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SODIUM DODECYL SULFATE - INDUCED CONFORMATIONAL AND ENZYMATIC CHANGES OF MULTICATALYTIC PROTEINASE

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SUMMARY: Chymotrypsin-like activity of the multicatalytic proteinase (MCP) purified from eggs of the ascidian *Halocynthia roretzi* was activated by the addition of SDS. Complete activation was achieved simultaneously at the time of SDS addition, and this activity decreased as a function of time. Autonomous fluorescence of MCP also increased rapidly at the time of SDS addition and then decreased at a rate that depended on the SDS concentration. The decrease of autonomous fluorescence induced by SDS preceded that of the activity. These results suggest that a rapid conformational change of MCP induced by SDS results in the enhancement of chymotrypsin-like activity, followed by the decrease of this activity because of the lability of the activated conformation. © 1989 Academic Press, Inc.

The multicatalytic (high-molecular-weight) proteinase (MCP) (1) is an enzyme complex composed of several components and shows multiple protease activities (2). This enzyme complex has been shown to be widely present in various tissues of many animals (2) and also in lower eukaryotic cells including yeasts (3). One interesting characteristic of MCP is that at least the chymotrypsin-like activity of MCP is activated by the addition of sodium dodecyl sulfate (SDS) (2, 4-10). This SDS-activation process has, however, not been well-characterized.

In the previous study of gamete proteases involved in the fertilization of the ascidian *Halocynthia roretzi*, we surveyed an egg chymostatin-sensitive chymotrypsin-like enzyme that possibly functions in the process

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Abbreviations: MCP, multicatalytic proteinase; SDS, sodium dodecyl sulfate; MCA, 4-methylcoumaryl-7-amide; AMC, 7-amino-4-methylcoumarin.

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leading to the cleavage of fertilized eggs and found that the enzyme has the characteristics of an MCP (11). The enzyme purified from ascidian eggs has properties similar to those of MCPs obtained from other cells and tissues (2, 3). The chymotrypsin-like activity of ascidian MCP was also activated by the presence of a low concentration of SDS or fatty acids.

In this study, we examined the effects of SDS on the structure and the chymotrypsin-like activity of ascidian MCP to define the structural basis for the SDS-activation process; we present evidence for an SDS-induced conformational change, as revealed by monitoring the autonomous fluorescence of proteins.

MATERIALS AND METHODS

MCP was purified from the eggs of *H. roretzi* as previously described (11) and stored at –20 °C in 25 mM Tris-HCl, pH 8.0, containing 75 mM NaCl, 0.0025 % Brij 35, and 50 % glycerol.

The chymotrypsin-like activity of MCP was measured at 25 °C in 50 mM Tris-HCl, pH 8.0, containing 10 mM $CaCl_2$ using Suc-Leu-Leu-Val-Tyr-4-methylcoumaryl-7-amide (MCA) (20 μM, Peptide Institute, Inc.) as the substrate (11). The time-dependent change of the activity in the presence of SDS was measured by adding SDS into a mixture of the enzyme (4 μg/ml) and the substrate and then monitoring the fluorescence due to 7-amino-4-methylcoumarin (AMC) using an excitation at 380 nm and an emission at 460 nm; the rate of the enzymatic reaction at a certain time of incubation was determined from the slope of the fluorescence vs. time curve at that time.

Autonomous fluorescence of MCP in the presence or the absence of SDS was measured with an excitation at 282 nm and an emission at 348 nm under the same conditions as those of the assay for chymotrypsin-like activity. SDS was added to a MCP solution without the substrate, and the time-dependent change of the fluorescence was measured.

RESULTS AND DISCUSSION

In a previous study (11), we demonstrated the SDS-activation of chymotrypsin-like activity of ascidian egg MCP by comparing the total amounts of the fluorescent reaction product AMC that accumulated during a defined duration of the enzymatic reaction in the absence and the presence of SDS (various concentrations). In this study, we first examined the effect of SDS on the activity by monitoring the rate of change of fluorescence increase due to AMC formation. The time-dependence of the rate in the absence or the presence of various concentrations of SDS is shown in Fig. 1. By the addition of SDS at a concentration higher than 0.008 % (Fig. 2), the enzyme activity increased promptly (during the very short time required to mix the SDS with the enzyme-substrate mixture),

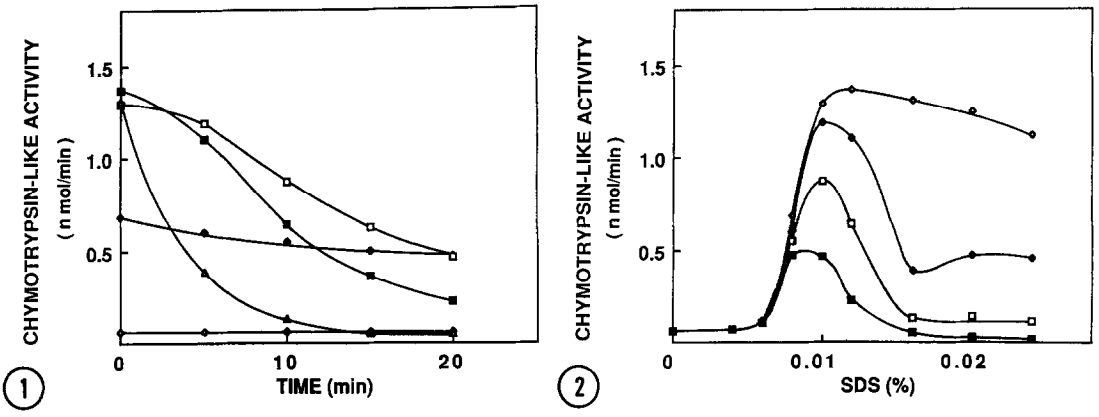

Fig. 1. Change of the chymotrypsin-like activity of ascidian egg MCP after addition of SDS at the final concentration of 0.016 % (Δ), 0.012 % (■), 0.01 % (□), 0.008 % (◆), or 0 % (◇). The activity was assayed at 25 °C with 20 μM Suc-Leu-Leu-Val-Tyr-MCA in 50 mM Tris-HCl, pH 8.0, containing 10 mM $CaCl_2$.

Fig. 2. Dependence on SDS concentration of the chymotrypsin-like activity of ascidian egg MCP measured at the time of SDS addition (◇) and after incubation with SDS for 5 min (◆), 10 min (□), or 20 min (■). The activity was assayed as described in Fig. 1.

and approximately 20-fold activation was observed at SDS concentrations higher than 0.01 %. However, the SDS-induced activity decreased as a function of time, and this was dependent on the SDS concentration (Fig. 1). Finally, it disappeared completely (data not shown). From the data shown in Fig. 2, the optimum SDS concentration for the enhancement of chymotrypsin-like activity was estimated to be about 0.008 - 0.01 % after a prolonged incubation. Thus, these results indicate that SDS-induced a temporary activation of the chymotrypsin-like activity of MCP, demonstrating that the SDS-activated activity is quite labile.

Next, we examined the alteration of autonomous fluorescence of the MCP induced by SDS to study the structural basis for the SDS-activation of this chymotrypsin-like activity. As shown in Fig. 3 (A), the fluorescence, probably emitted by tryptophan residues, decreased slightly during a prolonged incubation (20 min, 25 °C) even in the absence of SDS. These results may be relevant to the fact that the chymotrypsin-like activity decreased to the level of 75 % of the initial activity after incubation for 20 min in the absence of the substrate. It can be considered that the activity may be unstable without the substrate. On the other hand, a remarkable reduction of fluorescence took place after a prolonged incubation (20 min, 25 °C) in the presence of 0.01 % SDS (Fig. 3 (B)). By using 348 nm as the emission wavelength, where the maximum change of fluorescence was

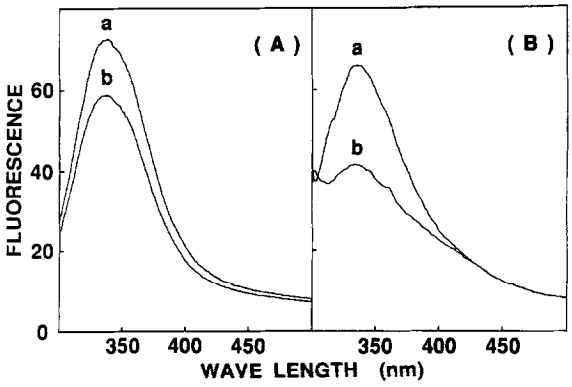

Fig. 3. Fluorescence emission spectra of ascidian egg MCP at the incubation time of 0-2 min (a) or 20-22 min (b) in the absence (A) or the presence of 0.01 % SDS (B). The spectra were measured with excitation at 282 nm at 25 °C in 50 mM Tris-HCl, pH 8.0, containing 10 mM $CaCl_2$. The scanning rate was 100 nm/min .

observed, we followed the change occurring in the presence of various concentrations of SDS (Fig. 4). At the time of SDS addition (precisely, during the time required to mix the enzyme solution with SDS), the fluorescence increased promptly about 1.2-fold. Such a rapid increase of the fluorescence seems to be consistent with a rapid enhancement of the activity by SDS as shown in Fig. 1. After that time, the fluorescence decreased as a function of time, and the rate of the decrease was dependent on the concentration of SDS added. In the control experiments, SDS at the concentrations used in the experiment shown in Fig. 4 did not affect the fluorescence emitting from a tryptophan solution

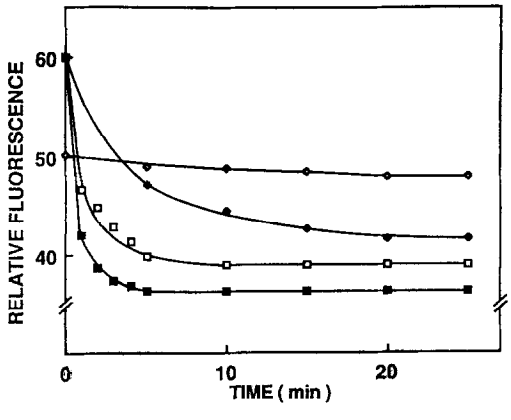

Fig. 4. Time-dependence of autonomous fluorescence of ascidian egg MCP in the absence (∘) or the presence of 0.008 % (•), 0.01 % (□), or 0.012 % SDS (■). Autonomous fluorescence was measured at the indicated time after addition of SDS with excitation at 282 nm and emission at 348 nm under the same conditions as those in Fig. 3.

(0.14 μM), the level of which was adjusted to be the same as that of the initial fluorescence of the MCP solution used in Fig. 4 (data not shown). Thus, these findings suggest that a conformational change occurs in a microenvironment near the fluorescing tryptophan residues at the time of SDS addition and also during the prolonged incubation with SDS. Comparison of the time-dependency between the change of chymotrypsin-like activity (Fig. 1) and that of autonomous fluorescence (Fig. 4) indicates that the change of the latter takes place more rapidly than that of the former: at 0.01 % SDS, for example, it takes only about 3 min for a 50 % reduction in the fluorescence, whereas about 15 min is required for a 50 % decrease in activity. Thus, the conformational change induced by SDS precedes the change of the chymotrypsin-like activity. We assume that the rapid SDS-activation may be directly related to the rapid conformational change, while the lability of SDS-activated activity may be regulated partly by the rather slow conformational change shown in this study. Further studies are necessary to define factors controlling the lability of the SDS-induced activity.

Upon SDS-activation of MCP from rat skeletal muscle, Dahlmann *et al*. (4) have observed increased susceptibility to the proteinase inhibitor chymostatin and reduced interaction with the antibody raised against the native enzyme, and they suggested that there is a conformational change of the enzyme in the activation process. A similar result with the increased susceptibility to chymostatin induced by SDS has been reported with the MCP of human kidney (8). However, SDS-induced conformational change of MCP molecules on the physicochemical basis has not been demonstrated. Ishiura and Sugita (6) has examined the chromatographic behavior of rabbit reticulocyte MCP in the presence of SDS on a gel filtration column and showed that the subunits of MCP bind tightly together even in the presence of SDS. Our preliminary study on the gel filtration of ascidian egg MCP gave a similar result; that is, the enzyme emerges in the presence of SDS at the same elution volume as that in the absence of SDS. In conclusion, this is the first report giving direct evidence for SDS-induced conformational change in MCP that results in the regulation of enzymatic activity.

In addition to SDS, fatty acids have been reported to enhance the activity of MCP (2, 4-5, 7, 10-14). The chymotrypsin-like activity of ascidian egg MCP was also enhanced by fatty acids such as myristic acid and stearic acid (11). Our preliminary studies on the fatty acid-activation process of this enzyme indicate that autonomous fluorescence decreases during the duration of incubation with fatty acid, accompanied by a decrease of the activity, in a similar manner to the case of SDS-activation,

although to a less extent. These results imply that the activity, at least the chymotrypsin-like activity, of MCP may be controlled by naturally-occurring fatty acids such as arachidonic acid and its metabolites through a conformational change of the enzyme molecule. Further studies are necessary to define natural regulators and clarify the mechanisms for the regulation of MCP activities by these substances.

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