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Fiber Molecular Model of Collagen Triple Helix and DNA Double Helix Complex in Aqueous Solution

David V. Svintradze and George M. Mrevlishvili
Department of Physics, Tbilisi State University, Tbilisi, 0128, Georgia

Abstract: The present study demonstrated the one example of biologically important and molecular self-assembly system is a collagen-DNA ordered aggregates which spontaneously forms in aqueous solution. DNA binds to collagen directly to form DNA-collagen complex. Interaction between the collagen and DNA leads to destruction of the hydration shell of the triple helix and stabilization of the double helix structure. From a molecular biology point of view this nano-scale self-assembling superstructure could increase the stability of DNA against the nucleases during collagen diseases and the growth of collagen fibrils in the presence of DNA. In addition the complex is one of the most useful carrier material for new drug and gene delivery systems as well as for gene therapy. The model suggests that DNA, containing well-arranged phosphate groups, helps the collagen to make ordered aggregates-extraordinary twisted fiber complex. The form of this complex can be controlled by variations of DNA's molecular weight and types. This result indicates that by choosing weight and type of DNA, containing various gene vectors, can be controlled the shape of the complex. The cell recognizes the shape of DNA-collagen complex, and effectively takes up complexes of certain shapes. Therefore, it is expected that when the correlation between the shape of complex and uptake of the complex into the cell is clarified, the efficiency of introduction of DNA into cells with the use of collagen will increase even more. Such extraordinary complex induces distraction hydration shell of collagen triple helix, stabilization hydration shell of ds-DNA, stability of DNA against the nucleases and this complex between collagen and DNA determines unusual properties of collagen fibers.

Key words: Collagen-DNA complex, molecular model, fiber

The living cell exists as a result of processes that generate complex, multi-component, functional structures by self-assembly. Molecular self-assembly is a process in which molecules (or part of molecules) spontaneously form ordered aggregates and involves no human intervention. The interactions involved usually are non-covalent (such as metal-ligand bonds, hydrogen bonds, or van der Waals' forces-hydrophobic interactions) (Whitesides and Boncheva, 2002). In this paper is demonstrated that one example of the biologically important and molecular self-assembling complex system is a collagen-DNA ordered aggregates spontaneously formed in aqueous solutions.

Usually DNA remains as a giant molecule packed in the cells nucleus and is composed of proteins in the chromatin fibers. However, during some pathological processes, in particular, during collagen diseases, DNA is released from the nucleus and is placed in the matrix between the cells-in the connective tissue (Poverenny, 1968). According to molecular biology data, the assembly of the

Corresponding Author: Dr. David V. Svintradze, Department of Physics, Tbilisi State University, Tbilisi, 0128, Georgia Tel: +995 32 290834

complex between collagen and DNA determines the stability of DNA against the nucleases and indicates a development of autoimmune reaction (Poverenny, 1968). In addition, collagen is one of the most useful carrier materials for new drug and gene delivery systems (Ruszczak and Friess, 2003; Gelse *et al*., 2003; Maeda *et al*., 1999; Olsen *et al*., 2003; Sano *et al*., 2003; Wallace and Rosenblatt, 2003). Accordingly it is very important study the principles of organization of the molecular complex between collagen and DNA. As demonstrated in (Kitamura *et al*., 1997; Kaya *et al*., 2005) growth of collagen fibrils in the presence of DNA was more rapid than in the absence of DNA. These results suggest that DNA not only absorbs to collagen but induces the extraordinary fibrillogenesis of collagen. Mrevlishvili and Svintradze (2005a, b) is demonstrated that one example of biological important and molecular self-assembly complex system is the collagen-DNA ordered aggregate which is spontaneously formed in aqueous solution. Interaction between the collagen and DNA leads to destruction of the hydration shell of the triple helix and stabilization of the hydration shell and structure of the double helix of DNA (Mrevlishvili and Svintradze, 2005a). According to the molecular model (Mrevlishvili and Svintradze, 2005b) DNA containing well-arranged phosphate groups helps to make this complex and these groups and water layers play key roles during collagen-DNA ordered aggregation in aqueous solution.

Collagen is the major structural protein in mammals. Collagen triple helix consists of three polyproline-II like chains wrapped around a common axis (Bella *et al*., 1994). The three chains present a repetitive sequence X-Y-Gly, where X and Y are often amino or imino-acids (usual proline (Pro) or 4(R)-hydroxyproline (Hyp) residues). As a model is discussed collagen with repetitive sequence Pro-Pro-Gly, which has CH groups besides the helix (in our opinion these CH groups make H-bonds with DNA). Collagen triple helix in aqueous solution presents as a stiff, rod-like structure, about L(collagen) = 300nm (280 nm) in length and d(collagen) = 1.5nm in diameter (Fraser *et al*., 1979; Privalov, 1982). According to the DSC (Privalov, 1982; Mrevlishvili, 1974), low-temperature calorimetry and NMR data (Mrevlishvili, 1998; Sharimanov *et al*., 1979), the stabilization of collagen and the enormously large enthalpy of disruption of its structure can be mainly the result of extensive hydrogen bonding network inside the triple helix and in the hydration shell of molecule. According to high resolution X-ray diffraction analysis each triple helix is surrounded by a cylinder of hydration, with an extensive hydrogen bonding network between water molecules and peptide acceptor groups (Bella *et al*., 1994). Thus H-bond network in the hydration shell of triple helix determines not only stability of this structure but the mechanism of interaction between molecules in the hierarchical assembly of helices at different levels of organization.

With respect to the role of bound water on the energetic and stabilization of the native macromolecule conformations in aqueous media, the triple helix of collagen and double helix of DNA have many common features. The DNA double helix in aqueous solution stabilized by H-bounds between complementary base pairs (A = T, G = C), stacking interactions along the helix axis and interactions with surrounding water layers. Hydration plays a major role not only in the stability of the 3D structure of double helix, but in the assembly of different forms (A-, B-, Z-) of ds-DNA and their conformational dynamics (Bloomfield *et al*., 2000; Mrevlishvili *et al*., 2002;). It is well known that the most principal conformation for DNA in aqueous solution is B-form, with 10 base pairs per turn and diameter of d(DNA) = 2 nm (height of a turn is 3.6 nm). This bound water in the multi-layer hydration shell of double helix (Bloomfield *et al*., 2000; Mrevlishvili *et al*., 2002; Arai *et al*., 2005; Bastos *et al*., 2004;), having two possible orientations (depending on content of base pairs (Arai *et al*., 2005)), plays an important role in the duplex stability and as is demonstrated in Mrevlishvili and Svintradze (2005a) disrupts hydration shell of the triple helix of collagen. The model for ds-DNA in

Table.1: Thermodynamic parameters of Collagen and DNA complex melting

	Collagen melting			DNA melting		
	Temperature	Enthalpy	Heat capacity increment	Temperature	Enthalpy	Heat capacity increment
Sample	T/°C	$\triangle H/Jg^{-1}$	$\triangle Cp/Jg^{-1}K^{-1}$	T/°C	$\triangle H/Jg^{-1}$	$\triangle Cp/Jg^{-1}K^{-1}$
Native	36±0.1	75±7	0.2±0.02	74.3±0.1	44±4	0.25±0.03
Complex	40±0.1	22±2	0.6±0.06	74.4±0.1	45±5	0

diluted aqueous solution is the worm-like chain. This chain represents the behavior intermediate between the rigid rod (like collagen) and the random coil, thus taking into account the local stiffness but long-range flexibility of the double helix (Bloomfield *et al*., 2000). The wormlike chain is defined by its contour length L (measured along the helix axis) and its persistence length α: $L = Nb = 2N\alpha$, $\alpha \ll L = 50$ nm, N is the number of statistical segments; diameter of ds-DNA = 2 nm.

From a condensed state physics point of view, the most interesting process in the diluted solutions of collagen and DNA is the "triple helix↔3 random coil" and "double helix↔2 random coil" phase transitions. These reactions, accompanied by drastic changes of many physical properties of solution, including the heat capacity and excess heat capacity change in the temperature intervals of biopolymers transitions (Fig. 1a and b).The thermodynamic parameters of these conformational transitions, obtained by using of DSC- DASM-4, is shown in the Table 1. In both cases, disruption of ordered H-bounded network in the hydration shells of the macromolecules determines the high value of the enthalpy of transition for collagen triple helix (Privalov, 1982) and composes a 70% of the enthalpy value of the transition of the DNA double helix (Mrevlishvili *et al*., 2002; Chalikian *et al*., 1999). The solution condition (0.015 M citric buffer, 0.15 M NaCl, pH = 4.0) for preparation of a molecular self-assembling complex of collagen-DNA determines the excellent and simultaneous solution of both components and ordered aggregates are spontaneously formed in this aqueous solution, without macroscopic aggregation of components (concentration is - 0.5 mg mL^{-1} for collagen and 0.6 mg $mL^{-1}$for DNA). The temperature dependence of the heat capacity (Fig. 1c) reveals the following characteristics of the collagen-DNA complex "phase" transition: (1) the heat capacity of the collagen-DNA complex is a nonlinear function of temperature in the range from 0 to 35°C; (2) in the temperature range of "collagen transition" the heat absorption peak indicates, that the enthalpy of triple helix melting significantly decreases ($\Delta H = 22\pm0.2$ J g^{-1}, Table 1); also the heat capacity increment significantly increases $\Delta cp_{,Collagen} = 0.6 \pm 0.06$ J $g^{-1}K^{-1}$ after this transition; (3) on the contrary, the DNA duplex melting thermodynamic parameters in the complex do not change in comparison with the "free" DNA in solution (Table 1), $\Delta Cp_{,DNA} \approx 0$. This indicates, that the heat capacity increment during the complex phase transition is determined by the conformational changes on the first stage of transition, when collagen triple helix melts.

As is known, the water molecules strongly bound to the collagen are the main contributors to the enthalpy and the entropy of collagen melting (Privalov, 1982). The total amount of water bound by the collagen ("unfreezable water") is about 0.5 g g^{-1} of collagen (Mrevlishvili *et al*., 1974; Mrevlishvili, 1998). Recalculating it per triplet, under a sufficiently strong influence of collagen there are about 7-8 water molecules. It is evident that not all these molecules interact equally with collagen as only the strongly bound water molecules (0.30±0.01 g g^{-1} of collagen or 5-6 water molecules per triplet) are released at collagen denaturation (Mrevlishvili, 1998; Sharimanov *et al*., 1979). Important results have been obtained in calorimetric studies of collagen melting in the presence of a limited amount of water (Privalov, 1982; Luescher *et al*., 1974; Monaselidze and Bakradze 1969). With a decrease in water

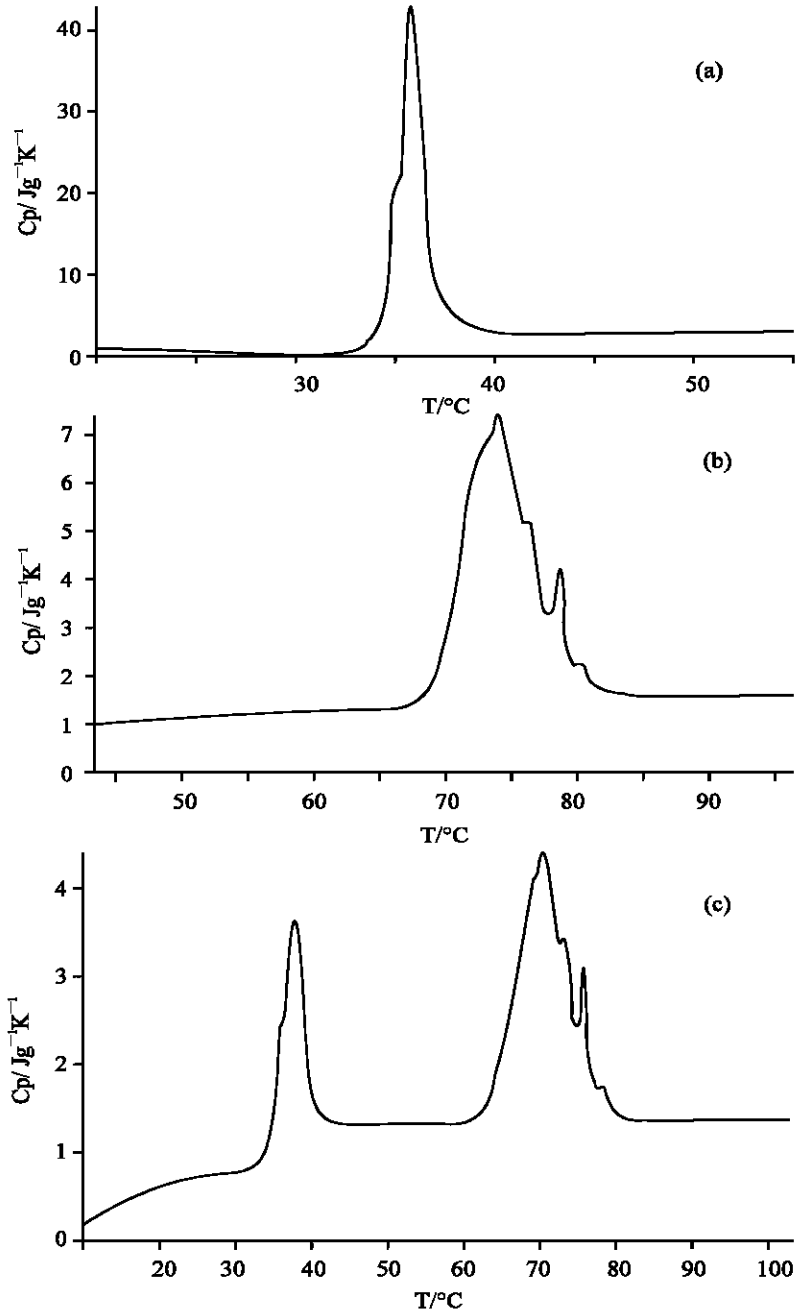

Fig. 1: Temperature dependencies of the partial heat capacity (Cp) of collagen (rat skin) (a), DNA (calf thymus) (b) and for complex collagen-DNA (c);(0.015 M citric buffer, 0.15 M NaCl, pH = 4.0); (concentration in mixture is - 0.5 mg mL^{-1} for collagen and 0.6 mg mL^{-1}for DNA). (a),(b)-Cp recalculated per gram of collagen and DNA, and (c)-gram of complex, respectively;

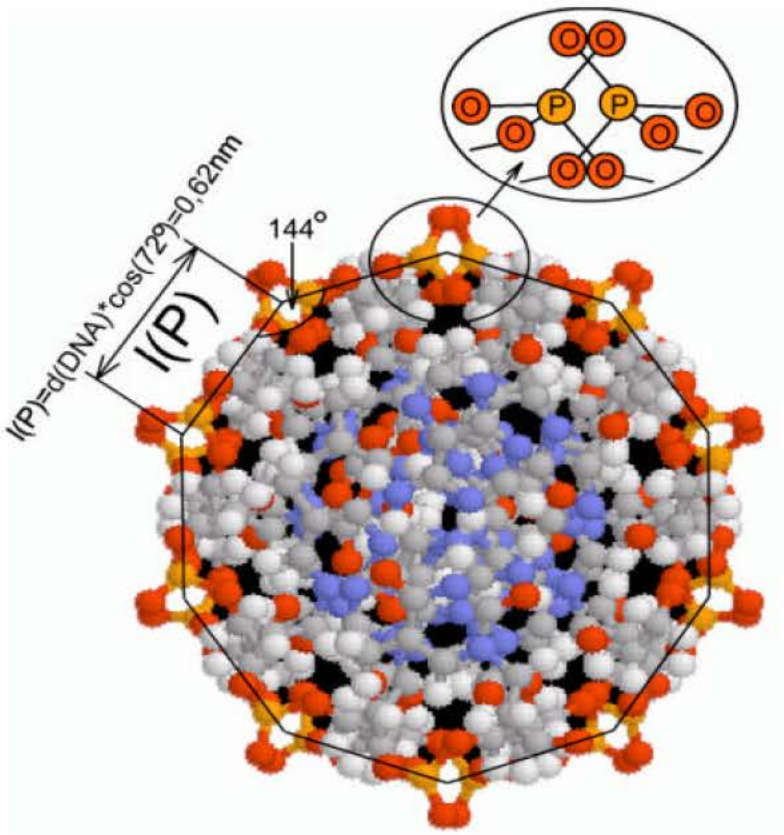

Fig. 2: The cross-section of transverse to the common axis of DNA

concentration below the critical value (about 3 water molecules per triplet) the enthalpy of collagen melting rapidly falls, while the melting temperature increases, i.e. at a water deficit the entropy of the collagen transition in the disordered state decreases. It follows that a small value of the enthalpy of melting of triple helix in the collagen-DNA mixture (Fig. 1c and Table 1), indicates disruption of the ordered H-bounded water network during the self-assembling of collagen-DNA ordered aggregates. The significant value of the $\Delta Cp_{collagen}$ on the first step of the complex transition also suggests this type of rearrangement in the hydration shell of triple helix in the collagen-DNA superstructure. At the same time the stability of the DNA double helix increases (Table 1). It follows that ordered water spines and clusters in the grooves of double helix maintain its structure.

As it was mentioned, in the B form DNA has 10 base pairs per turn, thus cross-section of transverse to the common axis is regular ten-angle inscribed into the circle with diameter of 2 nm (d(DNA) = 2 nm). Phosphate groups are located at the vertexes of the ten-angle (as the sum of the angles in n-angle is 180·(n-2) consequently each angle of the cross-section will be $\frac{180^0 \cdot (n-2)}{n} = \frac{180^0 \cdot (10-2)}{10} = 144^0$ Fig. 2). The side length of this ten-angle will be

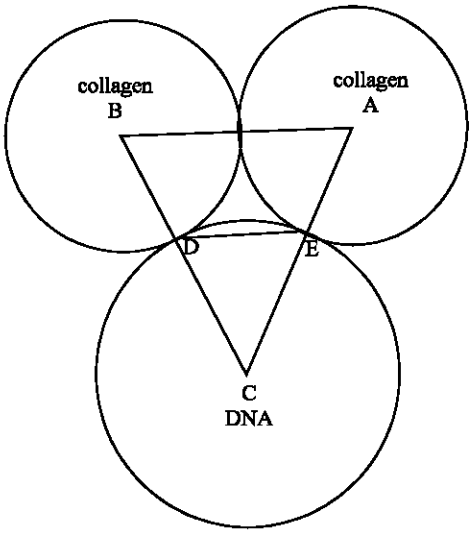

Fig. 3: The distance ED = l(collagen) between two neighboring H-bond locations of collagen with DNA by the condition that collagen molecules are in touch with each other; AB = d(collagen) = 1,5 nm; AC = d(DNA) + d(collagen)/2; EC = d(DNA)/2 (radius of DNA). Triangles ΔABC and ΔEDC are similar triangles (ΔABC~ΔEDC), i.e., ED/AB = EC/AC or l(collagen) = d(DNA).d(collagen)/d(collagen) + d (DNA) => l(collagen) ≈ 0.86 nm

l(P) = d(DNA). cos144°/2 ≈ 0.62 nm (l(P) projection of distance between phosphate groups on the plane transverse to the common axis). In our opinion the complex arises due to H-bonds between CH-groups of collagen and phosphate groups of DNA, i.e., it has to be calculated the distance between two neighboring H-bond locations of collagen with DNA by the condition that collagen molecules are in touch with each other (Fig. 3).

$$l(\text{collagen}) = \frac{d(\text{DNA}) \cdot d(\text{collagen})}{d(\text{DNA}) + d(\text{collagen})} \approx 0{,}86\text{nm} > 0{,}62\text{nm} = l(\text{P})$$

According to this relation it can be considered following model of DNA-collagen complex (Fig. 4), where collagen makes hydrogen bonds with four phosphate groups of a turn of DNA. If it is taken into account the length of collagen L(collagen) = 300 nm, the collagen will have H-bonds with $4 \cdot (L(\text{collagen})/3{,}6) \approx 332$ phosphate groups (hydrogen bonds are created directly: collagen(CH)---DNA(PO_4), or by water molecules: collagen(CH)---H_2O---DNA(PO_4)). According to the calculation, five collagen molecules arrange around each segment of DNA (length of segment equals to collagen length). Number of such segments depends on molecular weight of DNA (for example: for DNA with molecular weight of about $5 \cdot 10^6$ Dalton having counter length L≈3000 nm there exist ten segments (Mrevlishvili and Svintradze, 2005a). Thus there arises the structure similar to collagen fibrils, where DNA plays the role of polymer matrix.

According to all these results it can be proposed the model of self-assembling complex of collagen-DNA spontaneously formed in aqueous solution (Fig. 5). In this model, the worm-like chains of DNA duplex, with the persistent length α = 50 nm and a contour length L ≈ 3000 nm, interact with the rigid rod–like structure of triple helix of collagen (l = 300 nm) by electrostatic forces using overlap

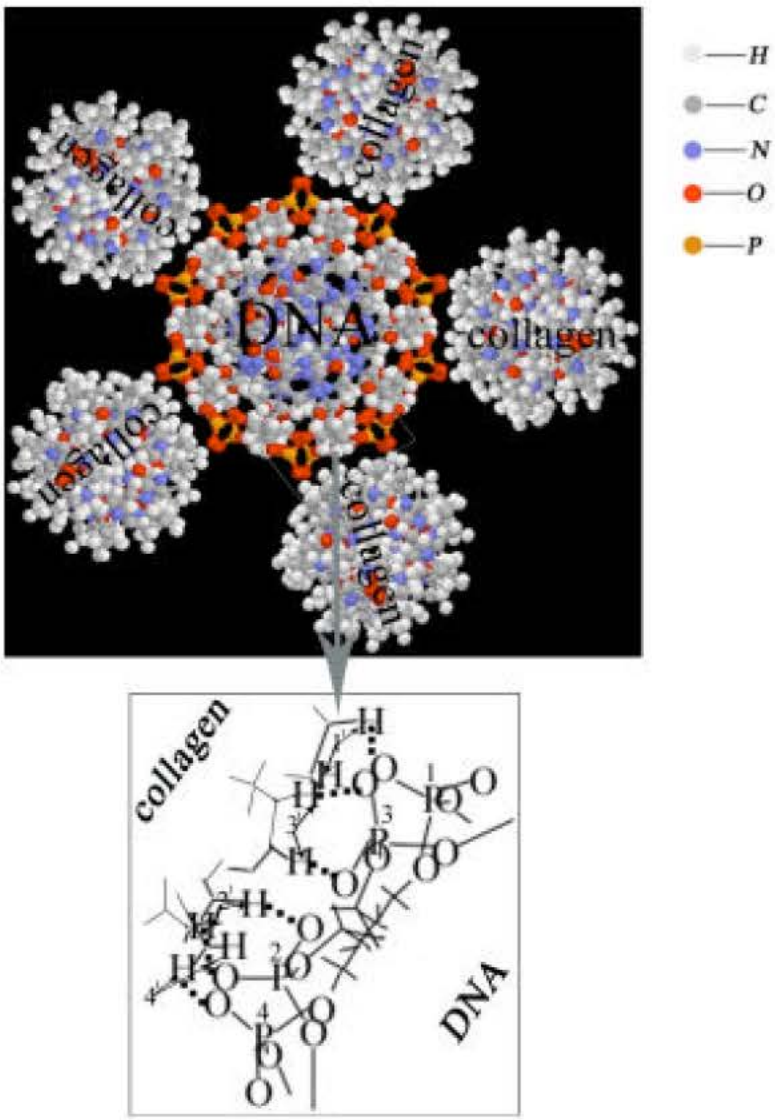

Fig. 4: The molecular model of collagen-DNA complex. Hydrogen bonds arise between CH and phosphate groups. 1,3 phosphate groups are situated on the first chain of ds-DNA and the others 2,4 phosphate groups (which are below the 1,3 phosphate groups) are situated on another chain. H-bonds are created between 1,3 ↔ 1^1,3^1 and 2,4 ↔ 2^1,4^1 groups (1^1,3^1,2^1,4^1 are CH groups of Gly and Pro amino acids situated on the triple helix of collagen and 2^1,4^1 CH groups are situated below of 1^1,3^1 CH groups. Such H-bonds are created directly or by water molecules, which aren't shown in the picture)

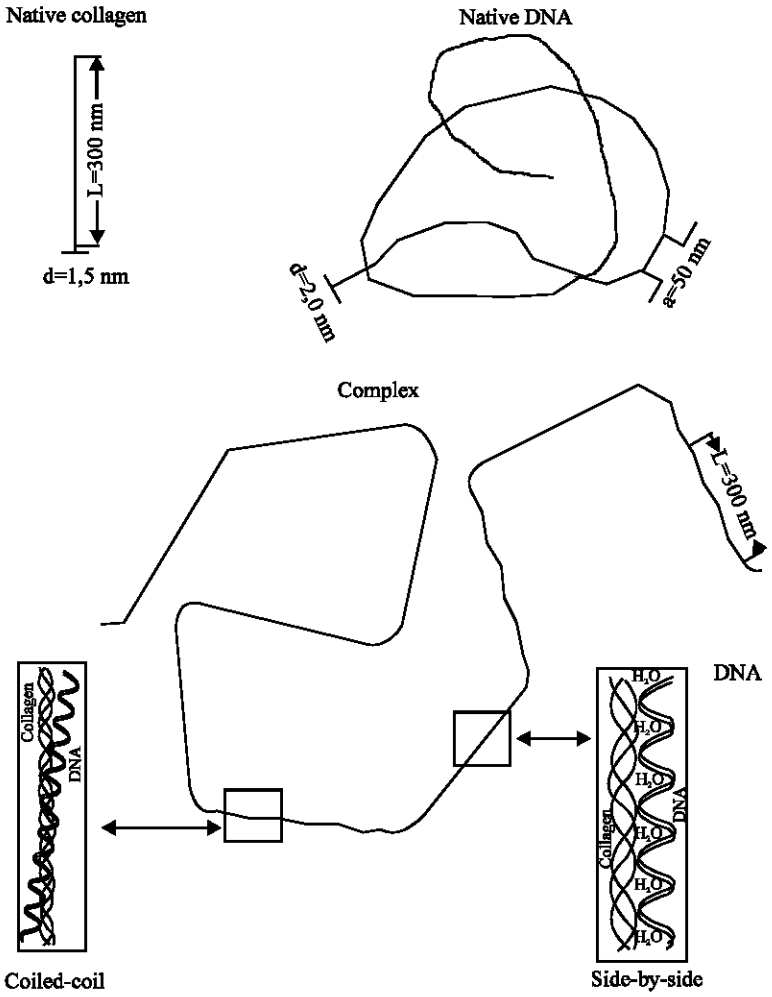

Fig. 5: The model of self-assembling complex of collagen-DNA spontaneously formed in aqueous solutions

of the hydration shells of these structures. There are about 10 such segments per mol of DNA (with molecular weight about $5 \cdot 10^6$ Dalton). This interaction leads to destruction of the hydration shell of the triple helix, and to stabilization of the double helix structure, including the hydration shell of the DNA. From the point of view of molecular biology this nano-scale self-assembling superstructure can determine the rising of the stability of DNA against nucleases during collagen diseases (Poverenny, 1968) and the extraordinary fibrillogenesis of native collagen in the presence of DNA (Kitamura *et al*., 1997; Kaya *et al*., 2005). Strong support for the importance of this collagen-DNA superstructure from the viewpoint of the molecular biology of collagen fibrillogenesis was also evidenced in that the initial micro-unfolding of the triple helix would trigger self-assembly of collagen fibers where the helices are protected from complete unfolding (Leikina *et al*., 2002).

The molecular model suggests that DNA, containing well arranged phosphate groups, helps the collagen to make fibrils by water molecules. Regularly arranged phosphate groups in the DNA molecule play key roles, such as an excellent polymer matrix, in the effect on the collagen fibrillogenesis. The shape of the complex depends on the molecular weight and type of DNA. This result indicates that by choosing weight and type of DNA, containing various gene vectors, can be controlled the shape of the complex. The cell recognizes the shape of DNA-collagen complex, and effectively takes up complexes of certain shapes (Sano *et al*., 2003). Therefore, it is expected that when the correlation between the shape of complex and uptake of the complex into the cell is clarified, the efficiency of introduction of DNA into cells with the use of collagen will increase even more. It has been proved that DNA-collagen complex has segmented arrangements and number of such segments can be controlled by variations on weight and type of DNA. It means that shape of the complex depend on weight and type of DNA. Such extraordinary complex induces disruption hydration shell of collagen triple helix, stabilization hydration shell of ds-DNA, stability of DNA against the nucleases and this complex between collagen and DNA determines unusual collagen fibrils properties.

References

Arai, S., T. Chatake, T. Ohhara, K. Kurihara, I. Tanaka, N. Suzuki, Z. Fujimoto, H. Mizuno and N. Niimura, 2005. Nucleic Acids Research, 9: 3017-3024.

Bastos, M., V. Castro, G. Mrevlishvili and J.Teixeira, 2004. Hydration of ds-DNA and ss-DNA by neutron quasielastic scattering. Biophy. J., 86: 3822-3827.

Bella, J., M. Eaton, B. Brodsky and H.M. Berman, 1994. Crystal and Molecular Structure of a Collagen-like Peptide at 1.9A Resolution. Science, 266: 75-81.

Bloomfield, V., D. Crothers and I. Tinoco, 2000. Jr. Nucleic Acids. Sausalito. California.

Chalikian, T.V., J. Volker, G.E. Plum and K.J. Breslauer, 1999. A more unified picture for the thermodynamics of nucleic acid duplex melting: A characterization by calorimetric and volumetric techniques. Proc. Natl. Acad. Sci. USA, 96: 7853-7858.

Fraser, R.D.B. and T.P. MacRae and E. Suzuki , 1979, Chain conformation in the collagen molecule. J. Mol. Biol., 129: 463-481.

Gelse, K., E. Pöschl and T. Aigner, 2003. Collagens-structure, function, and biosynthesis. Advanced Drug Delivery Reviews, 55: 1531-1546.

Kaya, M., Y. Toyama, K. Kubota, Y. Nadasaka, M. Ochiai, M. Nomizu and N. Nishi, 2005. Effect of DNA structure on the formation of collagen-DNA complex. Intl. J. Biol. Macromolecules, 35: 39-46.

Kitamura, H., C. Iwamoto, N. Sakai, S. Tokura and N. Nishi, 1997. Marked effect of DNA on collagen fibrillogenesis *in vitro*. Intl. J. Biol. Macromolecules, 20: 241-244.

Leikina, E., M.V. Mertts, N. Kuznetsova and S. Leikin, 2002. From the Cover: Type I collagen is thermally unstable at body temperature. Proc. Natl. Acad. Sci. USA, 99: 1314-1318.

Luescher, M., M. Ruegg and P. Schindler, 1974. Biopolymers, 13: 2489-2503.

Maeda, M., S. Tani, A. Sano and K. Fujioka, 1999. Microstructure and release characteristic of the minipellet, a collagen-based drug delivery system for controlled release of protein drugs. J. Controlled Release, 62: 313-324.

Monaselidze, D.R. and N.G. Bakradze, 1969. Melting heat of collagen, Dokl. Akad. Nauk SSSR, 189: 899-901.

Mrevlishvili, G.M., 1998. Low-temperature heat capacity of biomacromolecules and the entropic cost of bound water in proteins and nucleic acids. Thermochimica Acta, 308: 49-54.

Mrevlishvili, G.M., A.P. Carvalo and M. Ribeiro da Silva, 2002. Low-temperature DSC study of the hydration of ss-DNA and DS-DNA and the role of hydrogen-bonded network to the duplex transition thermodynamics, Thermochimica. Acta, 394: 73-82.

Mrevlishvili, G.M., 1974. Thermodynamic and proton relaxation parameters in collagen water solutions. Dokl. Akad. Nauk SSSR, 215: 457-459.

Mrevlishvili, G.M. and D.V. Svintradze, 2005a. Complex between triple helix of collagen and double helix of DNA in aqueous solution. Intl. J. Biol. Macromolecules, 35: 243-245.

Mrevlishvili, G.M. and D.V. Svintradze, 2005b. DNA as a matrix of collagen fibrils. Intl. J. Biol. Macromolecules, 36: 324-326.

Olsen, D., C. Yang, M. Bodo, R. Chang, S. Leigh, J. Baez, D. Carmichael, M. Perala, E.R. Hamalainen, M. Jarvinen and J. Polarek, 2003. Recombinant collagen and gelatin for drug delivery. Advanced Drug Delivery Reviews, 55: 1547-1567.

Poverenny, A.M., 1968. Destructive processes of connective tissue and its role in development of autoimmune reactions, Connective Tissue In Health and Diseases, "Nauka". Siberean Branch. Novosibirsk. USSR, pp: 254.

Privalov, P., 1982. Stability of Proteins. Proteins which do not present a single cooparative system, Adv. Prot. Chem., 35: 1-104.

Ruszczak, Zb. and W. Friess, 2003. Collagen as a carrier for on-site delivery of antibacterial drugs. Advanced Drug Delivery Reviews, 55: 1679-1698

Sano, A., M. Maeda, S. Nagahara, T. Ochiya, K. Honma, H. Itohc, T. Miyatac and K. Fujiokaa, 2003. Atelocollagen for protein and gene delivery. Advanced Drug Delivery Reviews, 55: 1651-1677.

Sharimanov, Y., L. Buashvili and G.M. Mrevlishvili, 1979. Spin-spin and spin lattice relaxation in collagen solution. Biofizika, 24: 606-610

Wallace, D.G. and J. Rosenblatt, 2003. Collagen gel systems for sustained delivery and tissue. Advanced Drug Delivery Reviews, 55: 1631-1649.

Whitesides, G.M. and M. Boncheva, 2002. Supramolecular Chemistry And Self-assembly Special Feature: Beyond molecules: Self-assembly of mesoscopic and macroscopic components. Proc. Natl. Acad. Sci. USA, 99: 4769-4774.