CD study of the actin DNase I complex

Katalin Ajtai* and S.Yu. Venyaminov

*Department of Biochemistry, Eötvös Lóránd University, H-1088 Budapest, Puskin u.3, Hungary and Institute of Protein Research, USSR Academy of Sciences, 142292 Poustchino, Moscow Region, USSR

Received 23 November 1982

DNase I, a specific actin binding protein, forms a stable complex with actin. CD spectroscopy was used to study the question whether the structure of actin and DNase I in their complex are identical with those of the individual components. Far and near UV analysis was used to study the secondary structure and the environment of aromatic chromophores. CD spectroscopic results on actin, DNase I and on their complex in solution are presented which show that no structural change takes place as a result of actin–DNase I complex formation and indicate the absence of aromatic chromophores on the interface of the actin and DNase I in their complex. CD spectroscopy proved to be a convenient technique for studying the interactions between actin and actin binding proteins in solution.

Actin *DNase I* *Circular dichroism*

1. INTRODUCTION

Several biochemical processes in eukaryotic cells are regulated by actin–protein interactions. Many 'actin binding' proteins have been identified but the mechanism of these interactions is not yet well known [1,2].

One of these proteins is DNase I which prevents actin polymerization by forming a specific complex with a binding constant of about 10^8 M^{-1} [3–6]. The inhibition of actin polymerization made the crystallization of the G-actin–DNase I complex possible, and an X-ray analysis at 6 Å resolution has been done [7]. However, it is not known whether the structure of the two molecules in the complex are identical with those of the individual components as they have not yet been crystallized.

It was of interest to study this structure in solution using CD spectroscopy. The advantage of this technique is that it permits individual structures to be compared to that of the complex, and thus to detect any conformational changes which might occur during complex formation [8,9]. Far and near UV analysis was used to study the secondary structure and the environment of aromatic chromophores, respectively.

CD spectroscopic results on actin, DNase I and on their complex in solution are presented which show that no structural change takes place as a result of actin–DNase I complex formation.

Abbreviations: CD, circular dichroism; DNase I, deoxyribonuclease I (EC 3.1.4.5); SDS–PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis

2. MATERIALS AND METHODS

Actin was isolated from acetone-dried rabbit skeletal muscle powder according to [10]. The powder was extracted with 0.2 mM ATP, 5 mM Tris–HCl pH 7.6 at 0°C and G actin was polymerised by addition of 50 mM KCl. For further purification the method of [11] was used. Purity of actin was analysed by SDS–PAGE [12].

Bovine pancreatic DNase I was from Sigma, chromatographically purified grade. DNase I enzymic activity was measured according to [13], DNA (high polymer from chicken erythrocytes) was from Reanal.

The inhibition of DNase I activity was completed after addition of actin in a 1:1 molar ratio. CD spectra of the complex were measured on the

Published by Elsevier Biomedical Press
00145793/83/0000—0000/$3.00

same preparation used for testing the inhibition of enzymic activity.

Protein concentration was determined either as in [14], assuming the nitrogen content to be 16%, or spectrophotometrically using $A^{0.1\%}_{1\,cm,280\,nm}$ = 1.23 for DNase I and $A^{0.1\%}_{1\,cm,290\,nm}$ = 0.63 for actin [15]. The spectra were recorded by a Hitachi EPS-3T instrument over 360–230 nm. Correction for turbidity was carried out according to [16].

Circular dichroism spectra were measured on a Jasco 41A spectropolarimeter at a spectral slit-width of 1 nm under constant nitrogen flush. The instrument was calibrated according to [17]. The thickness of the cell was 0.093 or 0.185 mm for the far UV and 10.0 mm for the near UV regions. All the measurements were made at 12°C. In each experiment the baseline for the solvent as well as that of the CD spectra for protein solutions, were scanned two times. The data were expressed in terms of mean residue ellipticity (deg . cm^2 . dmol^{-1}) taking the mean residue weights to be 115 and 112 for DNase I and actin, respectively.

3. RESULTS AND DISCUSSION

Fig.1 shows the CD spectra of actin and DNase I in the far and in the near UV regions.

In fig.2 the CD spectra of the actin–DNase I complex are presented together with the superimposed spectra of the individual components. There is almost no difference between the calculated CD spectrum of actin plus DNase I and the experimental spectrum of the complex (fig.2). Any substantial change in the backbone conformation of the components or in the surroundings of the aromatic sidechains would be reflected by CD spectra in the far and near UV region, respectively (unless an exact compensation of opposite changes takes place). The observed additivity in the spectra means that in spite of the tight interaction and dramatic effect of complex formation on the functional properties of each molecule, their intramolecular structure remains essentially unchanged. The coincidence of the CD curve of the complex with the additive curve in the near UV region does not only indicate the absence of changes in the tertiary structure of the two proteins, but also shows the absence of aromatic amino acids on the interface of actin and DNase I in their complex.

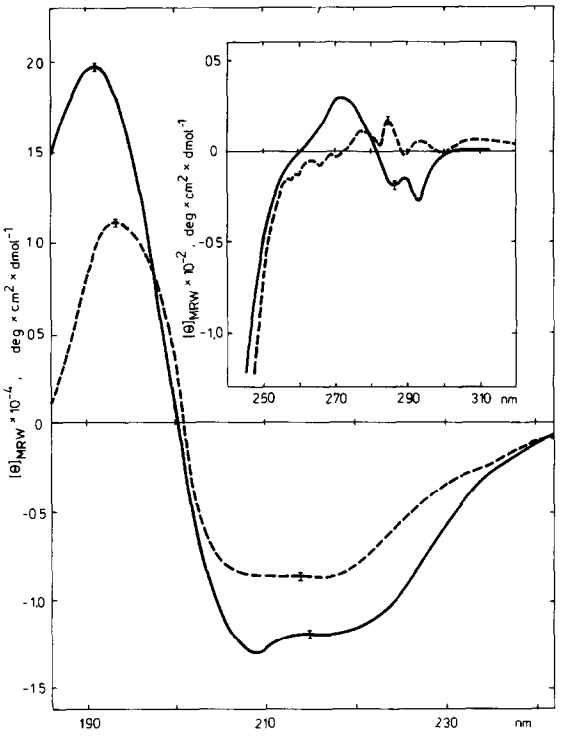

Fig.1. CD spectra of actin and DNase I in the far and near (insert) UV region: DNase I (-----) 0.935 mg/ml (30 μM) in 5 mM Tris–HCl (pH 7.6); actin (———) 1.0 mg/ml (26 μM) in 5 mM Tris–HCl (pH 7.6), 0.1 mM ATP.

The conclusion that the strong binding between actin and DNase I does not alter the tertiary structure of actin participating in the complex is in accordance with the known fact that actin is a rather rigid protein molecule. If actin is exposed to a relatively high concentration of trypsin [18], cleavage occurs only at two points near the N terminal, the extremely resistant core remains unchanged. According to [19], even these two points, accessible to trypsin, are protected in the actin–DNase I complex. In this case DNase I also becomes resistant to trypsin in the complex.

These CD results together with the results of proteolysis experiments verify the assumption of [7] according to which the structure of actin in the actin–DNase I complex (deduced from X-ray crystallographic studies) is practically identical with that of actin in solution.

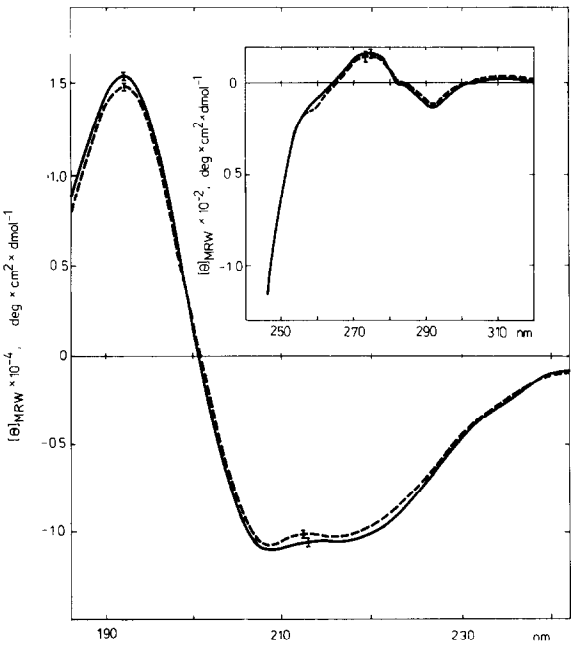

Fig.2. CD spectra of the actin–DNase I complex in the far and near (insert) UV region: (———) calculated curve (superposition of spectra in fig.1; (-----) experimental curve of the actin–DNase complex.

CD spectroscopy proved to be a convenient technique for studying the interactions between actin and actin-binding proteins in solution.

ACKNOWLEDGEMENTS

The authors thank Professor E.N.A. Biró and Dr G. Hegyi for support and critical reading of the manuscript. The experimental work was carried out at the Institute of Protein Research (Poustchino).

REFERENCES

[1] Weeds, A. (1982) Nature 196, 811–816.
[2] Craig, S.W. and Pollard, T.D. (1982) Trends Biochem. Sci. 7, 88–92.
[3] Lazarides, E. and Lindberg, U. (1974) Proc. Natl. Acad. Sci. USA 71, 4742–4746.
[4] Mannherz, H.G., Barrington-Leigh, J., Leberman, R. and Pfrang, H. (1975) FEBS Lett. 60, 34–38.
[5] Hitchcock, S.E., Carlsson, L. and Lindberg, U. (1976) Cell 7, 531–542.
[6] Mannherz, H.G., Goody, R.S., Konrad, M. and Nowak, E. (1980) Eur. J. Biochem. 104, 367–379.
[7] Suck, D., Kabsch, W. and Mannherz, H.G. (1981) Proc. Natl. Acad. Sci. USA 78, 4319–4323.
[8] Hennessey, J.P. jr and Johnson, W.C. jr (1981) Biochemistry 20, 1085–1094.
[9] Hennessey, J.P. jr, Johnson, W.C., Bahler, C. and Wood, H.G. (1982) Biochemistry 21, 642–646.
[10] Bárány, M., Biró, N.A., Molnár, J. and Straub, F.B. (1954) Acta Physiol. Acad. Sci. Hung. 5, 369–381.
[11] Mommaerts, W.F.H.M. (1951) J. Biol. Chem. 188, 559–565.
[12] Laemmli, U.K. (1970) Nature 227, 680–685.
[13] Lindberg, U. (1967) Biochemistry 6, 323–334.
[14] Jaenicke, L. (1974) Anal. Biochem. 61, 623–627.
[15] Houk, T.W. and Ue, K. (1974) Anal. Biochem. 62, 66–74.
[16] Winder, A.F. and Gent, W.L.G. (1971) Biopolymers 10, 1243–1251.
[17] Cassim, J.Y. and Yang, J.T. (1969) Biochemistry 8, 1941–1951.
[18] Jacobson, G.R. and Rosenbusch, J.P. (1976) Proc. Natl. Acad. Sci. USA 73, 2742–2746.
[19] Burtnick, L.D. and Chan, K.W. (1980) Life Sci. 26, 1323–1327.