Interaction between linker histone H1 and non-histone chromatin protein HMGB1

Alexander V. Fonin ^{a,b,*}, Olga V. Stepanenko ^a, Irina M. Kuznetsova ^a,
Konstantin K. Turoverov ^a, Elena I. Kostyleva ^a and Vladimir I. Vorobyev ^a

^a *Institute of Cytology of the Russian Academy of Sciences, St. Petersburg, Russia*

^b *St. Petersburg State Politechnical University, St. Petersburg, Russia*

Abstract. The possibility of interaction between linker histone H1 and non-histone chromatin protein HMGB1 was studied by intrinsic UV-fluorescence, far and near-UV CD and light scattering. The obtained data allow us to assume that the increase of histone H1 content in the HMGB1 solutions in a low ionic strength is accompanied by the destruction of HMGB1 associates. The interaction between proteins causes the increase of ordered regions in the protein molecules and the minor changes in their tertiary structure.

Keywords: Non-histone chromatin protein HMGB1, linker histone H1, chromatin, protein folding, intrinsically disordered proteins, intrinsic fluorescence of proteins, circular dichroism

1. Introduction

Recently it became evident that many proteins can form compact ordered structure only in contact with their partners, e.g. DNA or RNA molecules, different proteins, metal ions, etc. [13]. Such intrinsically disordered proteins being able to recognize and interact with different partners, play a crucial role in a variety of cell processes including regulation and control of cell processes. That is the reason of great interest to these proteins. Understanding of the structure and functions of these native partially or completely disordered proteins cannot be achieved without accurate knowledge of complexing processes and structure of protein complexes under formation [12]. Non-histone chromatin protein HMGB1 and linker histone H1 belong to such protein class. Both of them are the main chromatin proteins taking part in formation of high levels of chromatin structural organization. The HMGB1 tertiary structure contains two L-shaped DNA-binding domains and C-terminal acidic tail [2,9]. Histone H1 has central globular nucleosome-binding domain flanked by N and C-terminal basic shoulders [6,9]. It can not be excluded that HMGB1 and H1 both being the linker chromatin proteins can interact not only with DNA but also with each other. There are only few works devoted to this problem and there is no unanimous view on it in literature [1,4]. This paper is devoted to the study of interaction between non-histone chromatin protein HMGB1 and linker histone H1 in the wide range of ionic strength solution and different molar ratio of proteins.

*Corresponding author: A.V. Fonin, Institute of Cytology of the Russian Academy of Science, Tikhoretsky av. 4, 194064 St. Petersburg, Russia. Tel.: +7812 297 19 57; Fax: +7812 297 03 41; E-mail: alexfonin@gmail.com.

2. Materials and methods

2.1. Materials

Non-histone chromatin protein HMGB1 and linker histone H1 from calf thymus was obtained and purified as described earlier [7]. Histone H1 was extracted with 5% perchloric acid followed by precipitation with 3 volumes of acidic acetone at 20°C. Non-histone chromatin protein HMGB1 was isolated from the nuclei by 350 mM NaCl solution [3]. The purity of the proteins was evaluated by SDS-PAGE [5]. The concentration of HMGB1 was 0.3 mg/ml. Molar ratio of H1–HMGB1 was varied from 1:4 to 4:1. Measurements were performed in 10 mM TrisHCl buffer pH 7.0, at low salt concentration (15 mM NaCl), and under physiological conditions (150 mM NaCl).

Protein concentration was determined using extinction coefficient for HMGB1 at 280 nm $\epsilon_{280} = 18,920 \text{ M}^{-1} \text{ cm}^{-1}$, which was determined by the analysis of aromatic amino acid number in primary sequence of HMGB1 [2,8]. The extinction coefficient for histone H1 at 230 nm was taken as $41,000 \text{ M}^{-1} \text{ cm}^{-1}$ [4].

2.2. Fluorescence measurements

Fluorescence experiments were carried out using a homemade spectrofluorimeter with steady-state excitation [10]. Fluorescence was excited at the long-wave absorption spectrum edge 297 nm, where the contribution of tyrosine residues in the bulk protein fluorescence is negligible and at 280 nm where both tyrosine and tryptophan residues absorb. The position and form of the fluorescence spectra were characterized by the parameter $A = I_{320}/I_{365}$, where I_{320} and I_{365} are fluorescence intensities at $\lambda_{\text{em}} = 320$ and 365 nm, respectively [11]. The values of parameter A and of fluorescence spectrum were corrected by the instrument spectral sensitivity. Measurements were performed in micro cells 101.016-QS 5×5 mm (Hellma, Germany). All experiments were carried out at 23°C. Samples to be measured were held at the same temperature before mixing.

2.3. Circular dichroism measurements

CD spectra were obtained with Jasco-810 spectrophotometer (Jasco, Japan). Far UV CD spectra were recorded in 1 mm path length cell from 260 to 190 nm. Near UV CD spectra were recorded in a 10 mm path length cell from 320 to 250 nm. For all spectra, an average of two scans was obtained. CD spectra of the appropriate buffer solutions were recorded and subtracted from the protein spectra.

3. Results and discussion

Most clearly the interaction of non-histone protein HMGB1 with histone H1 is seen from comparison of absorption spectra of the HMGB1 in the low ionic strength solution with that of mixture of HMGB1 with low content of linker histone H1. Under these conditions absorption of the HMGB1 spectra are characterized by a significant level of light scattering (Fig. 1A). This effect can be explained by formation of the associates between HMGB1 molecules. However, the presence of histone H1 in the HMGB1 solution causes decrease of the light scattering, which can be explained by disintegration of associates between HMGB1 molecules due to electrostatic interaction with histone H1. The decrease of light scattering in HMGB1 solutions with the increase of ionic strength (Fig. 1A, *Insertion*) suggests that

Fig. 1. Interaction of non-histone chromatin protein HMGB1 and linker histone H1. Absorption spectra (panel A), fluorescence spectra at $\lambda_{\text{ex}} = 297$ nm (B), far-UV CD spectra (C) of HMGB1 (black solid lines) and H1 (black dashed lines) and mixture of these proteins with H1–HMGB1 molar ratio being equal to 1/3.4 (black dashed-dotted lines), 1/2.6 (gray lines), 1/1.7 (dark gray dashed dotted–dotted lines), 1/1.2 (black dotted lines) and 2.3/1 (gray dashed lines). All solutions were in 10 mM TrisHCl pH 7.0. Influence of ionic strength on the absorption spectra of HMGB1 (insert to panel A). Absorption spectra of HMGB1 in 10 mM TrisHCl, 15 mM NaCl and 150 mM NaCl are presented by solid, dashed and dotted lines, respectively. Comparison of experimentally recorded (solid lines) and calculated in the assumption of the absence of protein interaction (dashed lines) protein mixture spectra measured by tryptophan fluorescence (inserts to panel B) and far-UV CD (inserts to panel C). Molar ratio of linker histone H1 to non-histone protein HMGB1 was 2.3/1.

association of HMGB1 molecules in solution with low ionic strength is defined by interaction having electrostatic nature. So the significant decrease of HMGB1 light scattering in the presence of H1 in low ionic strength solutions can be regarded as the main evidence of protein interaction.

Circular dichroism spectra were used to determine the changes in secondary structure of both proteins upon interaction. The far-UV CD spectrum of HMGB1 has minima at 208 and 222 nm which is typical of proteins with high proportion of α -helical regions (Fig. 1C). At the same time far-UV CD spectrum of histone H1 corresponds to that of disordered proteins (Fig. 1C). Such far-UV CD spectrum of HMGB1 is obviously determined by presence of two structured DNA-binding domains, having three α -helices [8]. Histone H1 has only small, about 80 residues, folded core [1]. It was shown that the far-UV CD spectra of the HMGB1 become more pronounced in the presence of H1 under all experimental conditions (Fig. 1C). These changes of far-UV CD spectra suggest the increase of α -helical regions in the HMGB1 protein structure during interaction with linker histone H1.

Since histone H1 has no tyrosine residues in its structure data obtained by method of tryptophan fluorescence can be attributed to the changes of spatial structure of non-histone chromatin protein HMGB1 during investigation of protein interaction. Under all experimental conditions insignificant quenching of the HMGB1 tryptophan fluorescence was observed in solutions containing both proteins (Fig. 1B).

These data revealed only small changes of HMGB1 tertiary structure in the presence of linker histone H1. Near-UV CD spectra of HMGB1 solutions in the presence of H1 confirm data, obtained by analysis of intrinsic UV-fluorescence of protein (data are not presented).

The data obtained allow us to conclude that there is interaction between non-histone chromatin protein HMGB1 and linker histone H1. The protein interaction is accompanied by the increase of ordered regions in the protein molecules and by minor changes in protein tertiary structure.

Acknowledgements

This work was supported by Programs “Leading Scientific School of RF” (1961.2008.4) and MCB RAS, and RFBR Grant (07-04-01072).

References

- [1] L. Cato, K. Stott, M. Watson and J.O. Thomas, The interaction of HMGB1 and linker histones occurs through their acidic and basic tails, *J. Mol. Biol.* **384** (2008), 1262–1272.
- [2] C.H. Hardman, R.W. Broadhurst, A.R.C. Raine, K.D. Grasser, J.O. Thomas and E.D. Laue, Structure of the A-domain of HMG1 and its interaction with DNA as studied by heteronuclear three- and four-dimensional NMR spectroscopy, *Biochemistry* **34** (1995), 16596–16607.
- [3] E.W. Johns, *The HMG Chromosomal Proteins*, Academic Press, New York, 1982, pp. 43–45, 59–60.
- [4] L.A. Kohlstaedt and R.D. Cole, Specific interaction between H1 histone and high mobility protein HMG1, *Biochemistry* **33** (1994), 570–575.
- [5] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *Nature* **227** (1970), 680–685.
- [6] R. Misselwitz, D. Zirwer, H. Damaschun, G. Damaschun, H. Welfle, I.A. Zalenskaya, E.I. Ramm and V.I. Vorobyev, Marine invertebrate sperm-specific histones and histone-DNA interactions: circular dichroism and ultraviolet spectroscopy studies, *Int. J. Biol. Macromol.* **2** (1986), 194–200.
- [7] A.M. Polyanichko, E.V. Chikhirzhina, A.N. Skvortsov, E.I. Kostyleva, P. Colson, C. Houssier and V.I. Vorobyev, The HMG1 ta(i)le, *J. Biomol. Struct. Dynamics* **19** (2002), 1053–1062.
- [8] C.M. Read, P.D. Cary, C. Crane-Robinson, P.C. Driscoll, M.O.M. Carillo and D.G. Norman, The structure of the HMG box and its interaction with DNA, *Nucleic. Acids Mol. Biol.* **9** (1993), 222.
- [9] H. Triber, H. Bar, A. Walter, T.N. Osipova, E.I. Ramm, E.I. Kostyleva and V.I. Vorobyev, Structural differences between histone H1 molecules from sea urchin (*Strongylocentrotus intermedius*) sperm and calf thymus: hydrodynamic and CD studies, *Int. J. Biol. Macromol.* **11** (1989), 153–158.
- [10] K.K. Turoverov, A.G. Biktashev, A.V. Dorofeiuk and I.M. Kuznetsova, A complex of apparatus and programs for the measurement of spectral, polarization and kinetic characteristics of fluorescence in solution, *Tsitologiya* **40** (1998), 806–817.
- [11] K.K. Turoverov and I.M. Kuznetsova, Intrinsic fluorescence of actin, *J. Fluorescence* **13** (2003), 41–57.
- [12] K.K. Turoverov, V.N. Uversky and I.M. Kuznetsova, The protein kingdom extended: native globular and native partially or completely intrinsically disordered proteins, folding, formation of upmolecular complexes, aggregation, *Tsitologiya* **51** (2009), 190–203.
- [13] V.N. Uversky, C.J. Oldfield and A.K. Dunker, Intrinsically disordered proteins in human diseases: introducing the D2 concept, *Annu. Rev. Biophys.* **37** (2008), 215–246.