SPECTROSCOPIC EVIDENCE FOR THE INTERACTION OF PHALLOIDIN WITH ACTIN

Th. WIELAND, J. X. de VRIES, A. SCHÄFER and H. FAULSTICH
Max-Planck-Institut für medizinische Forschung, Abteilung Naturstoff-Chemie, D-69 Heidelberg, Jahnstr. 29, W. Germany

Received 10 March 1975

1. Introduction

Phalloidin, a bicyclic peptide from the toxic green deathcap Amanita phalloides [1] binds to muscle F-actin [2] thus stabilizing the micro-filaments against 0.6 M KI as well as against depolymerization by ATP [3] or by ultrasonic vibration [3a]. We have now further investigated the interaction of the toxin with this protein by difference spectroscopy in the uv region.

2. Materials and methods

Phalloidin and secophalloidin, a derivative which is nontoxic and does not stabilize F-actin [3], were samples from our laboratory. Bovine serum albumin was a product of Behringwerke, Marburg. F-actin, free from troponin and tropomyosin was a gift of P. Dancker and M. Hoffmann, who prepared it from rabbit sketetal muscle according to [4]. The F-actin pellets were homogenized in 0.1 M KCl to a final concentration of 1.48×10^{-5} M. Concentrations of actin and phalloidin were determined spectrophotometrically. For actin ϵ_M was determined at 280 nm as 4.16×10^4 based on Lowry's method [5] and on the molecular weight of the actin subunit of 45.000 daltons. The ϵ_M at 300 nm for phalloidin was 1.18×10^4 considering a correction of the original value of 1.10×10^4 [6] for 3 molecules of water in cristalline phalloidin. Spectroscopy was carried out in an Aminco DW-2UV-VIS spectrophotometer using two tandem cuvettes with a total length of 0.875 cm.

Initially both tandem cuvettes contained actin solution as described above in one compartment, and 0.70×10^{-5} to 1.48×10^{-4} M solutions of the cyclic peptide in the other. After recording the baseline, the solutions in the two compartments of one cuvette were mixed and the difference spectrum was measured.

3. Results and discussion

Fig.1 shows the uv spectra of phalloidin and of actin, and fig.2 the difference spectrum between actin/phalloidin and actin plus phalloidin. Clearly, at two wavelengths around 295 nm and 305 nm, the uv spectrum is changed by the interaction of the toxin with actin. In control experiments, phalloidin added to bovine serum albumin on one hand, and secophalloidin added to F-actin on the other, produced only minimal deviations from the zero line. These results indicate, that phalloidin does not interact with other proteins like albumin, and besides this, that secophalloidin, a monocyclic derivative of phalloidin being devoid of toxicity no longer binds to actin. From these observations we conclude that there is a specific interaction between phalloidin and actin. The fact that changes in optical density were observed also above 300 nm, suggests that these changes originate from the indolylthioether moiety of the toxin, which evidently participates in the interaction with the protein. This is strengthened by the observations of Herskovits and Sorensen [7], who produced difference spectra by solvent perturbations of acetyl tryptophan-ethylester very similar to that of phalloidin in interaction with actin. Though nearly identical in shape to the difference spectrum of the tryptophan derivative the difference spectrum of phalloidin/actin was found at longer wavelengths

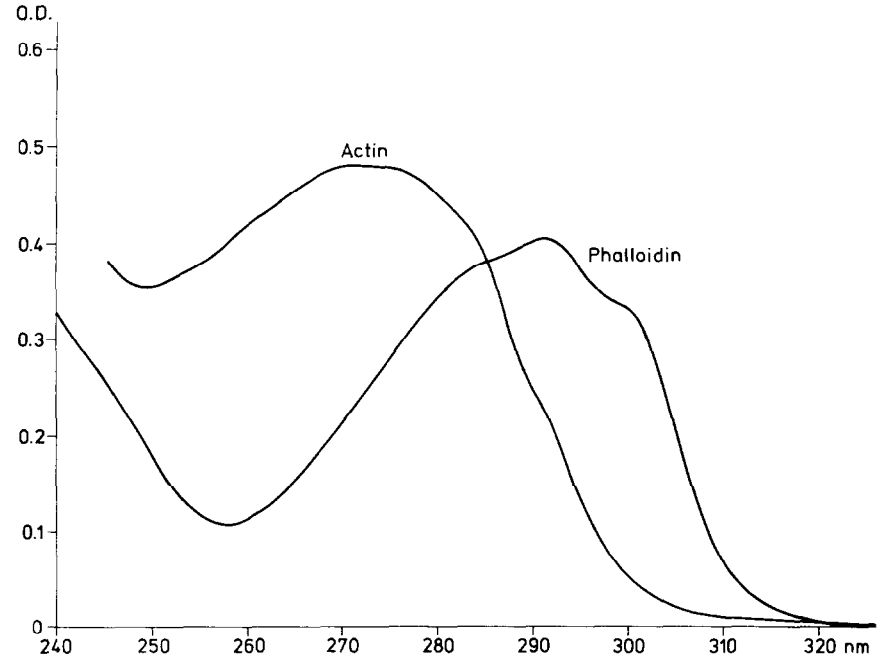

Fig.1. uv absorption spectrum of actin (2.65×10^{-5} M) and phalloidin (7.85×10^{-5} M).

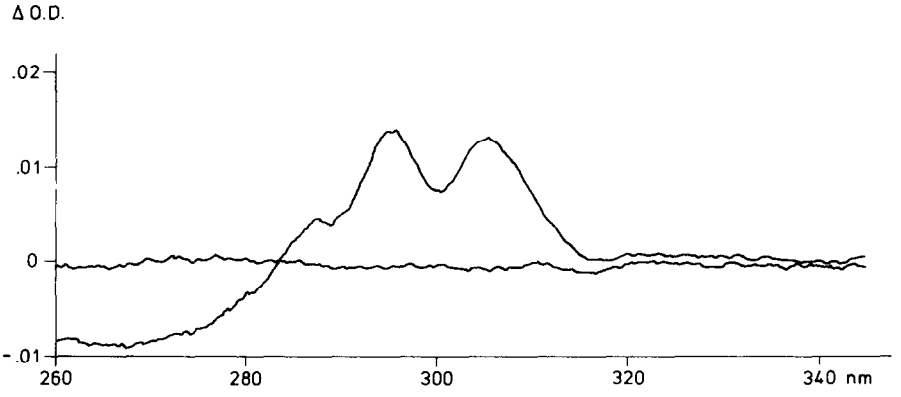

Fig.2. Difference spectrum of the combination of actin and phalloidin, each 1.09×10^{-5} M.

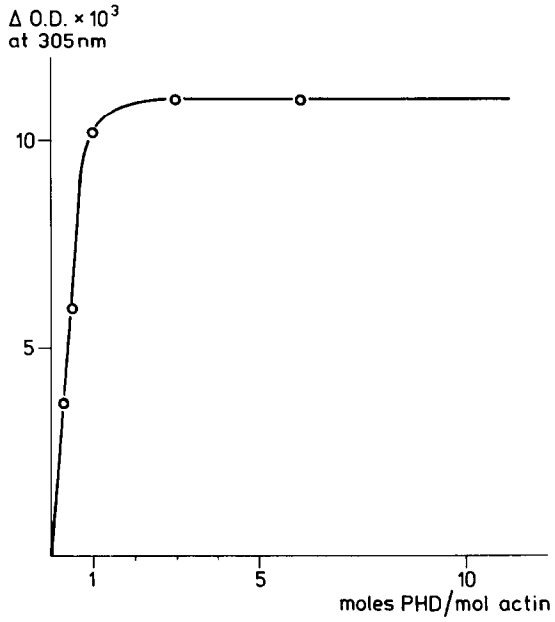

Fig.3. Difference in optical density (305 nm) of actin (1.09×10^{-5} M) plus increasing amounts of phalloidin.

corresponding to the 10 nm shift in the absorption spectrum of tryptophan caused by an alkylthio-substitution in the α-position of the indole nucleus.

In fig.3 the absorptivity difference values of mixtures of phalloidin and actin are plotted against increasing ratios of phalloidin/actin maintaining the concentration of protein constant. Taking into account the uncertainties of protein determination and the limit of error in the spectrophotometric measurements the binding sites of actin appear saturated at molar ratios around 1. At ratio values below 0.5 the absorptivity difference values follow linearly the concentration of phalloidin. Since with an excess of the toxin over actin no further increase of the optical density in the difference spectrum was observed, we suggest a molar ratio of 1 molecule toxin to 1 actin unit. This ratio has not been reached in earlier binding studies using a labelled phallotoxin [2], is, however, in accordance to results obtained from quantitative measurements of the effect of phalloidin on the acceleration of polymerization of G-actin and on the stabilization of F-actin against ultrasonic vibration, which will be published elsewhere [3a].

Acknowledgements

The authors wish to thank Dr P. Dancker and Mrs M. Hoffmann, Department of Physiology of the Max-Planck-Institute for Medical Research, for providing them with F-actin pellets.

References

[1] Wieland, Th. (1968) Science 159, 946.
[2] Wieland, Th. and Govindan, V. M. (1974) FEBS Lett. 46, 351.
[3] Löw, I. and Wieland, Th. (1974) FEBS Lett. 44, 340.
[3a] Dancker, P., Löw, I., Hasselbach, W. and Wieland, Th. (1975) Biochim. Biophys. Acta, in press.
[4] Dancker, P. and Hoffmann, M. (1973) Z. Naturforsch. 28C, 401.
[5] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265.
[6] Faulstich, H., Georgopoulos, D. and Bloching, M. (1973) J. Chromatography 79, 257.
[7] Herskovits, Th. T. and Sorensen, S. M. (1968) Biochemistry 7, 2523.