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A study on the mechanism of interaction between fullerene and cycloheximide for the explanation of the beneficial effect of C_{60} on the processes of spatial memory restoration

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A probable mechanism is proposed for the restoration of spatial memory infringed by the addition of cycloheximide suppressing the protein synthesis in the central nervous system; the spatial memory is restored by the introduction of a C_{60}/PVP fullerene adduct where the fullerene acts as an efficient adsorbent of this inhibitor.

It was found[1] that a complex of C_{60} with poly(*N*-vinylpyrrolidone) (C_{60}/PVP) prevents the infringement of the formation of long-term memory, where such an infringement is caused by the suppression of protein synthesis in the brain by inhibitors interrupting the synthesis chain. The most common inhibitor of protein biosynthesis is cycloheximide, a chlutarimide antibiotic.[2,3] A preliminary microinjection of the C_{60}/PVP complex as a NaCl solution into hippocampus prevents (by more than 90%) the infringement of spatial memory provoked by the introduction of a high dose of cycloheximide suppressing the protein synthesis in the central nervous system.[1] We attempted to give a physical explanation for this experimental fact.

It is well known that poly(*N*-vinylpyrrolidone) is a filler in many medical preparations; for example, it is used to remove toxins. However, its adduct with fullerene, C_{60}/PVP, is more efficient.[4,5] Obviously, this originates from the fact that fullerene, which is known for its biological activity[6,7] and extremely high adsorptivity,[8] plays the role of an active adsorbent of harmful compounds, including cycloheximide. The structure of PVP includes cavities with sizes close to those of C_{60} molecules and is filled by the latter without covalent bonding and thus enables the access of insoluble fullerene molecules into the body.[5] It can be assumed that, upon introduction of the C_{60}/PVP adduct into the hippocampus, C_{60} interacts with cycloheximide introduced earlier. Cycloheximide incorporated in the protein synthesis chain interrupts it, which results in the infringement of the long-term spatial memory. When fullerene appears, the inhibitor is adsorbed on the surface of C_{60} molecules. Thus, the protein synthesis chain is restored; in turn, this results in memory restoration. If a microinjection of C_{60}/PVP was carried out beforehand, the introduction of cycloheximide did not cause appreciable negative consequences because the adduct introduced earlier created the so-called 'blockade' of the inhibitor. As a result, the significant fraction of cycloheximide that is adsorbed on the C_{60} surface cannot significantly affect protein biosynthesis.

In order to prove that the mechanism proposed can actually exist, we carried out numerical calculations on the interaction between cyclohexylimide (inhibitor of protein synthesis) and C_{60}. The calculations were carried out within the semi-empirical quantum-chemical MNDO scheme[9] that proved well in calculations for a huge number of multi-molecular compounds.

At the first stage, MNDO calculations for the cycloheximide molecule, *i.e.*, β-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-oxyethyl]-glutarimide (Figure 1), were carried out. It was found that the molecule is non-planar, which is quite consistent with available experimental data obtained by chemical methods and NMR spectroscopy.[10] It can be assumed that the *cis*- and *trans*-forms of cycloheximide exist (with respect to the arrangement of the Me groups at the aromatic ring). The studies performed have proved that both types of molecule can exist; the difference of the total energies of these modifications is 0.5 eV. We used only the *cis*-form of cycloheximide, which is more stable, for further calculations.

Two ways of binding an inhibitor molecule to the fullerene surface were considered: (*a*) orthogonal to the surface *via* one of the cycloheximide molecule centres, so-called single-centre interaction; (*b*) parallel to the surface, based on the steric conformity between the C_{60} carbon hexagons and cycloheximide aromatic rings, so-called multi-centre interaction. The latter might explain the relative selectivity of sorption and the specific choice of cycloheximine as a ligand by fullerene, taking into account that biosystems[1] contain other possible ligands, including reactive oxygen.

The first way of interaction between fullerene and cycloheximide is the most variable. It is possible to distinguish certain centres of the cycloheximide molecule that can act as possible active points for its adsorption on the C_{60} surface: first, the well-known radical centres of the molecule;[10] second, the oxygen atoms that can readily add various molecules due to the existence of double bonds with carbon atoms of aromatic rings; third, the binding sites of certain H atoms, which can be eliminated from the cycloheximide frame in an aqueous C_{60}/PVP solution, thus leaving active adsorption centres. These variable centres are shown in Figure 1. We performed MNDO calculations for the

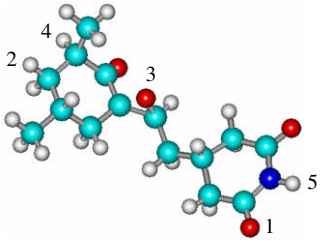

Figure 1 Possible adsorption centres of the cycloheximide molecule.

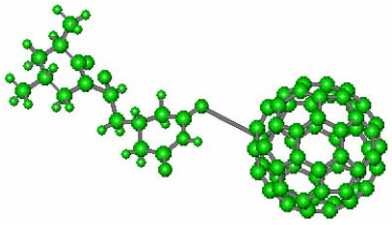

Figure 2 Orthogonal adsorption of a cycloheximide molecule on the fullerene surface, variant (1).

following variants of adsorption of an inhibitor molecule on the surface of a C_{60} fullerene molecule (Figure 2):

(1) a cycloheximide molecule binds orthogonally to the fullerene surface through active centre 1, that is, the oxygen atom of the hydrocarbon fragment;

(2) a cycloheximide molecule binds orthogonally to the fullerene surface through active centre 2, that is, the place where a hydrogen atom is eliminated from the molecule;

(3) a cycloheximide molecule binds orthogonally to the fullerene surface through active centre 3, that is, the oxygen atom at the bond between the hydrocarbon fragments;

(4) a cycloheximide molecule binds orthogonally to the fullerene surface through the well-recognised radical centre 4;

(5) a cycloheximide molecule binds orthogonally to the fullerene surface through centre 5, that is, the site where a hydrogen atom is eliminated from the hydrocarbon fragment.

Analysis of the results revealed that the adsorption of cycloheximide results in a 5% elongation of C–C bonds on the fullerene surface. Analysis of the charge distribution showed that addition of the inhibitor results in a distortion of the C_{60} surface; this distortion is considerable and extends up to the fifth interaction sphere.

Based on the calculated energies, we plotted the curves of potential energy (Figure 3). Analysis of the diagrams showed that each curve contains an energy minimum, *i.e.*, all adsorption variants are plausible. However, variants (2), (3) and (5) correspond to unstable states of the system. Variant (1) is the most energetically favourable (Table 1): $E_{ad} = 6.96$ eV. This is an expected result since the presence of the double bond at the oxygen atom enables cycloheximide binding to the C_{60} surface through a so-called oxygen bridge.

We also performed MNDO calculations for the second type of adsorption of an inhibitor molecule on the fullerene surface, where cycloheximide is oriented parallel to the surface (Figure 4). We found that this kind of adsorption interaction is also possible. In this case, the adsorption energy is $E_{ad} = 1.45$ eV. A comparison of this result with the adsorption energy calculated for variant (1) for single-centre adsorption allows us to state that the orthogonal single-centre interaction through an oxygen atom is more energetically favourable and hence more probable and more common. Moreover, it enables the simultaneous binding of several cycloheximide molecules to one fullerene molecule.

We also studied this multiple adsorption (Figure 5) and found that C_{60} binds several molecules simultaneously rather readily. In this case, the optimum adsorption distances do not exceed 3 Å.

Our calculation results can explain the experimental data obtained by Podolski *et al.*,[11] who found that about 200 μg of cycloheximide is required for the memory suppression effect to occur. The removal of this effect is observed upon addition of 1.75 μg of fullerene. Thus, the studies of multiple adsorption, which is most probable for single-centre interaction, prove the validity of the suggested mechanism of restoration of long-term memory formation.

Table 1 Optimum distances and adsorption energies of a cycloheximide molecule on the surface of C_{60}; charges on atoms forming the bond for different variants.

Variant	R_{ad}/Å	E_{ad}/eV	Charges on atoms forming the bond	
			Fullerene	Cycloheximide
1	3.3	–6.96	0.0226	–0.0909
2	3.3	11.07	–0.0003	–0.0067
3	3.2	10.48	0.0201	–0.1729
4	2.8	–3.56	0.0645	–0.4892
5	3.3	7.56	0.0725	–0.6073

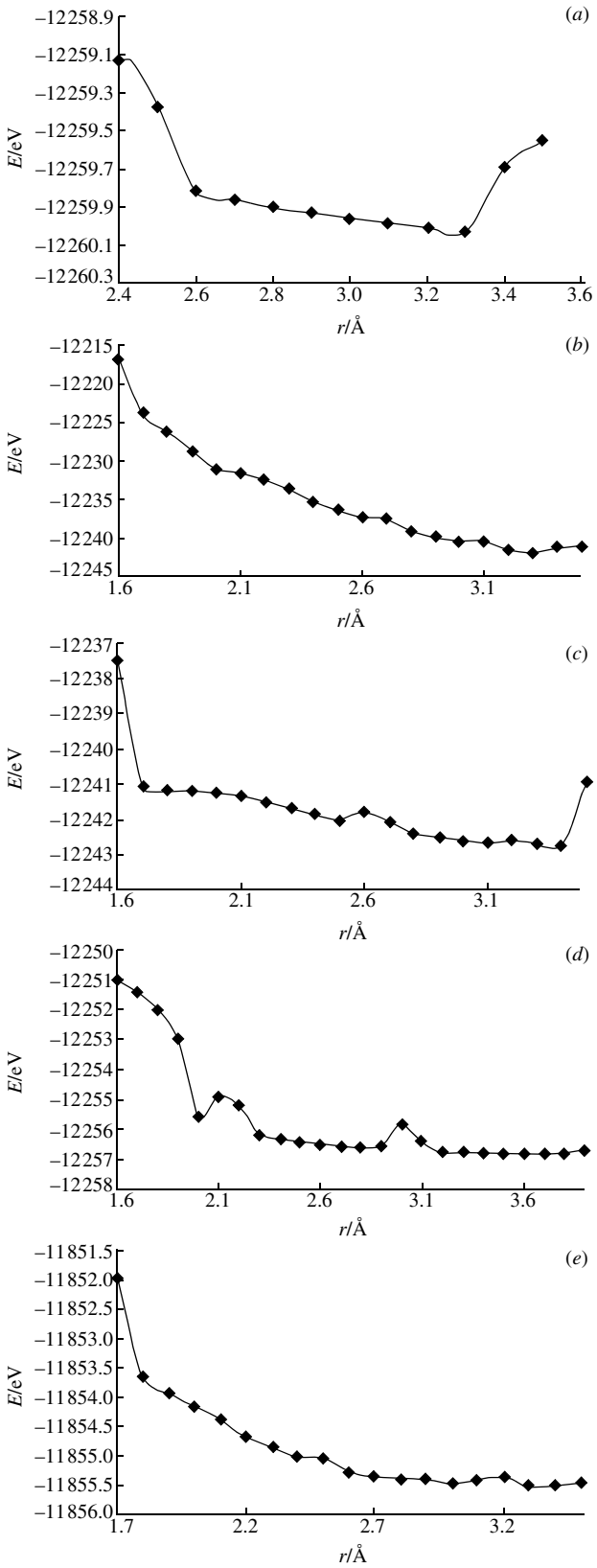

Figure 3 Total energy of single-centre interaction of a cycloheximide molecule with the C_{60} surface as a function of distance between bound atoms for different variants: (*a*) variant (1); (*b*) variant (2); (*c*) variant (3); (*d*) variant (4); (*e*) variant (5).

The geometry of cycloheximide was analysed and its charge and energy characteristics were found. The study of cycloheximide

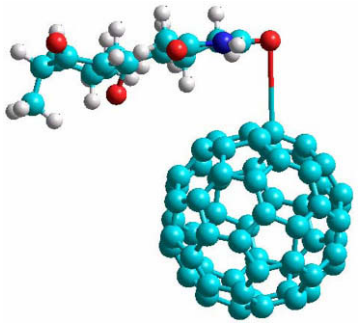

Figure 4 Parallel orientation of a cycloheximide molecule on the fullerene surface.

adsorption on the surface of C_{60} fullerene (perpendicular one-centre adsorption and parallel steric adsorption) allowed us to reveal the possible variants of adsorption, to find the optimum adsorption distances and charges on the interacting atoms of C_{60} and cycloheximide, and to determine the adsorption energies. The energy characteristics obtained allowed us to state that the surface of a fullerene molecule adsorbs cycloheximide rather readily and that it is able to adsorb several inhibitor molecules simultaneously. This can significantly affect the protein synthesis restoration in the central nervous system. To adsorb a cycloheximide molecule, fullerene withdraws it from the protein synthesis chain that has been interrupted by the preliminary addition of cycloheximide in order to simulate real memory loss processes; as a result, the integrity of the synthesis chain is restored. If fullerene is introduced in the neurosystem before cycloheximide, the former blocks the inhibitor by decreasing its active concentration considerably. In turn, this results in the restoration of the long-term memory.

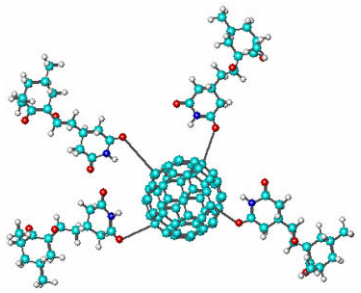

Figure 5 Single-centre binding of several cycloheximide molecules to a fullerene molecule.

Thus, the adsorption of a protein synthesis inhibitor, *viz.*, cycloheximide, on the surface of a C_{60} fullerene molecule is a probable mechanism of memory restoration.

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