PHALLOIDIN COUNTERACTS THE INHIBITORY EFFECT OF ACTIN ON DEOXYRIBONUCLEASE I

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

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1. Introduction

The naturally occurring inhibitor of pancreatic deoxyribonuclease I (DNase I) [1] has recently been found to be identical with actin [2]. From studies in our laboratories we know that the toadstool poison phalloidin accelerates the polymerization of actin [3,4] and stabilizes the microfilaments against 0.6 M KI, as well as against depolymerization under different conditions, e.g. by ATP [5], by ultrasonic vibration [6] or by cytochalasin B [7]. This prompted us to study the interaction of phalloidin with the system actin/DNase I.

2. Materials and methods

Phalloidin was a sample from our laboratory. DNase I (grade II) and DNS were from Boehringer, Mannheim. Actin, free from troponin and tropomyosin was a gift of P. Dancker and M. Hoffmann, who prepared it from rabbit skeletal muscle according to [8]. F-actin pellets were dissolved by homogenizing with 0.1 M KCl or depolymerized with 0.1 mM ATP. All concentrations were adjusted spectrophotometrically to 2×10^{-5} M. Concentrations of actin and phalloidin were determined as in [4], and DNase-I using ϵ_M 38·100. Hydrolysis of DNA was measured spectrophotometrically at 260 nm according to [9,10] using an Amino DW-2UV-VIS spectrophotometer. One unit corresponds to an enzyme activity which produces an increase in absorbance of 0.001/min measured in 1 cm cuvettes. Difference spectra were taken in two tandem cuvettes with a total length of 0.875 cm [cf. 4].

3. Results and discussion

G-actin (1.6 moles) inhibited the rate of hydrolysis of DNA by DNase I (1 mole) by about 36%. This figure confirmes the results obtained by Lazarides and Lindberg under similar conditions [2]. Moreover, we could give direct evidence for the interaction of the enzyme with G-actin by difference spectrophotometry. The interaction of the two proteins causes two distinct peaks at 287 nm and 295 nm which point to a participation of tryptophan residues in the complexing reaction. These two peaks were distinguished clearly in spite of a turbity, which causes an increasing absorption from 340 nm on to shorter wavelengths. The 36% inhibition of DNase I by G-actin was totally cancelled, if phalloidin was present in a ratio of only 0.25 mol toxin per 1 mol of G-actin. Even ratios lower than 0.25 counteracted the inhibitory activity of actin strongly (fig.1). In these experiments phalloidin was allowed to react with actin in presence of the substrate DNA 2 min before the addition of the enzyme.

According to Lazarides and Lindberg [2,9] the inhibitor, actin, acts in the monomeric form by binding tenaciously to the enzyme giving rise to a complex containing one molecule each of DNase and inhibitor, Since phalloidin causes polymerization of G-actin to filaments of extraordinary stability [5,6] the toxin possibility prevents the complex formation of the two proteins and hence inhibition of the enzyme by converting all actin monomers to filaments. This is in good agreement to the fact that only 0.3 mol of the toxin are necessary to produce 1 mol of actin filaments which withstand the depolymerization treatment with 0.6 M KI, a value, which is nearly identical to the 0.25 mol

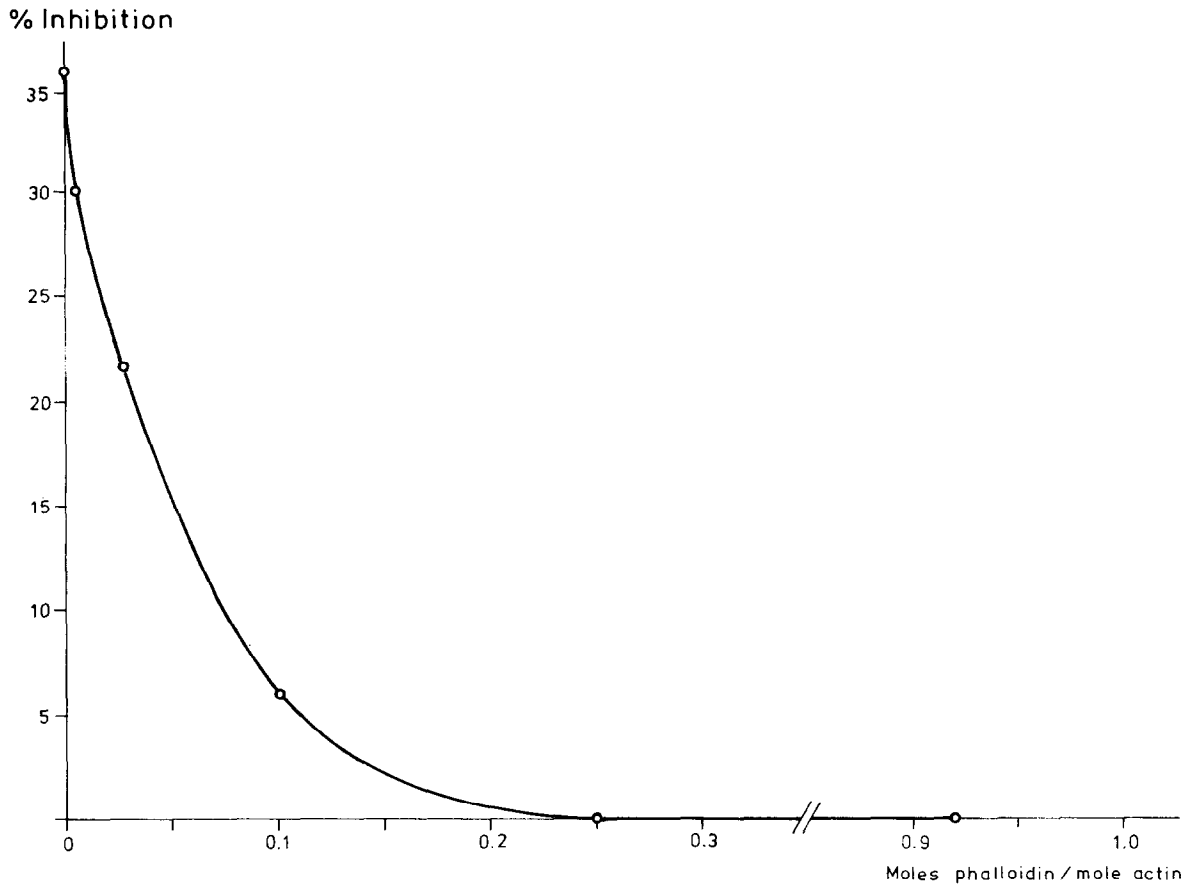

Fig.1. Decrease of the inhibitory effect (36%) of G-actin on DNase I with increasing concentrations of phalloidin. The values of inhibition were measured by following the rate of hydrolysis of DNA by monitoring the absorbance at 260 nm. The conditions of the assay were DNA (120 μg/3 ml), DNase I (0.76×10^{-6} M), G-actin (1.2×10^{-6} M), phalloidin (4.2×10^{-9} M – 1.1×10^{-6} M) in solutions of pH 7.0, 83 mM Na-acetate, 4.2 mM $MgSO_4$ and 0.02 M KCl at 20°C.

of toxin per mol of actin, which are necessary to counteract totally the inhibition capacity of actin. Since these inhibition experiments with actin were run under conditions where G-actin prevails (low ionic strength, 0.02 M KCl), where phalloidin, however, still induces polymerization of G-actin, further support is provided for the idea that counteraction of actin inhibitor capacity is achieved by simple polymerization of actin monomers.

In a second series of experiments we studied whether phalloidin is capable to reverse the inhibition of DNase I by actin when added after the addition of the enzyme. In these experiments F-actin (0.1 M KCl) was used, which under conditions of fig.2 (two-fold concentrations of the proteins) caused about 50% inhibition of DNase I. The inhibitory capacity of F-actin may be explained by the fact that F-actin also may give rise to 1:1 complexes with DNase I, while actin treated with phalloidin (Ph-actin) does not. When phalloidin was added to this system 2.5 min after the partially inhibited DNA hydrolysis was started (fig.2, B) the extent of inhibition could be reduced depending on the amount of toxin added (figs.2C and D). For example, an about 10-fold excess of phalloidin over actin reduced the inhibition within a few minutes to 25%. The high concentration of phalloidin necessary

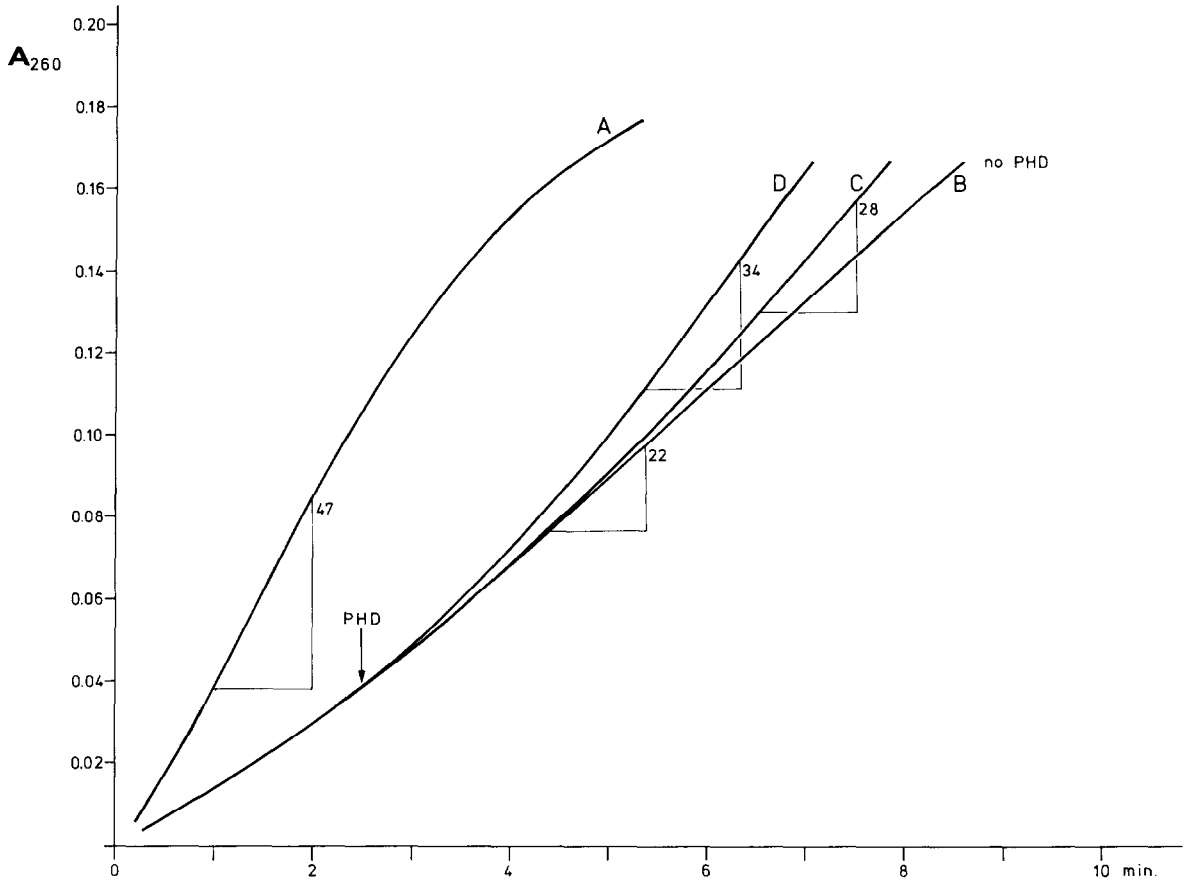

Fig.2. Rate of hydrolysis of DNA by DNase I. A: without F-actin, B: with F-actin, C and D: with F-actin and addition of phalloidin, 2.5 min after the addition of the enzyme. The concentrations in the assay mixture were DNase I (1.6×10^{-6} M), F-actin (2.5×10^{-6} M) DNA (120 μg/3 ml), phalloidin C (1.07×10^{-5} M) and D (2.8×10^{-5} M) in solutions of pH 7.0, 83 mM Na-acetate 4.2 mM $MgSO_4$ and 0.1 M KCl. The figures indicate the enzymatic activity by DNase I at 20 °C under the different conditions expressed in enzyme units (1 U = ΔA_{260} of 0.001 min^{-1}).

to reverse the actin inhibition may be explained by assuming that the dissociation of the actin – DNase complex even in the presence of phalloidin is a slow process.

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