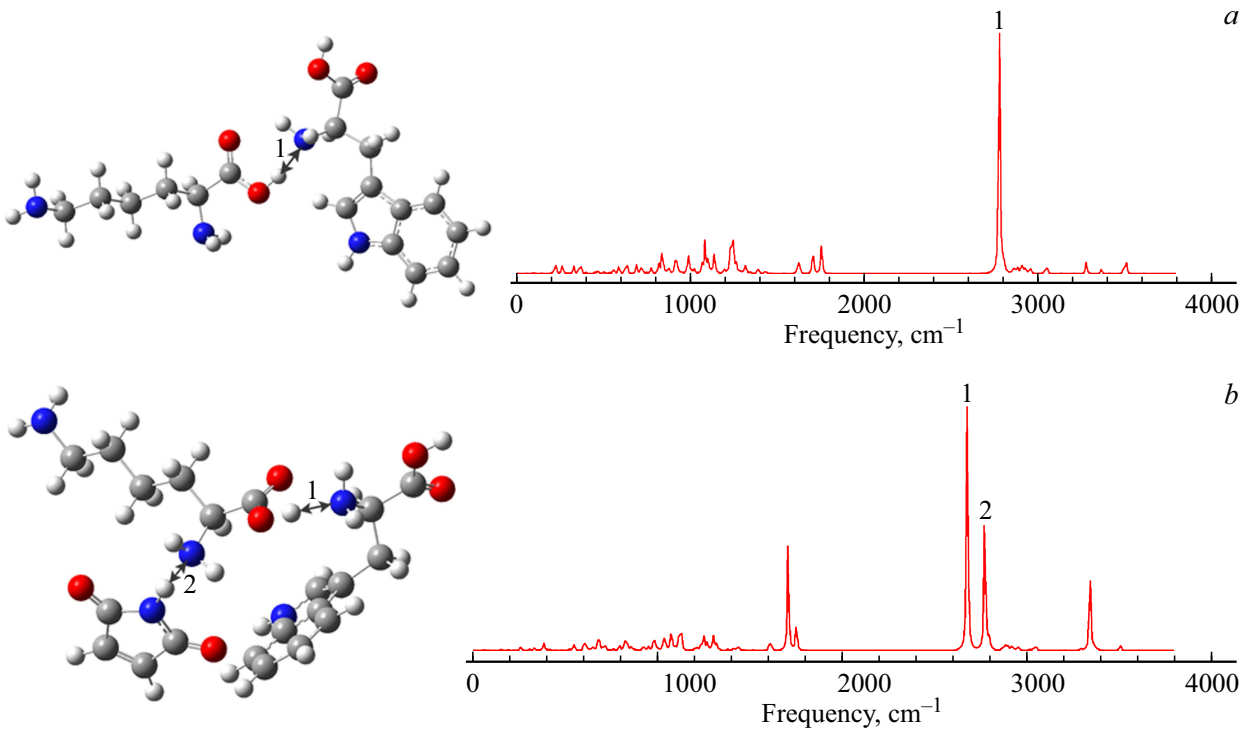

Figure 5. Calculated structures and IR spectra of molecular complexes of lysine and tryptophan (*a*) and maleimide, lysine, and tryptophan (*b*).

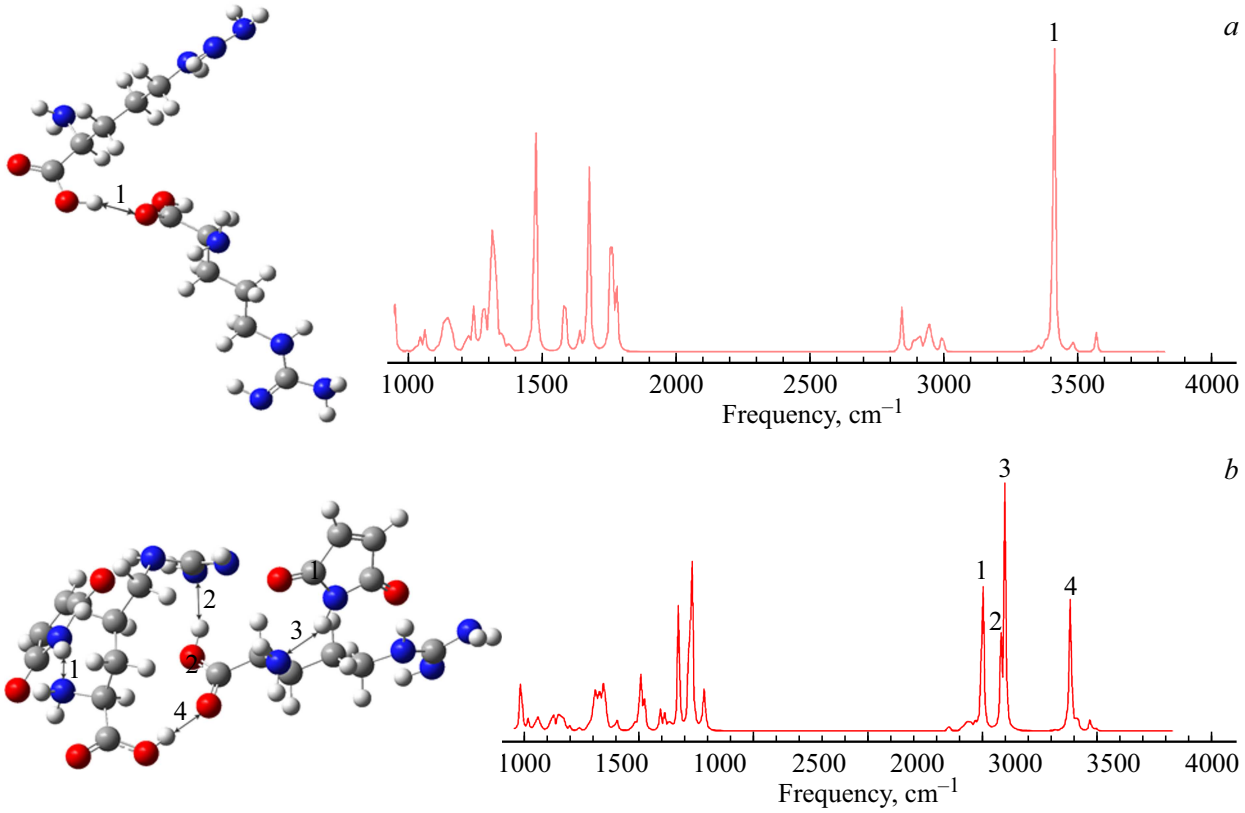

Figure 6. Calculated structures and IR spectra of molecular complexes of two arginine molecules (*a*) and arginine and maleimide (*b*).

Parameters of hydrogen bonds in molecular complexes of amino acids with maleimide (H — histidine, T — tryptophan, A — arginine, L — lysine, M — maleimide)

	Complex	Bond type	H-bond length, Å	Hydrogen bridge length, Å	Frequency, cm^{-1}	Frequency shift $\delta\nu$, cm^{-1}	Bond energy ΔH, kkal/mol	Intensity km/mol
h	H+T	O-H···N	1.68	2.71	2541	957	9.1	2008
i	H+T+M	O-H···N	1.65	2.68	2474	1024	9.4	2357
s	H+A	O-H···N	1.83	2.84	2961	537	6.7	1465
t	H+A+M	O-H···N	1.81	2.81	2921	577	6.9	1727
i	H+L	O-H···N	1.69	2.71	2672	826	8.4	2527
d	H+L+M	O-H···N	1.62	2.66	2363	1135	9.9	2761
i	H+H	O-H···N	1.71	2.73	2730	768	8.1	2247
ne	H+H+M	O-H···N	1.65	2.68	2499	999	9.3	2562
a	A+T	O-H···N	1.80	2.80	2988	552	6.8	1054
r	A+T+M	O-H···N	1.79	2.79	2949	591	7.0	1159
g	A+A	O-H···O	1.87	2.85	3415	148	3.1	715
i	A+A+M	O-H···O	1.87	2.85	3409	154	3.2	615
n	A+L	O-H···N	1.82	2.82	3034	506	6.5	1170
i	A+L+M	O-H···N	1.80	2.80	2986	554	6.8	1372
ne	A+H	O-H···N	1.82	2.82	3047	493	6.4	1080
	A+H+M	O-H···N	1.79	2.79	2957	583	7.0	2090
l	L+T	O-H···N	1.74	2.76	2771	726	7.9	1988
y	L+T+M	O-H···N	1.70	2.72	2673	824	8.4	2221
s	L+A	O-H···N	1.78	2.79	2903	594	7.1	1448
i	L+A+M	O-H···N	1.75	2.76	2827	670	7.5	1982
ne	L+L	O-H···N	1.74	2.75	2808	689	7.6	2016
	L+L+M	O-H···N	1.72	2.74	2741	756	8.0	2546
	L+H	O-H···N	1.76	2.77	2859	638	7.3	1347
	L+H+M	O-H···N	1.74	2.76	2789	708	7.8	2301
t	T+T	O-H···N	1.73	2.75	2756	742	7.9	2229
r	T+T+M	O-H···N	1.72	2.74	2736	762	8.1	1972
y	T+A	O-H···N	1.73	2.75	2769	729	7.9	1962
p	T+A+M	O-H···N	1.73	2.75	2748	750	8.0	2262
t	T+L	O-H···N	1.69	2.71	2651	847	8.5	2752
o	T+L+M	O-H···N	1.66	2.69	2553	945	9.0	3174
ph	T+H	O-H···N	1.72	2.73	2799	699	7.7	3247
a	T+H+M	O-H···N	1.69	2.71	2709	789	8.2	4036
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target protein and, consequently, improves capsule retention and ensures a more complete drug release. Thus, the reported data verify and provide theoretical justification for the results of earlier experimental studies [2,3] that revealed the feasibility of application of maleimide for bioconjugation of protein delivery capsules in targeted therapy.

Conflict of interest

The authors declare that they have no conflict of interest.

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