DNA—metal(II) ion—phosphatidylcholine complexes: structure calculations and stability estimates by molecular mechanics

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The structures and formation energies of nucleic acid—phospholipid complexes both in the absence and in the presence of Mg^{2+} ions were calculated taking double-stranded trinucleoside diphosphates NpNpN or heptanucleotides ApAp(NpNpN)pApA, composed of 64 possible combinations of genetic code, and phosphatidylcholine as model compounds. The dependence of intramolecular interactions on the primary structure of nucleic acid molecules and on the presence of a cationic bridge was revealed. The formation energies and structure of oligonucleotides were found by molecular mechanics calculations with the AMBER force field. The structures of phospholipid and $MgCl_2$ molecules were calculated by the semiempirical PM3 method, while the energies of phospholipid—oligonucleotide complexes were calculated by the molecular mechanics method. Calculations of complexes were carried out with consideration of solvation effects. Considerable gain in the formation energy of triple complexes (13—14 kcal mol^{-1}) is achieved due to the presence of the electroneutral metal bridge. A tendency toward increasing the stability of triple complexes containing guanosine- and cytidine-enriched triplets was revealed.

Key words: phospholipid, nucleic acids, complexes, structure, formation energy, computer simulation, molecular mechanics, quantum-chemical calculations.

Four main classes of biopolymers, namely, proteins, nucleic acids, polysaccharides, and lipids are known. Only lipid-protein and nucleic acid-protein interactions and corresponding complexes were studied in detail.[1] Interactions of nucleic acids with lipids and phospholipids have been poorly studied. Almost no information on structural-conformational and energy characteristics of nucleic acid—phospholipid complexes is available. At the same time, complexes of DNA with an internal nuclear membrane can be isolated and studied.[2,3] The formation of DNA and RNA complexes with phospholipid liposomes in model systems was studied in more detail.[4—6] It is known that lipids can affect stability of the double DNA helix by causing its local unwinding or the appearance of supercoiled DNA fragments.[7,8] Direct nucleic acid-phospholipid binding is hampered because of the electrostatic repulsion between negatively charged phosphate groups of lipids and DNA. However, it can be strengthened by introducing cationic bridges (*e.g.*, metal ions (M^{2+})) or positively charged fragments of polypeptides or proteins.[9,10]

The aim of this work is to calculate the structure and formation energies of nucleic acid—phospholipid complexes (with phosphatidylcholine (PC) as phospholipid) both in the absence and in the presence of the Mg^{2+} ion, as well as to reveal the dependence of intramolecular interactions on the primary structure of nucleic acid molecules and on the presence of the Mg^{2+} cationic bridge. Similar complexes of double-stranded nucleic acids with phosphatidylcholine liposomes have been studied turbidimetrically,[5] by differential scanning microcalorimetry,[7,11,12] and by spin and fluorescent labels.[6,7]

To calculate structural and energy characteristics of such complexes, we determined energetically favorable mutual arrangements of the lipid and oligonucleotides, as well as the formation energies of lipid—nucleic acid complexes between PC and oligonucleotides with different sequences of nitrogen bases using the molecular mechanics method. All calculations were performed under the assumption that nucleic acid molecules have the B-form and that the carbohydrate core has the 2'-*endo*-conformation. Of all cations present in the cell we chose

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the Mg^{2+} ion as the only divalent metal ion whose concentration in the nucleus can reach high values.

Calculation procedure

Calculations were performed using the HYPERCHEM program (Version 4.5). The formation energies and structures of oligonucleotides were found by the molecular mechanics method with the AMBER force field developed for proteins and nucleic acids.[13] The structures of phospholipid and $MgCl_2$ molecules were calculated by the semiempirical PM3 method,[14] whereas the energies of phospholipid—oligonucleotide complexes were calculated by molecular mechanics with fixed lipid geometry. The structures of the complexes were optimized using an original program written in the Visual Basic® programming language, which made it possible to move the lipid molecule (with retention of its preliminarily optimized geometry) with respect to nucleotides and cationic bridge. The complexation energies were determined as differences between the total energies of the complexes and corresponding isolated molecules.

Results and Discussion

We began the simulation of nucleic acid—phospholipid complexes with calculating the *O,O'*-diethyl phosphate—Mg^{2+}—diadenosine monophosphate (ApA) complex, whose components served as phospholipid, bridging metal ion, and nucleic acid models, respectively. It was assumed that such a model will to some degree reflect the interaction between the phosphate groups of the phospholipid and nucleic acid molecules and Mg^{2+} ions. The calculated formation energy of the complex was 392 kcal mol^{-1}. However, lipid-nucleic acid interactions belong to much weaker intermolecular interactions, so the value indicated seems to be overestimated. This is likely due to oversimplification of the bridge structure in this model. Structures with an electroneutral magnesium chloride bridge, which are described below, appeared to be more adequate to ideas of weak interactions. According to different estimates, the formation energies of such complexes do not exceed 1—2 kcal mol^{-1} (with respect to 1 mole of phospholipid per pair of bases).[7,13,14]

Next we performed a molecular mechanics simulation of nucleic acid—phospholipid complexes with PC as phospholipid. Phosphatidylcholine plays an important role in the DNA-phospholipid interaction (for simplicity, hydrophobic residues of PC were shortened to butyric acid fragments). Here, double-stranded trinucleoside diphosphates (NpNpN) composed of 64 possible combinations of guanosine (G), adenosine (A), cytidine (C), and thymidine (T), which are constituents of genetic code, were used as models of nucleic acid molecules. Na^+ ions were added to meet the condition of electroneutrality of the entire system. The complementary chain of nucleic acid molecule stabilizes the complex, thus making it possible to increase similarity between the model complex and that existing in solution. The parameters of (NpNpN)—PC and (NpNpN)—$MgCl_2$—PC complexes listed in Table 1 were calculated. Solvation effects were taken into account by placing the complex molecules into a cubic unit cell containing up to 1668 water molecules; the minimum distance between the complex and solvent molecules was 0.46 Å.

The most complex model corresponded to ApAp(NpNpN)pApA—$MgCl_2$—PC complexes. In this case, two adenylic acid molecules were added in positions 3′ and 5′ of NpNpN trinucleotide in order to reduce the influence of boundary effects on the formation energies of the complexes. The sequence of three neutral nitrogen base molecules was varied to obtain 64 possible heptanucleotides.

We calculated the formation energies of all complexes with the above-mentioned trinucleotides or heptanucleotides. The results of calculations are listed in Table 1. In each column of Table 1, NpNpN trinucleotides are listed in descending order of complex stabilities. From the data in Table 1 it follows that the NpNpN—$MgCl_2$—PC derivatives, in which nucleic acid-phospholipid interactions occur with participation of a bridging metal ion, are 13—14 kcal mol^{-1} more stable than NpNpN—PC complexes in which the choline group serves as an analogous bridge. It should be noted that the mutual arrangement of the constituents of a bridge-containing complex at the energy minimum corresponds to the location of the Mg^{2+} ion between phosphate groups of the PC and DNA molecules. Such a structure obtained as a result of computer experiments indicates the possibility of the formation of a DNA-phospholipid contact at which the phosphate groups of interacting molecules form chemical bonds with the Mg^{2+} ion, which plays the role of a bridge. This model requires that Mg^{2+} cations necessarily be shielded by anions (*e.g.*, Cl^-); otherwise it corresponds to extremely stable compounds similar to inorganic phosphates.

Depending on the structure of NpNpN trinucleotides, the formation energies of NpNpN—$MgCl_2$—PC and ApAp(NpNpN)pApA—$MgCl_2$—PC complexes differ by 1.7—2.6 kcal mol^{-1}. A tendency toward increasing the stability of PC complexes with guanosine-enriched triplets can be pointed out (see Table 1). For instance, complexes containing GGT, GGG, GGA, and GGC nucleotide sequences are by not less than 0.3 kcal mol^{-1} more energetically favorable than other complexes. The formation energies of ApAp(NpNpN)pApA—$MgCl_2$—PC complexes are 10—13 kcal mol^{-1} lower than those of NpNpN—$MgCl_2$—PC complexes. This is likely due to changes in the packing of acyl residues upon elongation of the oligonucleotide chain. From the data in Table 1 it follows with certainty that the phospholipid—nucleic acid structures containing oligonucleotides with three or two guanosine residues are the most energetically favorable.

Thus, we proposed models and estimated the stabilities of nucleic acid—phospholipid complexes with a bridging metal ion or without it. It was shown that a considerable gain in the formation energy of triple complexes (13—14 kcal mol^{-1}) is obtained because of

Table 1. Formation energies (E/kcal mol^{-1}) of nucleic acid—phosphatidylcholine complexes NpNpN—PC, NpNpN—$MgCl_2$—PC, and ApAp(NpNpN)pApA—$MgCl_2$—PC depending on the sequence of nitrogen bases in oligonucleotides and on the presence of a bridging metal ion (Mg^{2+})

NpNpN—PC		NpNpN—$MgCl_2$—PC		ApAp(NpNpN)pApA—$MgCl_2$—PC		NpNpN—PC		NpNpN—$MgCl_2$—PC		ApAp(NpNpN)pApA—$MgCl_2$—PC	
Triplet	$-E$	Triplet	$-E$	Triplet	$-E$	Triplet	$-E$	Triplet	$-E$	Triplet	$-E$
GCC	13.6	GTC	27.1	CCC	16.3	CAC	12.3	TAT	26.3	CAG	14.9
GTC	13.3	GGC	27.1	TCC	15.9	AAT	12.3	TCT	26.3	GGA	14.9
TCC	13.3	GAC	27.0	CCA	15.9	TAA	12.2	TTA	26.2	GTA	14.8
ACC	13.2	GCC	27.0	GCC	15.8	CCA	12.2	TGA	26.2	TAA	14.8
GCT	13.1	GTT	26.9	CTC	15.7	CTT	12.2	AAA	26.2	CAT	14.8
TTC	13.1	GGT	26.9	CCG	15.7	GGA	12.1	ACA	26.2	GAA	14.7
GAC	13.1	GAT	26.8	CCT	15.6	GTG	12.1	TAA	26.1	ACG	14.7
ATC	13.0	GCT	26.8	CGC	15.6	TGT	12.1	TCA	26.1	AGC	14.7
GCA	12.9	GTA	26.8	TCA	15.6	AAA	12.1	ATG	26.0	TGG	14.7
GTT	12.9	GGA	26.8	GCA	15.5	TCG	12.1	AGG	26.0	TTG	14.7
TCT	12.9	GAA	26.7	CAC	15.5	CGC	12.0	CTC	26.0	GGG	14.6
TAC	12.8	GCA	26.7	ACC	15.3	ACG	12.0	TTG	26.0	ATC	14.6
CCC	12.8	GTG	26.6	CGA	15.3	AGT	12.0	CGC	26.0	TGT	14.6
ACT	12.8	AGC	26.6	CTA	15.3	CTA	12.0	TGG	26.0	GTG	14.6
GGC	12.8	GGG	26.6	TCG	15.3	CAT	11.9	AAG	26.0	ACT	14.5
AAC	12.7	ATC	26.5	TGC	15.3	TGA	11.9	ACG	25.9	TTT	14.5
GTA	12.7	TTC	26.5	TTC	15.3	TTG	11.9	CAC	25.9	GGT	14.5
TCA	12.7	TGC	26.5	GGC	15.3	GAG	11.9	CCC	25.9	TAG	14.5
TTT	12.7	GAG	26.5	TCT	15.3	AGA	11.8	TAG	25.9	AAC	14.5
GAT	12.6	AAC	26.5	GCG	15.2	ATG	11.8	TCG	25.9	GTT	14.5
CTC	12.6	GCG	26.5	CAA	15.2	CAA	11.7	CTT	25.9	GAG	14.4
ACA	12.6	ACC	26.4	GTC	15.2	TAG	11.6	CGT	25.9	TAT	14.4
ATT	12.6	TAC	26.4	TAC	15.1	CGT	11.6	CAT	25.8	AGA	14.3
TGC	12.5	ATT	26.4	GCT	15.1	CCG	11.6	CCT	25.8	GAT	14.3
AGC	12.4	AGT	26.4	CTG	15.0	GGG	11.6	CTA	25.7	ATA	14.3
GAA	12.4	TCC	26.4	GAC	15.0	AAG	11.5	CGA	25.7	AAA	14.2
TTA	12.4	TTT	26.4	CGG	15.0	CGA	11.4	CAA	25.6	AGG	14.1
TAT	12.4	TGT	26.4	ACA	15.0	CTG	11.4	CCA	25.6	ATG	14.0
CCT	12.4	AAT	26.3	TGA	15.0	TGG	11.3	CTG	25.5	AAG	13.9
ATA	12.4	ACT	26.3	CGT	15.0	AGG	11.2	CGG	25.5	AGT	13.9
GGT	12.3	ATA	26.3	TTA	14.9	CAG	11.1	CAG	25.4	ATT	13.8
GCG	12.3	AGA	26.3	CTT	14.9	CGG	10.8	CCG	25.4	AAT	13.7

the presence of the electroneutral metal bridge as compared with the case of direct DNA fragment-phospholipid interaction; a tendency toward increasing the stability of complexes containing guanosine- and cytidine-enriched triplets was revealed.

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References

1. Yu. A. Ovchinnikov, *Bioorganicheskaya khimiya* [*Bioorganic Chemistry*], Nauka, Moscow, 1985 (in Russian).
2. F. A. Manzoli, S. Capitani, and N. M. Maraldi, in *Advances in Enzymology Regulation*, Ed. G. Weber, Pergamon Press, Oxford—New York, 1979, **17**, 175.
3. M. P. Moyer, *Int. Rev. Cytol.*, 1980, **61**, 1.
4. V. G. Budker, A. A. Godovikov, and L. P. Naumova, *Nucl. Acids Res.*, 1980, **8**, 2499.
5. V. V. Kuvichkin and A. G. Sukhomudrenko, *Biofizika*, 1987, **32**, 628 [*Sov. Biophys.*, 1987, **32** (Engl. Transl.)].
6. R. I. Zhdanov, L. A. Volkova, and A. I. Petrov, *Appl. Magn. Reson.*, 1994, **7**, 115.
7. R I. Zhdanov and V. V. Kuvichkin, in *New Developments in Lipid-Protein Interactions and Receptor Function*, Ed. K. W. A. Wirtz, Plenum Press, New York, 1993, 249.
8. E. Palecek, M. Robert-Nicoud, and T. Jovin, *J. Cell Sci.*, 1993, **104**, 653.
9. A. Koiv, J. Palvimo, and P. K. J. Kinnunen, *Biochemistry*, 1995, **34**, 8018.
10. R. I. Zhdanov, L. A. Volkova, and V. V. Rodin, *Appl. Magn. Reson.*, 1994, **7**, 131.
11. E. E. Bichenkov, V. G. Budker, and I. K. Korobeinikova, *Biol. Membrany*, 1988, **5**, 843.
12. V. Kuvichkin, S. Kuznetsova, and R. Zhdanov, *J. Mol. Med.*, 1997, **75**, No. 5, B33.
13. S. J. Weiner, P. A. Kollman, D. A. Case, U. C. Singh, C. Ghio, G. Alagona, S. Profeta, and P. Weiner, *J. Am. Chem. Soc.*, 1984, **106**, 765.
14. J. J. P. Stewart, *J. Comput. Chem.*, 1989, **10**, 209.
15. B. Fedorov, L. Lopukhov, A. Merkin, P. Dyachkov, and R. Zhdanov, *J. Mol. Med.*, 1997, **75**, No. 5, B28.

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