THE EFFECT OF EDTA ON SPECTRAL PROPERTIES OF ATP-, ADP-, AND ITP-G-ACTIN*

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Globular actin prepared under the usual conditions contains one mole of tightly bound nucleotide (Straub and Feuer, 1950; Laki, Bowen, and Clark, 1950; and Mommaerts, 1952) and one mole of calcium per mole of protein (Tonomura and Yoshimura, 1961; Maruyama and Gergely, 1961; and Barany, Finkelman, and Therattil-Antony, 1962). The bound ATP is dephosphorylated to ADP during polymerization (Straub and Feuer, 1950; Laki, Bowen, and Clark, 1950; and Mommaerts, 1952). Other nucleotides (Martonosi and Gouvea, 1961: Strohman and Samorodin, 1962) including ADP (Szent-Györgyi, 1951; Pragay, 1957; Hayashi and Rosenbluth, 1960) and ITP can be substituted for ATP.

A previous spectroscopic investigation (Higashi and Oosawa, 1965) suggested that changes take place in the environment of tyrosyl and tryptophyl residues, as well as in the peptide backbone, either on polymerization, or on binding of nucleotide to nucleotide-free actin.

The loss of the bound nucleotide, as a consequence of the removal of the divalent cation by chelating agents (Martonosi and Gouvea, 1961; Strohman and Samorodin, 1962), leads to loss of polymerizability and denaturation, accom-

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panied by irreversible conformational changes in the protein (Nagy and Jencks, 1962). We therefore investigated the effect of EDTA on the 240-400 mμ spectral region of G-actin with either ATP, ADP, or ITP as the bound nucleotide.

F-actin pellets were prepared and stored as previously described (West, Nagy, and Gergely, 1967) except that free nucleotide was removed before polymerization by treatment of each ml of G-actin solution with 0.2 ml of a 50% (v/v) suspension of Dowex-1 X-2-Cl. ATP-, ITP-, and ADP-G-actin were prepared from the F-actin pellet by homogenizing it in a solution containing 1 mM Tris, pH 8.0, or 1 mM N-tris(hydroxymethyl) methyl-2-aminoethane sulfonic acid (TES), pH 7.4, and 1 mM of the appropriate nucleotide. In the case of ATP-G-actin the homogenate was dialyzed for 17 hours against the same buffer containing only 0.2 mM ATP. Solutions of G-actin were centrifuged at 105,000 x g for 20 minutes and the supernatant was treated with Dowex-1 to remove free nucleotides. The resin was removed after four minutes by filtration through a Millipore filter (0.45μ pore size) under pressure. Protein concentration was calculated from the extinction at 280 mμ with the use of $E^{1\%}_{1_{cm}} = 11.08$ based on the amino acid composition of actin (Carsten, 1963) and on micro Kjeldahl nitrogen determination. Difference spectra were measured with a Beckman Model-DK-2 spectrophotometer with the use of split compartment mixing cells (Yankeelov, 1963). Optical rotation was measured in a Jasco spectropolarimeter. Temperature, if not stated otherwise, was maintained below 4°.

Addition of 1 mM EDTA produces the difference spectra shown in Fig. 1. The spectrum of ATP-actin has a positive peak at 256 mμ, a negative double peak at 281 and 292 mμ with trough at 289 mμ, and a positive peak at 305 mμ. The difference spectrum in the 280-295-mμ region can be attributed to changes in the environment of tryptophyl and tyrosyl residues (Wetlaufer, 1962). The peak at 256 mμ, present also in the difference spectrum of ADP-actin, is close to the absorption maximum of the adenine ring; this suggests that changes in the absorption of the ATP released on addition of EDTA are, at least in part, responsible for this region of the difference spectrum.

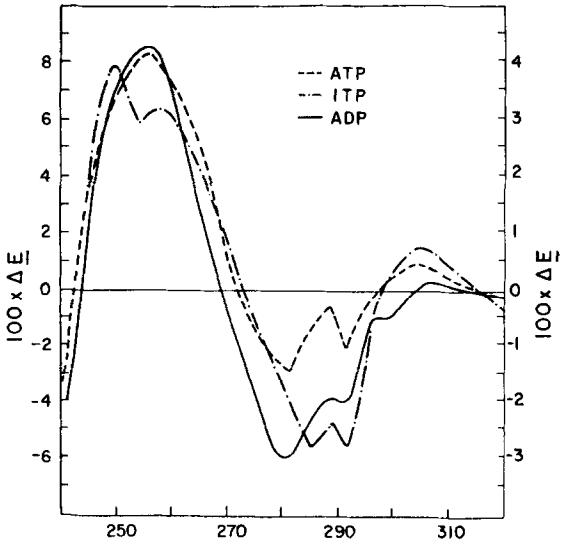

Fig. 1. Comparison of difference spectra of ATP-G-actin, ADP-G-actin and ITP-G-actin. All difference spectra were obtained by adding 1 mM EDTA at 2°. Key: — — — , ATP-G-actin, 1.3 mg/ml in 1 mM TES, pH 7.4 (l.h.s. ordinate); —·— , ITP-G-actin, 0.74 mg/ml in 1 mM tris, pH 8.0 (r.h.s. ordinate); ——— , ADP-G-actin of final concentration, 1.3 mg/ml in 1 mM tris, pH 8.0 (l.h.s. ordinate).

The suggestion that changes in the absorption of the originally bound nucleotide upon release by EDTA contribute to the difference spectrum is supported by the fact that, in the case of ITP-actin, a peak appears around 249 mμ, where ITP has its absorption maximum. Whether the 256-mμ peak also present in ITP-actin is due to some ATP, or ADP-actin (preliminary results suggest less than 20%) present in the ITP-actin preparation, or whether there is a 256-mμ peak attributable to a change in the proteins is at present uncertain.

The ratio of the negative peak in the 280-300-mμ region to the positive-difference spectrum in the nucleotide region is smaller for ATP-actin than for either ADP- or ITP-actin. For ADP-actin there is an indication of a negative peak at 296-300 mμ and a reduction of the positive peak at 306 mμ seen in both ATP-actin and ITP-actin. The difference spectra of ATP-actin and of ADP-actin include a negative peak at 281 mμ which is greater than that

at 292 mμ. In ITP-actin, on the other hand, in the place of a 281-mμ peak one of essentially equal magnitude with that at 292 mμ is observed at 285 mμ. The difference spectrum of ATP-actin is distinguished from those of ADP-actin and ITP-actin by a better resolution of the two peaks in the 280-300-mμ complex.

The characteristic features of the EDTA-difference spectra for the three types of actin described above were also observed if actin was slowly denatured by exposure to 27° or by treatment with apyrase (Straub and Feuer, 1950; and West, Nagy, and Gergely, 1967).

Difference spectra of all three types of preparations studied showed a broad negative peak at 350 mμ, and a corresponding peak could also be detected in the absorption spectrum of the native actins. The 350-mμ peak, however, showed some variability from one actin preparation to another, and its significance in relation to the structure of actin is not clear at present.

At 0° the polymerizability of actin, on addition of EDTA, is lost relatively slowly (Martonosi and Gouvea, 1961; Maruyama and Gergely, 1961; Strohman and Samorodin, 1962) in contrast with the rapid loss of polymerization when EDTA is added at 25°. On adding EDTA to ATP actin at 25° the difference spectrum was fully developed within 5 min. In contrast, at 2° the difference spectrum continued to grow for more than 1/2-hour. The spectral changes developed at different rates in different parts of the spectrum. It appears that the change in the nucleotide region proceeds at a faster rate than in the band of the tyrosyl-tryptophyl residues (Fig. 2); the apparent halftimes are cir.2 and 8 min, respectively. EDTA also produces a change in the ORD spectrum of actin, and the rate of change in absorption at 292 mμ parallells the rate of change in optical rotation at 232 mμ. The preparation whose kinetic behavior is depicted in Fig. 2 was ADP-actin. Similar differences between various spectral regions in the rate of the development of the difference spectra were also found with ATP-actin and with ITP-actin.

There is a well demonstrated hypochromicity in the π-π^* transition of the purine and pyrimidine bases in nucleotide interactions, which may be due

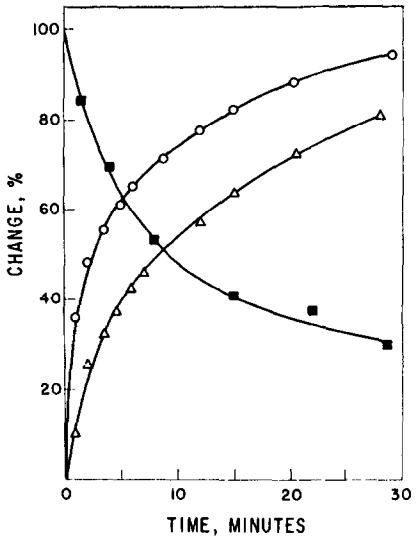

Fig. 2. Time course of changes in extinction at 256 and 292 mμ and in optical rotation at 232 mμ. Final actin concentration 1.5 mg per ml, 1 mM Tris, pH 8; 1 mM EDTA added at 0 time. Measurements were made over a period of one hour, until no further changes were apparent. Ordinate: changes expressed as % of the maximal change. Key: ■ $[\alpha]_{232}$; 0, E_{256}; △, E_{292}.

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either to forming hydrogen bonded complexes (Thomas and Kyogoku, 1967) or to parallel, stacking intereactions between the chromophores (Laland, Lee, Overend, and Peacocke, 1954). Hydrogen bonding to amino acid side chains or "stacking" interaction with aromatic amino acid residues may exist in protein-nucleotide base combinations.

The hyperchromicity exhibited by the release of actin-bound nucleotide is a direct indication of the involvement of the base in the protein-nucleotide interaction, which was suggested previously by differences in affinities of the various nucleotides to actin (Martonosi and Gouvea, 1961). The fact that there is nucleotide specific variation in the difference spectrum, attributable to the tyrosyl and/or tryptophyl chromophores, upon release of the bound nucleotide, suggests as one alternative a direct interaction of these chromophores with the nucleotide base. One objection to this suggestion is that the time course of the changes in the part of the difference spectrum attributable to

nucleotide and that to tyrosyl and/or tryptophyl residues differ significantly. This variation in the tyrosyl-tryptophyl region of the difference spectrum may be due to a change in the environment of chromophores depending on whether ATP, ITP, or ADP is the bound nucleotide. However, the effect should be rather well localized since no nucleotide specific changes can be found in the optical rotatory dispersion of actin in the 225-300-mμ spectral region either with bound nucleotides or after EDTA denaturation.

The slower change in the tyrosyl-tryptophyl band, which parallels the change in optical rotation reflecting a conformational change in the protein, as compared with the spectral change in the nucleotide region, suggests that at least part of the change in protein structure takes place after the release of the nucleotide.

References

Barany, M., Finkelman, F., and Therattil-Antony, T., Arch. Biochem. Biophys. 98, 23 (1962).
Carsten, M. E., Biochem. 2, 32 (1963).
Higashi, S. and Oosawa, F., J. Mol. Biol. 12, 843 (1965).
Laki, K., Bowen, W. F., and Clark, A., J. Gen. Physiol. 33, 437 (1950).
Laland, S. G., W. A. Lee, W. G. Overend, and A. R. Peacocke, Biochem. Biophys. Acta 14, 356 (1954).
Martonosi, A. and Gouvea, M. A., J. Biol. Chem. 236, 1345 (1961).
Maruyama, K. and Gergely, J., Biochem. Biophys. Res. Comm. 6, 245 (1961).
Mommaerts, W.F.H.M., J. Biol. Chem. 198, 469 (1952).
Nagy, B. and Jencks, W. P., Biochem. 1, 987 (1962).
Straub, F. B. and Feuer, G., Biochim. Biophys. Acta 4, 455 (1950).
Strohman, R. C. and Samorodin, A. J., J. Biol. Chem. 237, 363 (1962).
Thomas, G. F. and Kyogoku, Y., J. Am. Chem. Soc. 89 4224 (1967).
Tonomura, Y. and Yoshimura, J., J. Biochem. (Tokyo) 50, 79 (1961).
West, J. J., Nagy, B., and Gergely, J., J. Biol. Chem. 242, 1140 (1967).
Wetlaufer, D. B., Advanc. Protein Chem. 17, 303 (1962).
Yankeelov, J. A., Jr., Anal. Biochem. 6, 287 (1963).