The activation of cellulases from different sources by actin

Anatole A. Klyosov, Alla V. Bolobova, Marina V. Ostrovskaya and Boris F. Poglazov

A.N. Bach Institute of Biochemistry, USSR Academy of Sciences, Moscow 117071, USSR

Received 11 April 1985; revised version received 7 May 1985

Actin has been shown to be a potent activator of the enzymatic hydrolysis of cellulose, its addition to the reaction mixture leading to an increase in the hydrolysis rate of up to 6–7 fold. The action of actin is directed primarily towards endoglucanases of cellulase complexes. The degree of activation varies in relation to the cellulases from different microbial sources. The activation of the enzymatic hydrolysis of an insoluble cellulose by actin does not affect the Michaelis constant but increases the maximum velocity of the reaction. Increasing the actin concentration leads to a linear increase in the activation effect which indicates a rather poor binding of actin with the cellulases under study.

Actin *1,4-β-D-Glucan 4-glucanohydrolase (endoglucanase) activation* *Hydrolysis* *Cellulose degradation*

1. INTRODUCTION

Owing to recent achievements in genetic engineering, a series of quite unexpected parallels have been elucidated in the biosynthesis of compounds which are, at first glance, unrelated. Thus, during the screening of *Schizophyllum commune* genes, which are responsible for the synthesis of cellulolytic enzymes and during the construction of the corresponding cDNA library on the pBR322 plasmid, the fragment was recovered at 660 bp and identified as actin [1]. However, the part played by actin in the enzymatic hydrolysis of cellulose remains unknown.

On the other hand, there is an increasing amount of data to the effect that actin, the basic structural protein of muscle filaments and cytoskeleton of non-muscle cells, interacts with many enzymes and is a regulatory agent in relation to a number of metabolic processes in the cell [2].

The present work has shown that actin is a potent activator of the enzymatic hydrolysis of cellulose, and leads to an increase as great as 6–7-fold in the hydrolysis rate.

2. MATERIALS AND METHODS

2.1. *Purification of actin*

Globular (G) actin was prepared from acetone powder of rabbit skeletal muscle according to Spudich and Watt [3] with the modification of Maclean-Fletcher and Pollard [4]. The prepared G-actin was polymerized into F-actin by addition of KCl (to a final concentration of 50 mM) and $MgCl_2$ (to a final concentration of 2 mM) for 30 min at room temperature [5].

F-Actin and G-actin were repeatedly purified by the method of polymerization-depolymerization [5]. The homogeneity of actin preparations was monitored by polyacrylamide gel electrophoresis in the presence of SDS [6]. The initial protein concentration in the actin preparations was 3–7 mg/ml.

2.2. *Cellulase preparations and their activity*

Cellulase preparations from *Trichoderma viride* (Meicelase, Meiji Seika, endoglucanase activity 6000 IU/g), *T. reesei* (purified by affinity chromatography on cellulose [7], 3300 IU/g), *Myrothecia verrucaria* (260 IU/g), *Sporotrichum dimorphosporum* (Rapidase, 360 IU/g) were used. The molar concentration of endoglucanase was

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

calculated assuming the specific activity of homogeneous endoglucanase to be 70 IU/mg, and its M_r 50000 [8].

Endoglucanase (1,4-β-D-glucan glucanohydrolase) activity was determined by the viscometric method [9] using 1.0% CM-cellulose (Serva), pH 6.0, 40°C. The activity was expressed in μmol glucosidic bonds split · min^{-1}.

Reducing sugars in the course of the enzymatic hydrolysis of CM-cellulose (1.0%, pH 6.0, 20°C) were measured by the modified Somogyi-Nelson method [10]. In the absence of cellulolytic preparations, the addition of actin to CM-cellulose did not result in a decrease in the viscosity of the solution, or in the formation of reducing sugars.

A dyed amorphous cellulose (NPO 'Ferment', Vilnius) was used as an insoluble cellulosic substrate for studying the enzymatic hydrolysis (pH 6.0, 20°C); the absorbance of the supernatant was recorded at 490 nm.

3. RESULTS

3.1. *Effect of actin on endoglucanase activity from* A. foetidus *and* S. dimorphosporum

F-Actin (0.3 mg/ml, or 7.1×10^{-6} M) activated endoglucanase of *A. foetidus* by 2.0 ± 0.3-times, but not endoglucanase of *S. dimorphosporum* under the same conditions. In both cases, the concentration of cellulase preparations was 0.0025 mg/ml, or about $2–3 \times 10^{-10}$ M calculated for the endoglucanase concentration in the preparations; thus, actin was in great molar excess over endoglucanases in the reaction mixture.

3.2. *Effect of actin on cellulase activity in relation to the insoluble cellulose*

F-Actin (0.25 mg/ml) activates the hydrolysis of the insoluble dyed cellulose (10 mg/ml) under the action of cellulase preparations from the following sources: *S. dimorphosporum*, 2.8-times; *A. foetidus*, 2.3-times; *T. viride*, 1.3-times; *M. verrucaria*, 1.2-times. Further studies have shown that actin (0.25 mg/ml) does not affect the Michaelis constant for the hydrolysis by *T. viride* (K_m 6.6 mg/ml) and *S. dimorphosporum* (K_m 67.0 mg/ml), but the corresponding V_m values are 2.5-times (*T. viride*) and 6.6-times (*S. dimorphosporum*) higher. Fig.1 shows that the activation effect on the enzymatic hydrolysis of the dyed cellulose increases linearly with the concentration of F-actin.

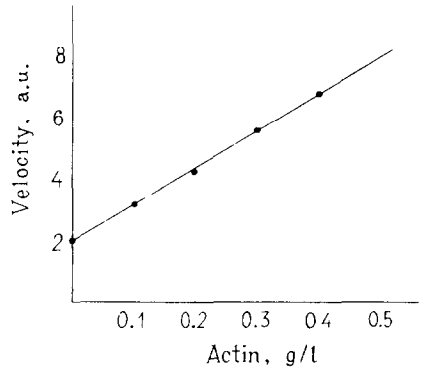

Fig.1. Effect of actin concentration on the rate of hydrolysis of insoluble dyed cellullose by a cellulase complex from *T. viride* at 0.05 g/l. Conditions: pH 6.0; 20°C; cellulose, 10 g/l.

3.3. *Dependence of the activation effect on preincubation of actin with cellulases*

Actin was preincubated with cellulases at pH 6.0 and 20°C in the absence of substrate. The results showed that activation by actin changed gradually with time, the character of the change varying according to the sources of the cellulases. Thus, for *S. dimorphosporum* activation by actin was not observed after the first 30 min, during the next 60 min endoglucanase activity decreased by 35–40% (fig.2), and, finally, after 10 h storage at 4°C the activity increased 2.3-times over the blank solution and remained unchanged thereafter. On the other hand, for *T. viride* with actin the endoglucanase activity increased 1.5-times over the control in the first 30 min, returned to the initial value during the next 30 min of incubation, and after 10 h of storage at 4°C again increased 1.5-times and remained constant.

3.4. *Effect of actin on the formation of reducing sugars in the course of CM-cellulose hydrolysis*

In this case, the activation by actin also varied for different microbial sources of cellulases. For cellulases of *S. dimorphosporum* the addition of actin (0.31 and 0.48 mg/ml) did not result in a change in formation of reducing sugars for CM-cellulose hydrolysis for at least 40 min. This obser-

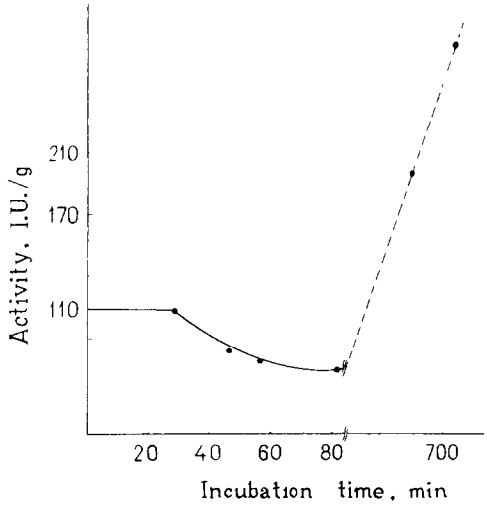

Fig.2. Activation of endoglucanase from *S. dimorphosporum* by actin at 0.6 g/l for different preincubation periods. The enzymatic activity was determined viscometrically (CM-cellulose hydrolysis at pH 6.0, 40°C). The concentration of the cellulase preparation was 0.3 g/l in the incubation mixture, and $4–5 \times 10^{-3}$ g/l in a viscometer.

vation is in agreement with previous data, in which the hydrolysis of CM-cellulose by the same cellulase preparation was followed using viscometry.

For cellulases of *T. viride* addition of actin (0.5 mg/ml) to the mixture with CM-cellulose resulted in a time lag of 15 min in formation of the reducing sugar (fig.3a), then the rate of hydrolysis increased 2.5-times over the control, and decreased again 40–50 min later. These data are in agreement with those of previous experiments (see above) on the preincubation of *T. viride* cellulases with actin, in which the activation of the viscometric hydrolysis passed the maximum in the first 60 min. To verify these results, the same experiment was carried out with a sufficiently better purified cellulase preparation from *T. reesei* (fig.3b). It can be seen that the kinetic curves are basically the same.

3.5. *Formation of actin complexes with cellulases*

After mixing cellulases with actin (0.6–0.8 mg/ml) and ultracentrifugation of the solution (5°C, 3.5 h, 38000 rpm) with the subsequent determination of endoglucanase activity in the supernatant

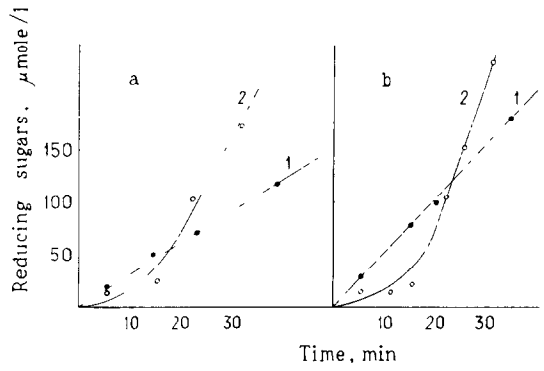

Fig.3. The kinetics of the formation of reducing sugars from CM-cellulose at 5.0 g/l under the action of (a) a commercial cellulase complex from *T. viride* at 0.014 g/l, (b) cellulase complex from *T. reesei* purified by affinity chromatography at 0.008 g/l. (1) No actin; (2) actin at 0.5 g/l (a), 0.31 g/l (b). Conditions: pH 6.0, 20°C.

and residue, quite different results were obtained for different cellulases.

For *S. dimorphosporum* endoglucanase activity in the supernatant was 15% lower than in the control, but the major activity was found in the residue in a complex with actin. In contrast, the same experiments with the cellulase of *T. viride* and the purified cellulase of *T. reesei* showed that the residue did not possess endoglucanase activity. Instead, the viscometric activity of endoglucanases in the supernatant after the precipitation of highly polymeric actin increased 2.0 ± 0.2-times in both cases. The same degree of activation (2.2 for *T. viride* and 1.8 for *T. reesei*) was also observed for the hydrolysis of dyed insoluble cellulose.

4. DISCUSSION

Our results show that F-actin is a fairly effective activator of cellulases leading to as much as 6–7-fold acceleration of the enzymatic hydrolysis of cellulose. The data also demonstrate the distinct differences in behaviour of actin towards endoglucanases from different microbial sources. Thus, the activation of endoglucanase of *T. reesei* (and *T. viride*) is apparently induced solely by relatively low-M_r fragments of actin, as follows from the ultracentrifugation data. In the latter case, an endoglucanase–highly polymeric actin

complex is either inactive or does not exist at all.

As far as the activation of endoglucanase of *S. dimorphosporum* is induced by highly polymeric actin, formation of the corresponding complex apparently takes a certain time. This feature may explain the rather prolonged time lag in activation of *S. dimorphosporum* endoglucanase in relation to the hydrolysis of both soluble CM-cellulose and the insoluble substrate (fig.2). On the other hand, for the action of endoglucanases from *A. foetidus*, *T. viride* and *T. reesei*, where the activation is apparently caused by low-M_r actin, there was no detectable lag period in the activation. In general, the time pattern of the activation effect of actin is rather complicated and may indicate complex conformational processes which develop between the enzymes and polymeric actin.

Finally, the finding that an increase in F-actin concentration (up to 5 mg/ml, or 1.2×10^{-4} M) leads to a linear increase in the activation effect (fig.1) points to a rather poor affinity between actin and cellulases. This correlates well with the relatively low transfer of endoglucanase from the solution into the precipitating endoglucanase-actin complex, as shown by ultracentrifugation. This, in turn, might indicate that in the actin-endoglucanase complex the enzyme is activated at least several dozen times more strongly than would follow directly from the kinetic data.

Thus, the observation that the actin gene is expressed simultaneously with the cellulase genes in response to the addition of cellulose to the media [1] may not be accidental, and the present data indicate the possible co-ordinated regulation of biosynthesis and the functioning of compounds such as actin and cellulases which appear at first glance to be quite unrelated.

REFERENCES

[1] Seligy, V.L., Barbier, J.R., Dimock, K.D., Dove, M.J., Moranelli, F., Morosoli, R., Williok, G.E. and Yaguchi, M. (1983) in: Gene Expression in Yeast, Proceedings of the ALKO Yeast Symposium, Helsinki, pp.167–185.
[2] Poglazov, B.F. (1983) Biochem. Int. 6, 757–765.
[3] Spudich, J.A. and Watt, S. (1971) J. Biol. Chem. 246, 4866–4871.
[4] Maclean-Fletcher, S. and Pollard, T.D. (1980) Biochem. Biophys. Res. Commun. 96, 18–27.
[5] Silonova, G.V., Livanova, N.B., Andreeva, I.E., Solovjeva, N.B., Eronina, T.B., Ostrovskaya, M.V. and Poglazov, B.F. (1984) Biokhimiya (in Russian) 49, 127–135.
[6] Laemmli, U.K. (1970) Nature 227, 680–685.
[7] Martianov, V.A., Rabinovich, M.L., Klyosov, A.A., Mjagkich, I.V. and Gernet, M.V. (1983) Prikl. Biokhim. Mikrobiol. (in Russian) 19, 513–520.
[8] Chernoglazov, V.M., Klyosov, A.A. and Ermolova, O.V. (1983) Biokhimiya (in Russian) 48, 1617–1623.
[9] Klyosov, A.A. and Rabinowitch, M.L. (1980) in: Enzyme Engineering Future Directions (Wingard, L.B. et al. eds) pp.83–165, Plenum, New York.
[10] Klyosov, A.A., Rabinovich, M.L., Sinitsyn, A.P., Churilova, I.V. and Grigorash, S.Yu. (1980) Bioorg. Khim. (in Russian) 6, 1225–1242.