Backbone Dynamics of Triple-helical Collagen-like Structure

YU.A. LAZAREV, A.V. LAZAREVA and V.M. KOMAROV

Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino, Moscow Reg.

Abstract. Some details of the backbone dynamics in the collagen-like triple helix is discussed and the role of backbone dynamics in functioning collagen proteins is illustrated. On a series of oligotripeptides synthetic analogs of collagen formation of high-frequency vibrational backbone dynamics and low-frequency nonlinear backbone dynamics upon stepwise elongation of peptide chain have been described using infrared spectroscopy and hydrogen-exchange method. In the fully completed triple helix the level of high-frequency backbone dynamics is regulated firstly by contact interactions of adjacent atoms and chemical bounded groups, while the level of low-frequency large-amplitude backbone dynamics depends mainly on cooperative interactions attributed by conjugation of interpeptide hydrogen bonds. In native collagens the nonlinear large-amplitude dynamics following by non-denaturational micro-unfolding of the triple-helical structure appears to be under the natural selection control delivering an optimal condition for formation, functioning and utilization of collagen fibrils.

Key words: Triple helix, Backbone dynamics, Collagen, Natural selection

Molecular dynamics as generally accepted is an important element of molecular structure which is closely linked to physico-chemical properties and biological functions of biopolymers. The internal motion in biopolymers as well known is rather complex and may proceed in different time scale. There is a very large frequency spectrum of internal motion from nanosecond till millisecond and even second time periods [1]. The high-frequency backbone motion of atoms and valent-bounded groups in nanosecond and picosecond time scale reveals particularly in infrared and Raman vibration spectra and NMR spectra and can be described by these methods [2, 3]. The low-frequency backbone dynamics in millisecond time scale reveals firstly in hydrogen exchange kinetics of peptide groups [4]. It can be described by spectroscopic methods and by other physical methods such as fluorescence- and spin-label methods [5]. Nonlinear large-amplitude motions of high-frequencies stipulate strong interaction between different normal vibration modes and lead to very fast energy degradation of an excited mode to the average energy level of heat induced motion. The study of nonlinear effects of high-frequency dynamics is very important to clarify the molecular ways and molecular mechanisms of translocation of electronic and vibration energy in biological systems especially by photobiology processes [6]. The low frequency backbone mobility detected by the hydrogen exchange method is nonlinear by its nature. The exchange process may proceed only by high-amplitude mobility followed by non-denaturational micro-unfolding

of protein structure and by disruption of interpeptide hydrogen bonds [1, 3, 4]. The low frequency dynamics of biopolymers proves to be the most important for biological functions to provide the normal recognition and metabolic processes in living organisms [1, 3, 7].

In this communication some details of the backbone dynamics in the collagen-like triple helical structure will be discussed and the role of backbone dynamics in functioning collagen proteins will be illustrated.

The triple helix is the main structural component in fibrillar collagens as well as an important structural motif found now in a set of globular proteins with host-defense function and in several membrane proteins. The strict amino acid sequence repeat with glycine residue in every third position $(\text{Gly-X-Y})_n$ is the feature of this conformation. Collagen molecule consists of three peptide chains in conformation of polyproline II type, which are tightly bound and supercoiled around general axis and stabilized by a set of interchain hydrogen bonds [8, 9]. Now several series of oligo- and polytripeptides synthetic-analogs of collagen are designed. By the use of this models very important information about folding, stabilizing forces, conformational features, backbone dynamics of the triple helical structure is obtained [3, 10–16]. Interesting data concerning formation of high- and low-frequency backbone dynamics have been taken from the study of oligotripeptides with stepwise elongating chain length [12–16]. In this work the main results are illustrated on the row $\text{Z-(Gly-Pro-Pro)}_n\text{-OMe}$ ($n = 1, 2, 3 \dots 8, \dots$ polymer). Carbobenzoxy Z- and OMe are the protective end groups removing the perturbation action of terminal charges in the peptide chain. This oligomer row provides the most favorable conditions for triple helix formation and may be regarded as a model of the nucleation stages of collagen folding. The polytripeptides have been studied in the solid state and in solutions by the use of X-ray diffraction, infrared spectroscopy, circular dichroism, hydrogen-exchange and microcalorimetry methods [12–16]. In the series of our papers the detailed picture of the nucleation process have been presented. It was shown step-by-step formation of one full turn of collagen-like superhelix, when during elongation of peptide chain the main structural parameters of triple helix adopt at last the values very close to that of the polymer [12–16].

Formation of the high-frequency vibration dynamics in the oligomer series may be illustrated by formation of infrared spectra, which reflect not only formation of structural elements of triple helix but formation of the whole system of chain interactions. The main results are following.

Formation of the main elements of infrared spectra- (formation of the spectra of vibration modes) or another words formation of the main elements of high-frequency small-amplitude vibration dynamics is completed already at initial steps of the nucleation ($n = 2\text{--}3$ triplets). Just at the steps when the main role in helix formation (in helix integrity and helix stability) plays only local and contact interactions [14–16] The subsequent chain elongation, bond conjugation, including cooperative interactions affect slightly on the high-frequency vibration dynamics. The formation of low-frequency dynamics of non-denaturational micro-unfolding

type on the contrary is completed at final steps of the nucleation ($n=8$ triplets). Just at the steps when the cooperative block of nucleation is form, which includes one full turn of the superhelix [14–16]. There is distinct correlation between the formation of the low-frequency large-amplitude dynamics followed by kinetic data of hydrogen exchange of peptide groups and the formation of cooperative interactions followed by the thermodynamic data of denaturation transitions [14–16]. After formation of cooperative block of nucleation further chain elongation reflects on the low-frequency dynamics markedly weaker.

Thus in formation of the vibration high-frequency small-amplitude backbone dynamics local interactions play the main role, while in formation of the low-frequency large-amplitude backbone dynamics the main role belongs to cooperative interactions.

Not only for synthetic models, for native proteins as well cooperative interactions proves to be the dominant factor determining the level of the low-frequency backbone dynamics. In our researches [17] we have dealt with collagens from different organisms, very different amino acid composition and residue sequence, different rigidity and thermostability. Hydrogen exchange data along with X-ray diffraction and microcalorimetric data show that in collagen fibrils the level of low-frequency backbone dynamics depends directly on protein crystallinity and stability [17]. It is indeed clear, the more rigid and stable the triple helix is, the more unfavorable conditions are for the micro-unfolding process. At the same time the vibrational high-frequency backbone dynamics in the collagens remains very close to each other as followed from the coincidence of frequencies, shapes, halfwidth of the amide bands of collagen IR-spectra [13].

About fifty years ago Nasonov and Alexandrov [7] put forward an idea that a definite level of molecular backbone dynamics in millisecond or subsecond time scale is quite important to maintain the need level of metabolic activity in organism. They proposed that this dynamics must be under direct natural selection control. If that is the case collagens from different organisms would be expected to differ not far in levels of conformational dynamics at physiological temperatures or at habitat temperatures of species. It is indeed the fact for collagens. The hydrogen exchange experiments carried out in our laboratory and at the Institute of Protein of RAS by Privalov with colleagues [18] support the Nasonov-Alexandrov conception. The levels of the micro-unfolding backbone dynamics of collagens from very different organisms being compared under physiological temperatures show really close values. It means that cod-fish living among Arctic ice-floes and lizard living among African burn sands must have the comparable level of metabolic activity and protein conformation mobility.

The need (sufficiently high) level of collagen conformational mobility in living systems is provided by peculiar properties of collagen proteins. The denaturation temperature of collagens appears to be very close to the upper limit of habitat temperature of species [19]. This collagen properties secure optimal favorable conditions for fibril formation. The fibril formation is a recognition process [8]. The rate

of recognition process as known depend directly on the level of low-frequency conformational dynamics [3]. Not only the rate of selforganization processes, but the rate of degradation processes as well proves to be linked to the level of conformational dynamics. Stability to digestion action depends directly on the level of conformational mobility [7]. For example the triple-helical domain of native collagens are stable to the proteinase action excepting digestion action of very special enzymes-collagenases. Some of collagenases can cut collagen just at the middle of the chain, inducing only slight changes in stability and mobility of the triple helix in both remained parts. But this changes appear to be sufficient to make the triple helix accessible to the proteolytic action of ordinary enzymes. Thus, in native collagens the nonlinear large-amplitude dynamics following by non-denaturational micro-unfolding of the triple-helical structure proves to be under the natural selection control delivering an optimal condition for formation, functioning and utilization of collagen fibrils.

This work was supported by a grant 97-04-49021 from Russian Fund of Fundamental Researches.

References

1. Yakushevich, L.V.: Nonlinear dynamics of biopolymers: theoretical models, experimental data. *Quarterly Reviews of Biophysics* **26** (1993), 201–223.
2. Cantor, C.R. and Schimmel P.R.: Biophysical chemistry. Part 2. Chapt 7–9. W.H. Freeman and Company. San Francisco. 1979.
3. Fan, P., Li, M.-H., Brodsky, B. and Baum, J.: Backbone-dynamics of (Pro-Hy-Gly)₁₀ and designed collagen-like triple-helical peptide by ¹⁵N NMR relaxation and hydrogen-exchange measurements. *Biochemistry* **32** (1993), 13299–13309.
4. Hvidt, A. and Nielsen, S.O.: Hydrogen exchange in proteins. *Adv. Prot. Chem.* **21** (1966), 287–386.
5. Lichtenstein, G.I.: Spin-label method in molecular biology. Chapt. 7–8. 'Nauka' Publishers. Moscow. 1974.
6. Kohen, E., Santus, R. and Hirschberg, J.G.: 'Photobiology'. Chapt.2, 11. *Acad. Press*. New York, Boston, London. 1994.
7. Alexandrov, V.Ya.: Cell reactivity and proteins. Chapt. 7–8. 'Nauka' Publishers. Leningrad. 1985.
8. Frazer, R.D.B. and MacRae, T.P.: Conformation in fibrous proteins. *Acad. Press*. New York. 1973.
9. Jones, E.Y. and Miller, A.: Analysis of structural design features in collagen. *J. Mol. Biol.* **218** (1991), 209–219.
10. Bella, J., Eaton, M., Brodsky, B. and Berman, H.M.: Crystal and molecular structure of collagen-like peptide at 1.9 Å resolution. *Science* **266** (1994), 75–81.
11. Li, M.-H., Fan, P., Brodsky, B. and Baum, J.: Two-dimensional NMR assignments and conformation of (Pro-Hyp-Gly)₁₀ and designed collagen-like triple-helical peptide. *Biochemistry* **32** (1997), 7377–7387.
12. Lazarev, Yu.A., Grishkovsky, B.A., Khromova, T.B., Lazareva, A.V. and Grechishko, V.S.: Bound water in collagen-like triple-helical structure. *Biopolymers* **32** (1992), 189–195.
13. Lazarev, Yu.A., Lazareva, A.V., Shibnev, V.A. and Esipova N.G.: Infrared spectra and structure of synthetic polytripeptides. *Biopolymers* **17** (1978), 1197–2214.

14. Lazarev, Yu.A., Lobachev, V.M., Grishkovski, B.A., Shibnev, V.A., Grechishko, V.S., Figenova, M.P., Esipova, N.G. and Rogulenkova, V.N.: Formation of the collagen-like triple-helical structure in oligopeptides during elongation of the molecular chain. *Biopolymers* **17** (1978), 1215–1234.
15. Lazarev, Yu.A., Lazareva, A.V. and Grechishko, V.S.: Influence of molecular chain length on the formation of a collagen-type trihelical complex in solution. The role of water in the formation of the triple helix. *Biofizika* **42** (1997), 54–67.
16. Lazarev, Yu.A., Lazareva, A.V., Khromova, T.B. and Grechishko, V.S.: Thermodynamic investigations of the collagen-type trihelical structure in oligotripeptides on successive lengthening of the molecular chain. *Biofizika* **42** (1997), 326–333.
17. Lazarev, Yu.A. and Esipova, N.G.: Spectral studies of collagen structure ordering. *Dokl. Akad. Nauk.* **209** (1973), 478–481.
18. Privalov, P.L., Tiktopulo, E.I. and Tischenko, V.M.: Stability and mobility of the collagen structure. *J. Mol. Biol.* **127** (1979), 203–216.
19. Rigby, B.J.: Thermal transitions in invertebrate collagens and their relation to amino acid content and environmental temperature. In W.G. Grewther (ed.) *Symposium on Fibrous Proteins*. Butterworths. Sydney. 1968. pp. 217–225.