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CHANGES IN OD AT 235 nm DO NOT CORRESPOND TO THE POLYMERIZATION STEP OF ACTIN

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SUMMARY. Discrepancies were observed when the polymerization of rabbit muscle actin was monitored by ΔOD_{235} and viscometry (η). For example, in the presence of (β,γ)-methyleno ATP, the ΔOD signal was as large as with ATP although polymerization was very poor (η = 1.1, compared with η = 1.7 in the presence of ATP). Furthermore, when monomeric actin, kept for 1 h in the presence of a stoichiometric equivalent of ADP, was exposed to conditions favoring polymerization (addition of $MgCl_2$), a considerable ΔOD_{235} signal appeared, although the actin had completely lost its polymerizability (η = 1.0). We conclude that the observed changes in OD_{235} cannot reflect polymerization itself, but must be caused by another reaction preceding the assembly. Under normal conditions, this reaction is supposed to be the slowest step of filament formation and so to determine the velocity of the whole process. In conclusion, monitoring of actin polymerization by ΔOD_{235} is a valid method only when polymerization has been assessed by another, independent method.

In 1965 Higashi and Oosawa (1) showed that polymerization of rabbit muscle actin was associated with a spectral change in the U.V. absorption of the protein. Difference spectra were observed in the region of 275-295 nm, where aromatic side chains make the main contribution, and between 230 and 240 nm indicating that possibly also the chromophores of the peptide backbone are involved. In the years following ΔOD at 235 nm was used by several laboratories for monitoring polymerization of actin, (see for example refs. 2,3,4,5,6,7). However, since the method has been introduced by Higashi and Oosawa its general validity has never

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ABBREVIATIONS: APPCP, (β,γ)-methyleno ATP.

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been tested. By chance we detected in polymerization studies with analogs of ATP and ADP that the data obtained with the ΔOD_{235} method were not consistent with those obtained by viscometry. The present study shows that positive spectral changes of OD at 235 nm can be obtained with actin preparations which have completely lost their polymerizability.

MATERIALS AND METHODS

$ATPNa_2$ and $ADPNa_2$ were from Boehringer, Mannheim, APPCP from Serva, Heidelberg. Purity of APPCP was examined by the luciferase assay (Lumac, Dusseldorf). We found that ATP, but not ADP, was absent in all cases. ADP was removed from the nucleotide by purification on anion exchanger QAE-Sephadex as described elsewhere (Stournaras et al., manuscript submitted). Actin was isolated from rabbit muscle as described by Faulstich et al. (8). G-actin was prepared by gentle homogenization of the pellets in a Teflon potter at 4°C with TrisHCl (1 mM) buffer, pH 7.4, containing either 0.02 mM ATP, or 1.0 mM APPCP, or no nucleotide. The concentration of G-actin was determined spectrophotometrically using $\epsilon_{290} = 26.460\ mol^{-1}\ cm^{-1}$. Polymerization was started by the addition of 5 µl $MgCl_2$ (0.1 M) to one of the 2 mm (0.5 ml) quartz cuvettes in an Aminco double beam spectrophotometer, and optical density at 235 nm was monitored against the reference cuvette. In parallel, relative viscosity was measured in an Ostwald viscometer (Canon 175) as described in ref. (8).

RESULTS AND DISCUSSION

It is generally accepted that measuring relative viscosity and monitoring the change of optical density at 235 nm are equivalent methods of following actin polymerization. When monomeric actin was prepared in the presence of ATP and polymerized by the addition of Mg ions, the increase of OD_{235} did indeed parallel the increase of η values (not shown). Such congruence was not seen when the non-hydrolyzable (ß,γ)-methyleno ATP was used instead of ATP. Here a complete polymerization was suggested by ΔOD_{235}, with maximum values and a time course being very similar to those obtained with ATP (fig. 1). However, viscometric measurements performed in parallel revealed that in this case polymerization was nearly absent. Typical η values were in the range of 1.1 to 1.2, compared to $\eta = 1.74$ with ATP. Moreover, maximum viscosity was reached only after a long time (ca. 1 h), while by the optical method the maximum value was reached as fast as in the presence of ATP (ca. 10 min). Obviously, in the presence of this ATP analog the two effects were not correlated.

Inconsistent results were also found when monomeric actin, prepared in the presence of APPCP, was stored for 1 hour at 4°C. This preparation was unable to polymerize at all ($\eta = 1.0$) but

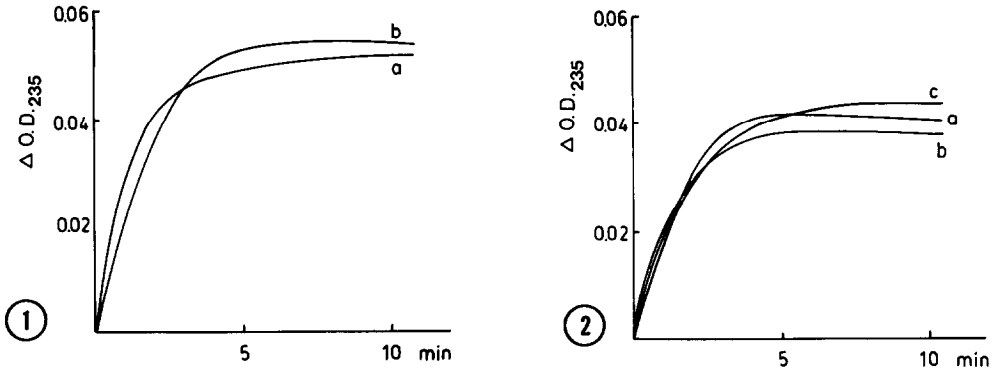

Fig. 1
Changes in OD at 235 nm measured for a solution of 23 µM G-actin in buffer containing 0.02 mM ATP (a) and 1.0 mM APPCP (b), upon the addition of $MgCl_2$ to a final concentration of 1 mM. The relative viscosity values η were 1.74 (a) and 1.1 (b).

Fig. 2
Changes in OD at 235 nm induced by $MgCl_2$ (final concentration 1 mM) in G-actin (20 µM) depolymerized in the presence of 1 mM APPCP. Sample (a) was stored for 60 min at 4° before $MgCl_2$ was added (η = 1.00). Sample (b) same as (a) but ATP (0.1 mM) was added before $MgCl_2$ (η = 1.05). To sample (c) ATP (0.1 mM) and $MgCl_2$ were added immediately after depolymerization (η = 1.66).

on the addition of $MgCl_2$ gave the full ΔOD_{235} signal (E = 0.04) (fig. 2a). By the optical method it was indistinguishable from a corresponding sample to which ATP had been added immediately after depolymerization and which exhibited nearly full polymerization (η = 1.66, fig. 2c). In this case too the spectral change was clearly independent of the polymerization process. Another actin sample prepared in the presence of APPCP was assayed after 60 min. storage at 4°C for its proportion of native actin. For this, ATP was added and polymerization was induced by $MgCl_2$. We found that only a residual amount of actin was still present in the native form (η = 1.05). This sample likewise gave an ΔOD_{235} signal of ca. 0.04 (fig. 2b).

In a third series of experiments, actin was depolymerized in the absence of added nucleotide. The resulting monomer is able to polymerize, although very slowly and incompletely (η = 1.25). Obviously denaturation processes are in competition with the assembly reaction. Despite the poor and slow polymerization the ΔOD_{235} signal after 10 min. was higher than that of a fully polymerizable sample (η = 1.74), to which ATP had been added soon after depolymerization. Moreover, ΔOD was still increasing and showed no tendency to level off as usually observed with samples exhibiting full polymerization. Also this kind of monomeric actin

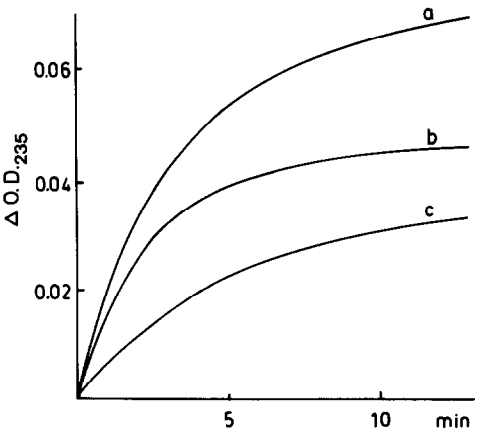

Fig. 3
Changes in OD at 235 nm induced by $MgCl_2$ (final concentration 1 mM) in G-actin (23 μM) prepared in the absence of any added nucleotide. Sample (a) immediately treated with $MgCl_2$ (η = 1.25); sample (b) immediately treated with ATP (0.1 mM) and $MgCl_2$ (η = 1.74); sample (c) treated with $MgCl_2$ after 60 min storage at 4°C (η = 1.0).

lost polymerizability completely on storage. Nevertheless it exhibited a considerable spectral change upon the addition of Mg ions. Already after 10 minutes it was as high as 0.03 and may have reached the maximum value obtained with polymerizable samples (0.04) later on (fig. 3c).

These experiments show that examples exist where following optical density at 235 nm is not a valid method for monitoring polymerization of actin. On the other hand, the method has been successfully used by several workers and has proved useful for studying the polymerization kinetics of actin in all cases where polymerization actually occurred. How is the breakdown of the method in the present study to be explained?

In the past years it has become obvious that actin polymerization is not a single reaction but rather a sequence of events. Any one of these, if slow enough, could determine the rate of the whole process. This means that following the kinetics of the partial step producing the known shift of optical density, can be employed for monitoring polymerization itself, provided this step is rate-limiting, and polymerization actually occurs. However, the possibility arises that under particular conditions the polymerization step fails. In this case the optical method would not be suited for following the polymerization reaction.

We have not checked for the formation of only very short filaments, which possibly will not contribute to viscosity. However,

ηvalues of 1.00,as found in two kinds of our experiments, strongly suggest the unpolymerizability of these samples; if so, the spectral change at 235 nm exhibited by these samples upon the addition of Mg ions may indeed not be a consequence of the polymerization, but may rather be associated with an event normally conditioning the actin molecule for assembly.

This event is most probably a change in actin conformation. Conformational changes induced by the addition of Mg or K ions have been proposed by Rich and Estes in order to explain the high proteolytic stability of actin in the presence of salts (9). Further investigations by Rouayrenc and Travers (10) showed that this actin species contains uncleaved ATP and will exist as a monomer at concentrations below the critical concentration. These authors suggested that the conformational change as documented by a difference spectrum around 280 nm may result from the neutralization of a polyanionic region on the actin surface. This region is probably identical with the low affinity binding sites for cations postulated by Barany et al (11) who further suggested that saturation of these binding sites with cations is one prerequisite for actin polymerization. So it seems possible that it is by the interaction with salts that a conformational change -together with a spectral change at 235 nm- is induced, by which actin monomers adopt a conformation suitable for polymerization. Under normal conditions the assembly will occur in one of the subsequent steps all of them being faster than the conformational change. However, under unfavorable conditions, for example at concentrations lower than the critical concentration, or with unsuited ATP analogs such as APPCP, the polymerization may fail to occur.

From the practical point of view it is important to note that the ΔOD_{235} method should not be used in ill-defined actin systems. It should also not be used to distinguish whether actin polymerizes or not. On the other hand it still represents,by its simplicity and by its independence of filament length, a very valuable tool for monitoring the kinetics of actin polymerization in all cases, for which polymerizability has been proved by other independent methods (for a review of these methods see Cooper and Pollard (5)).

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