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Effects of Detergents on Catalytic Activity of Human Endometase/Matrilysin-2, a Putative Cancer Biomarker†

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Abstract

Matrix metalloproteinases (MMPs) are a family of hydrolytic enzymes that play significant roles in development, morphogenesis, inflammation, and cancer invasion. Endometase (matrilysin 2 or MMP-26) is a putative early biomarker for human carcinomas. The effects of the ionic and nonionic detergents on catalytic activity of endometase were investigated. The hydrolytic activity of endometase was detergent concentration-dependent exhibiting a bell-shaped curve with its maximum activity near the critical micelle concentration (CMC) of nonionic detergents tested. The effect of Brij-35 on human gelatinase B (MMP-9), matrilysin (MMP-7), and membrane-type 1 MMP (MT1-MMP) was further explored. Their maximum catalysis was observed near the CMC of Brij-35 (~90 μ M). Their IC_{50} values were above the CMC. The inhibition mechanism of MMP-7, MMP-9, and MT1-MMP by Brij-35 was mixed-type as determined by Dixon's plot, however, that of endometase was non-competitive with a K_i value of 240 μ M. The catalytic activities of MMPs are influenced by detergents. Monomer of detergents may activate and stabilize MMPs to enhance catalysis, but micelle of detergents may sequester enzyme and block substrate binding site to impede catalysis. Under physiological conditions lipid or membrane microenvironment may regulate enzymatic activity.

Keywords

Matrix metalloproteinases (MMPs); MMP-26; ionic and nonionic detergents; critical micelle concentration; enzyme kinetics; enzyme inhibition mechanisms; regulation of catalytic activity; peptide hydrolysis; inhibition constant; putative cancer biomarker; homology modeling; hydrophobic interaction; lipids and membrane microenvironment; detergent-enzyme interaction; detergent-substrate interaction

Introduction

Detergents are soluble amphiphiles used for membrane [1] and membrane protein solubilization [2,3]. Soft detergents (nonionic detergents or bile salts) do not interact

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significantly with most water-soluble proteins except for those that can accommodate small amounts of detergents on their hydrophobic surface. This hydrophobic surface is the means for a protein to interact with detergent molecules. The hydrophobic tail of the detergent molecule will interact with the hydrophobic sites of the protein, whereas the hydrophilic head is exposed to water. By the mediation of detergent molecules, the unfavorable interaction between hydrophobic residues on the surface of a protein and water molecules can be minimized, and the aggregation of protein molecules can be prevented [1-3].

One of the outstanding characteristics of detergents is the ability to form a micelle (an aggregate of detergent molecules). In solutions, both micelle and monomer states of a detergent are in equilibrium. The formation of micelles is facilitated by increasing the concentration of the detergent in solution. Therefore, the higher concentration of the detergent may not mean that there is an increase of monomer concentration but actually an increase of micelle concentration occurs. Critical micelle concentration (CMC) is defined as the total detergent concentration in a solution where maximum number of monomer molecules are present [4]. Because of the equilibrium between monomer and micelle states in detergents, proteins can interact either with monomer or micelle of detergents in use. The extensive interaction of protein with detergent molecules may affect the functions of the protein.

Matrix metalloproteinases (MMPs) belong to the metzincin superfamily of proteases [5]. They are inhibited by transition metal chelators, such as EDTA and orthophenanthroline and specifically by tissue inhibitors of matrix metalloproteinases (TIMPs) in biological systems. Their main biological function is believed to be cleaving extracellular matrix (ECM) and shedding cell surface receptors and growth factors [6,7]. MMP-26 is one of the smallest members of the MMP family composed of only pro- and catalytic domains [8]. The substrate specificity of MMP-26 is similar to that of MMP-2 and MMP-9 [9]; the structure of S_1 ' pocket resembles that of the MMP-8 with an intermediate S_1 ' pocket at the enzyme active site [10]. A recent study has revealed that MMP-26 has two calcium ions with different binding affinities. The low affinity calcium ion modulates enzymatic activity by changing the tertiary structure of MMP-26 [11]. Moreover, biological and pathological importance of MMP-26 has also been studied. Cancer tissue at a pre-invasive stage shows high expression of MMP-26 as compared to cells in normal and invasive cancer tissues [12-15].

For enzyme kinetic analyses of MMPs 0.05% of Brij-35 is generally used to maintain the catalytic activity at low enzyme concentrations [16,17]. Kinetic study of endometase has shown that the catalysis of endometase is affected by the concentration of Brij-35. In the case of Brij-35, the optimum concentration was lower than 0.05%, which was the concentration commonly used for MMP analysis. The role of detergents during the catalysis of MMPs has not been clearly understood. The study presented here, investigates the effects of ionic and nonionic detergents (Figure 1) on the hydrolysis of peptide substrates by endometase (MMP26). The study also provides a clarification of the relationship between the catalytic activity and CMCs of detergents.

Materials and Methods

Chemicals and Reagents

Peptide substrates, Mca-Pro-Lys-Pro-Leu-Ala-Leu-Dap(Dnp)-Ala-Arg-NH₂ and Mca-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH₂, were purchased from Bachem Chem., Inc. Brij-35 and Tween-20 from Fisher Scientific, Co., Triton X-100 and TTAB were purchased from Sigma Chemicals, Co. All reagents were of enzyme grade. ANS, 8-Anilino-1-naphthalene-sulfonate, was purchased from Aldrich Chemicals Co. GM6001 was purchased from Calbiochem (San Diego, CA). All of metal salts were purchased from Fisher Scientific Co.

Preparation of Enzyme

Since prodomain of MMP-26 is necessary for proper folding of the enzyme, proMMP-26 was expressed in the form of inclusion bodies from transformed *E. Coli* cells [8]. The activation mechanism of MMP-26 is still unclear, but is likely to involve auto-activation [9,18]. Recombinant MMP-26 protein was prepared as described previously [8]. The inclusion bodies were isolated and purified using B-PER™ bacterial protein extraction reagent according to the manufacturer's instructions. The insoluble protein was dissolved in 8 M urea to about 5 mg/ml. The protein solution was diluted to ~100 µg/ml in 8 M urea and 10 mM (DTT) for 1 hour, dialyzed in 4 M urea, 1 mM DTT, 50 mM HEPES or Tricine, at pH 7.5 at least 1 hour, then folded by dialysis in buffer containing 50 mM HEPES, 0.2 M NaCl, 10 mM CaCl₂, 20 µM ZnSO₄, 0.05 % Brij-35, pH 7.5 for 12 hour three times. During dialysis enzyme was partially auto-activated. The total enzyme concentration was measured with $\epsilon_{280} = 57130 \text{ M}^{-1}\text{cm}^{-1}$ calculated by Genetics Computer Group (GCG) software. Because endometase was not fully activated, the concentration of active endometase was further determined by titration with synthetic inhibitor GM6001. MMP-7/matrilysin and MT1-MMP/MMP-14 were kindly provided by Dr. Harold E. van Wart (Roche Diagnostics) and professor Harald Tschesche (Bielefeld University), respectively.

Enzyme Kinetic Assays

The peptide substrates were prepared as 100, 50, or 25 µM stock solution in 1:1 dimethyl sulfoxide (DMSO) and water. Routine assays were performed with 1 µM of peptide substrate concentration. Fluorescent assays were measured at $\lambda_{\text{excitation}} = 328 \text{ nm}$ and $\lambda_{\text{emission}} = 393 \text{ nm}$ using a Perkin Elmer LS 50B Luminescence Spectrometer connected with a constant-temperature water bath. Assays were performed at 25 °C in pH 7.5 assay buffer containing 50 mM HEPES, 0.2 M NaCl, and 10 mM CaCl₂ in the presence or absence of 0.01 % or 0.05 % Brij-35 [9-11]. Nonionic and ionic detergent concentrations ranged from 0.001% to 0.96% and 1 µM to 512 µM, respectively. The active enzyme concentrations in the assays were 10, 1, 0.5, and 0.2 nM for endometase (MMP-26), human neutrophil gelatinase (HNG, gelatinase B, MMP-9), catalytic domain (cd) MT-1 MMP (MMP-14), and matrilysin (MMP-7), respectively. Initial linear hydrolysis rates were monitored for 10 minutes for the kinetic measurement. Mca-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH₂ was used with endometase, and Mca-Pro-Lys-Pro-Leu-Ala-Leu-Dap(Dnp)-Ala-Arg-NH₂ was used with MMP-7, MMP-9, and MT1-MMP.

Determination of Critical Micelle Concentration (CMC)

The CMC of nonionic detergents used were determined as described previously [19]. Nonionic detergents were prepared as 1% stock solutions in the assay buffer, well above their CMCs. Ionic detergents were prepared as 1% stock solutions in water. The stock solutions were diluted with 2X buffer and water to give constant ionic strength. The stock solutions of fluorescent probes, 8-anilino-1-naphthalene-sulfonate (ANS), were prepared 500 µM in water. Accurate ANS concentration was determined by using the molar absorption coefficient, ϵ_{350} , of 5000 M⁻¹.cm⁻¹ [20,21]. The final concentration of ANS for the determination of CMC was 4 µM and 200 nM, respectively. The changes in fluorescence intensity were determined at 25 °C using a Perkin Elmer Luminescence Spectrophotometer LS 50B connected with a constant temperature water bath. The excitation wavelength was 370 nm and the emission wavelength was 490 nm.

Homology Modeled Structure of the MMP-26 Catalytic Domain

Homology modeled structure of the MMP-26 catalytic domain was generated based on MMP-12 (PDB access code 1JK3) structure and the outcome structure was minimized with an Amber forcefield as described previously [10,11]. Hydrophobic surfaces areas around the

catalytic site of MMP-26, MMP-7 (PDB access code 2DDY), MMP-9 (PDB access code 1L6J), and MMP-14 (PDB access code 1BQQ) were examined for the comparison.

Test of Detergent-Enzyme and Detergent-Substrate Interactions

To test if the enzyme inhibition observed at detergent concentration higher than the critical micelle concentration could be due to enzyme or substrate sequestering, the following experiments have been designed and performed. Firstly, detergent-enzyme interactions were tested; 20 μ L of 190 nM MMP-26 was mixed with 372 μ L assay buffer (50 mM HEPES, 0.2 M NaCl, 10 mM CaCl_2 , pH=7.5) containing no or different concentrations of Brij-35. After one hour incubation at 25 $^{\circ}\text{C}$ different reaction mixtures were centrifuged at 3,000 rpm for 5 minutes. Then, 196 μ L supernatant was transferred into a quartz cuvette. Substrate hydrolysis reaction was initiated by adding 4 μ L of 50 μ M substrate in the enzyme containing buffer. Secondly, detergent-substrate interactions were measured; 8 μ L of 50 μ M quenched fluorescence peptide substrate, Mca-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH₂, was mixed with 372 μ L assay buffer (50 mM HEPES, 0.2 M NaCl, 10 mM CaCl_2 , pH=7.5) with no or different concentrations of Brij-35. After one hour incubation at 25 $^{\circ}\text{C}$ different reaction mixtures were centrifuged at 3,000 rpm for 5 minutes. 190 μ L of supernatant was transferred into a quartz cuvette. Substrate hydrolysis reaction was initiated by adding 10 μ L of 190 nM of MMP-26. The changes in fluorescence intensity were determined at 25 $^{\circ}\text{C}$ using a Perkin Elmer Luminiscence Spectrophotometer LS 50B connected with a constant temperature water bath. The excitation wavelength was 328 nm and the emission wavelength was 393 nm.

Total Substrate Hydrolysis and Determination of Conversion Factor of Fluorescence

To test if there is any change in the relative conversion factor (fluorescence intensity unit/nM substrate hydrolyzed) when the detergent concentration is changed the following experiments were carried out. MMP-26 and the quenched fluorescence peptide substrate, Mca-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH₂, in the absence or presence of different concentrations of Brij-35 were mixed and incubated at 25 $^{\circ}\text{C}$ for 2 days. Each reaction mixture contained 10 μ L of 190 nM MMP-26 and 4 μ L of 50 μ M substrate with no or different concentrations of Brij-35, and 186 μ L reaction buffer. Each experimental mixture was diluted to containing 125, 62.5, 31.25 and 15.625 nM of substrate with 50 mM HEPES, pH 7.5, 10 mM CaCl_2 , 0.20 M NaCl, in total 200 μ L reaction mixture to measure fluorescence intensity. The changes in fluorescence intensity were determined at 25 $^{\circ}\text{C}$ using a Perkin Elmer Luminiscence Spectrophotometer LS 50B connected with a constant temperature water bath. The excitation wavelength was 328 nm and the emission wavelength was 393 nm.

Results and Discussions

Critical micelle concentration (CMC) is the narrow concentration range of detergents below which no micelles are detected and above which virtually all additional detergent molecules form micelle [4]. This property was determined because the self association of detergent molecules at the critical micelle concentration may correlate with the ability of compounds to interact with biological molecules [2]. The formation of micelles may influence the catalysis of peptide hydrolysis by endometase.

CMCs of detergents (Figure 1) were determined and the results are summarized in Table 1. CMCs of Brij-35, Tween-20, and Triton X-100 were estimated by measuring the intensity of fluorescence from ANS in the assay conditions for MMP-26. The anionic dye, ANS, is almost nonfluorescent in water but becomes highly fluorescent in organic solvents or when bound to hydrophobic surfaces or macromolecules [21]. This property of ANS was also used to measure conformational change of MMP-26 [11]. Figure 2 shows the increase in ANS fluorescence with the increasing concentration of the detergent. Two straight lines can be drawn through

the points; their intersection is taken as the concentration at which the detergents aggregate to form micelles. The determined CMC values of the nonionic detergents were in good agreement with the literature [22,23]. The order of CMCs among the nonionic detergents were Tween 20 < Brij-35 < Triton X-100. The results confirm that CMC of nonionic detergents were not significantly affected by the ionic strength of the detergent [2-4].

The interactions between surfactants and proteins in aqueous solutions can be rather specific, and the enzyme activity is dependent on the nature of both the surfactant and the enzyme. For instance, it has been found that nonionic detergents and bile salts are capable of stimulating catalytic activity of some enzymes. Brij-35 stimulates cytoplasmic glycerol-3-phosphate dehydrogenase [24], and the addition of Tween 20 results in a 3 to 6 fold increase in the activity of mitochondrial carnitine palmitoyltransferase [25]. However, they may be inhibitory as found in protein kinase C [26]. In MMP-26 (endometase/matrilysin 2) catalysis, the peptide hydrolysis was inhibited by 0.05% (417 μ M) Brij-35, which is commonly used in the assay of MMPs. Thus the influence of surfactants on MMP-26 catalysis was further investigated with three different types of detergents. Detergents that were examined are; three nonionic (Brij-35, Triton X-100, and Tween-20), one cationic (tetradecyl trimethyl ammonium bromide, TTAB), and one anionic (sodium dodecyl sulfate, SDS). Since proMMP-26 was not fully active, the exact concentration of active MMP-26 has been measured and determined by titration with GM6001. Figure 3 shows data fitting to the Morrison equation [27].

MMP-26 displayed bell-shaped concentration dependence for all of the detergents used in this study (Figure 4). Using nonionic detergents, the optimum activities of MMP-26 for peptide hydrolysis was observed at near CMC. The detergent that displayed the widest concentration range for optimum endometase activity was triton X-100 (maximum at 140 μ M), probably because of the highest CMC (220 μ M). Brij-35 (maximum at 56 μ M) and tween-20 (maximum at 79 μ M) displayed a narrower range than triton X-100 but broader than TTAB and SDS. The optimum concentrations of ionic detergents were about one order lower than those of the nonionic detergents. The reason may be that CMCs of these ionic detergents in our assay conditions may be close to the optimum concentration for MMP-26 catalysis, because they are generally much more sensitive to ionic strength [2-4]. The detergents behaved as inhibitors of endometase at concentrations above CMC. The IC_{50} values of the detergents for the peptide hydrolysis by endometase were measured by using the concentrations above CMC as summarized in Table 1. The results indicate that optimum activity of endometase in nonionic detergents was closely related to the CMCs of the detergents under the assay conditions.

The effect of Brij-35 on MMP peptide hydrolytic activity was further investigated with other human MMPs, MMP-9 (human neutrophil gelatinase, HNG), MMP-7 (matrilysin), and cd-MMP-14 (cd-MT1-MMP), and the results were compared with that of MMP-26. The results are shown in Figure 5 and summarized in Table 2. Their optimum activity ranges are also near the CMC of Brij-35. Thus, the catalysis of MMPs in the presence of Brij-35 may be at maximum near the CMC. Among the MMPs tested, MMP-7 and MMP-26 displayed the broadest and the narrowest optimum range with Brij-35, respectively. The optimum activity being near the CMCs, indicates that the interactions of monomeric detergent molecules with MMPs may be favorable and required for the optimum catalysis of MMPs. The monomeric interactions may reduce the unfavorable interactions of MMPs with water molecules by covering hydrophobic sites, thus stabilizing the folding structure and preventing the aggregation of enzyme. Micelles can also cover the hydrophobic sites. They might become so large that they block the active site or surround the substrate molecules resulting in inhibition. MMPs used in this experiment do not have any hydrophobic domain like membrane proteins, but a few hydrophobic interactions with detergents may occur on the surface of the protein. Although MMPs are active in the absence of detergents, their activities are sub-optimal. It seems that the detergent activation mechanism is not preventing non-specific enzyme sticking to test tubes or cuvettes

during the reaction. Detergents cannot be replaced by bovine serum albumin (BSA). In fact, under the conditions tested in the presence of 0.01%, 0.1%, and 1% BSA, the MMP-26 activity was reduced to 61.3%, 13.4%, and 0%, respectively, compared to 100% control in the absence of BSA, perhaps due to non-specific albumin binding of the enzyme.

The inhibition of MMP-9, MT1-MMP, and MMP-26 by Brij-35 was further investigated to determine the mechanisms of inhibition by Dixon's plot. Inhibition of MMP-9 and MT1-MMP by Brij-35 displayed a mixed type of inhibition (data not shown), but MMP-26 seems to be inhibited by Brij-35 noncompetitively (Figure 6). The mixed inhibition observed in the MMPs indicates that Brij-35 in solution can affect both the substrate binding and the turnover number at the same time. Inactivation due to an irreversible denaturation can be ruled out because when all of the MMPs in high concentrations of Brij-35 were diluted in substrate-containing assay buffer without Brij-35, their activities were restored. The decline of the catalysis rate above the CMC indicates that the inhibition of Brij-35 is related to the increase in the micelle concentration, suggesting that the increase in rate and catalytic activity near or at the CMC could be due to enzyme-detergent interaction. Further increase in the concentration of a detergent above the CMC decreases both the catalytic activity and the rate. It is possible that micelles may trap enzyme or substrate molecules or blocking the active site of MMPs. Hypothetically, competitive inhibition may be due to the trapping of the substrate. A micelle formed on the surface of MMPs near the active site may block the access of the substrate, which could cause a decrease in the effective enzyme concentration needed for the catalytic activity. As a result, the turnover number decreases. In both cases, the increase in the number of micelle in assay solution enhances the inhibitory effect of the detergent on the catalytic activity of MMPs. Among the MMPs tested here, endometase/MMP-26 is the most sensitive MMP to Brij-35. This could be an indirect evidence that endometase may have more hydrophobic surface around the enzyme active site than the other MMPs tested.

To predict structural aspect of the hydrophobic surface area around the catalytic site, overlapped structures of homology modeled MMP-26 structure and MMP-7 X-ray crystal structure (pdb code 2DDY) have been created by overlapping the catalytic histidine and glutamic acid residues. Figure 7 represents only the hydrophobic surface areas between MMP-26 and MMP-7 around the catalytic site. Dark gray wire mesh structure represents MMP-26 and light gray solid structure represents MMP-7. MMP-26 shows more hydrophobic surface area around the catalytic glutamic acid residue (top) than that of the MMP-7. It was indicated by Marchenko *et al.* that homology modeling of MMP-26 with MMP-7 (PDB code 1mmr) and MMP-3 (PDB code 1slm) predicted a narrower and more hydrophobic active site groove in MMP-26 than that in MMP-7 [28]. Thus, it is possible that the active site of endometase may recruit detergent molecules to form micelles at the surface and have stronger interactions with it through the hydrophobic groove. The extent of inhibition by micelles through blocking the enzyme active site might be greater than the trapping of substrate molecules.

To further test the hypothesis that the increased interactions between enzyme and micelles are the major factor for the inhibition by nonionic detergents, the following experiments were carried out. MMP-26 was incubated with different concentrations (0–25 mM) of Brij-35, the concentration ranges were much broader than what were tested previously in this work. Then, the reaction mixtures were centrifuged to precipitate micelles and enzyme molecules sequestered by micelles. The enzyme molecules soluble in supernatant were used to hydrolyze the quenched fluorescence peptide substrate, and the hydrolytic activities were measured. As shown in Figure 8, the reduced enzyme activity is statistically significant at 0.1% (0.83 mM) and higher concentrations of Brij-35 than that at 0.01% (0.08 mM, near CMC of 0.09 mM) ($P < 0.01$, 4 sets of experiments). At extreme high concentration of 1% (8.34 mM) and 3% (25.01 mM) of Brij-35, all the enzymes were inhibited or denatured by the detergent and no enzyme

activity was detected. These data support our hypothesis that detergent-enzyme interaction is the major factor for inhibition of MMP activity above CMC of detergents.

Because our experimental data and homology modeling predictions suggest that detergent-substrate interaction or the entrapment of substrate by micelles at the detergent concentration above CMC is not a major factor for the inhibition of MMPs by nonionic detergents, the following experiments were performed. The quenched fluorescence peptide substrate, Mca-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH₂, was incubated with different concentrations (0–25 mM) of Brij-35. Then, the reaction mixtures were centrifuged to precipitate micelles and substrate molecules sequestered by micelles. The substrate molecules soluble in supernatant were used to perform enzyme hydrolysis assays upon addition of MMP-26, and the hydrolytic activities were measured. As shown in Figure 9, the reduced enzyme activities are not statistically significant at 0.05% (0.42 mM) and 0.1% (0.83 mM) of Brij-35 than that at 0.01% (0.08 mM, near CMC of 0.09 mM) (3 sets of experiments). At extreme high concentration of 1% (8.34 mM) and 3% (25.01 mM) Brij-35, the vast majority of substrate molecules were sequestered or denatured by the detergent and little or no hydrolytic activity was detected. These data support our hypothesis that detergent-substrate interaction is not a major factor for inhibition of MMP activity above CMC of detergents.

Finally, to rule out there is any change in the relative conversion factor (fluorescence intensity unit/nM substrate hydrolyzed) when detergent concentration is changed the following substrate total substrate hydrolysis experiments were performed. MMP-26 and the quenched fluorogenic peptide substrate were incubated with different concentrations (0–25 mM) of Brij-35 for two days and the cleaved substrate molecules were detected based on fluorescence intensity. As shown in Table 3, the conversion factors were approximately the same (~4.5 fluorescence intensity units/nM of substrate hydrolyzed) under the following Brij-35 concentrations, 0, 0.08 (near CMC 0.09), 0.42, and 0.83 mM, demonstrating that detergent concentration change did not induce conversion factor change at a broad range of the detergent concentrations, and further verifying that detergent-substrate interaction was not a significant factor for the inhibition of MMPs by detergent at concentrations above CMC. At extreme high concentration of 1% (8.34 mM) and 3% (25.01 mM) Brij-35, the vast majority of enzyme and substrate molecules were sequestered or denatured by the detergent and little hydrolytic activities were detected.

In this investigation, the catalytic properties of MMPs in aqueous surfactants solutions have been determined. From our data it is apparent that the concentration of detergent used in MMP assays needs to be carefully selected. The most commonly used concentration of 0.05 % (0.42 mM) Brij-35 for MMP assays were not the optimum concentration for endometase and other MMPs tested. We have also found that Brij-35 maximally stimulated catalysis by MMPs around CMC. However, Brij-35 inhibited MMP catalysis above the CMC. Based on the inhibition kinetics of Brij-35 with MMPs tested and the correlation between CMC of nonionic detergents and the optimum concentration of MMP catalyses, the increase in interactions between enzyme and micelles may be a major factor for the inhibition by nonionic detergents.

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Abbreviations

ANS	8-anilino-1-naphthalene-sulfonate
cd	catalytic domain

CMC	critical micelle concentration
Dap	2,3-diaminopropionyl
Dnp	2,4-dinitrophenyl
ECM	extracellular matrix
EDTA	ethylenediaminetetraacetic acid
HEPES	N-(2-hydroxyethyl) piperazine-N'-2-ethanesulfonic acid
HNG	human neutrophil gelatinase (gelatinase B, MMP-9)
Mca	(7-methoxycoumarin-4-yl) acetyl
MMP	matrix metalloproteinase
MT1-MMP	membrane type 1 matrix metalloproteinase (MMP-14)
SDS	sodium dodecyl sulfate
TTAB	tetradecyltrimethylammonium bromide

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Brij-35 [dodecyl-poly(ethylene oxide-23) ether], $n = 23$

Tween 20, Poly (oxyethylene 20 sorbitan) mono laurate
 $W + X + Y + Z = 20$
 $R_1 = \text{CH}_2(\text{CH}_2)_9\text{CH}_3$

Triton X-100, Octylphenol-poly(ethylene glycol ether)_n
 $n = 9.5$

SDS, Sodium dodecylsulfate

TTAB, Tetradecyltrimethylammonium bromide

Figure 1.
 Structures of the detergents tested.

Figure 2. Determination of the critical micelle concentration (CMC) of Triton X-100. The intensity of fluorescence was measured in the presence of 4 μM of ANS. The CMC of Triton X-100 was measured to be 220 μM .

Figure3.

Titration of active MMP-26 with GM6001, a potent and broad-spectrum MMP inhibitor. MMP-26 was incubated with various concentrations of GM6001 for 20 min prior to the substrate addition. Enzyme inhibition assays were performed at 25 °C in pH 7.5 substrate-containing assay buffer containing 50 mM HEPES, 0.2 M NaCl, and 10 mM $CaCl_2$ in the presence of 0.01 % Brij-35. An experimental inhibition curve fitted with the Morrison equation calculates concentration of active MMP-26 to be 13.2 nM.

Figure 4.

Noionic (A) and ionic (B) detergent concentration dependence of endometase catalysis of the peptide substrate hydrolysis. Mca-PLGLDpa-AR-NH₂ (1 μ M) hydrolysis catalyzed by endometase (1 nM) was measured in the presence of different detergent concentrations ranging from 0.001 to 9 mM. The maximum fluorescence detected for the set of experiments was assumed to be the relative 100% enzyme activity.

Figure 5.

Brij-35 concentration dependence of MMP-7, MMP-9, and MT-1 MMP. The catalytic activity of MMPs (0.5 nM) catalyzing the hydrolysis of Mca-PKPLAL(Dpa)AR-NH₂ (1 μ M) was measured in the presence of different Brij-35 concentrations ranging from 0.008 to 4 mM. The maximum fluorescence detected for the set of experiments was assumed to be the relative 100% enzyme activity.

Figure 6.

Dixon's plot for inhibition of endometase catalysis by Brij-35. The hydrolysis of the peptide substrate was measured at three different substrate concentration 0.5, 1.0, and 2.0 μM in the presence of six different detergent concentrations ranging from 0.17 mM to 2.7 mM. [I] is the concentration of the inhibitor (Brij-35). The results show a noncompetitive inhibition mechanism.

Figure 7.

Overlapped catalytic domain structures of homology modeled MMP-26 and X-ray crystal structure of MMP-7 (pdb code 2DDY). Overlapped structures have been created by overlapping the catalytic histidine and glutamic acid residues of MMP-26 and MMP-7. Small spheres illustrate zinc ions and large spheres illustrate calcium ions. Only catalytic zinc bound histidines and glutamic acid residues are shown. Dark gray color represents for MMP-26, light gray color represents for MMP-7. Hydrophobic surface areas of MMP-26 (dark gray wire mesh) and MMP-7 (light gray solid) around catalytic site are analyzed. MMP-26 shows more hydrophobic surface area (top) around catalytic glutamic acid residue than that of MMP-7.

Figure 8.

Test of detergent-enzyme interaction. MMP-26 was incubated with different concentrations (0, 0.08, 0.42, 0.83, 8.34, and 25.01 mM) of Brij-35. Then, the reaction mixtures were centrifuged to precipitate micelles and enzyme molecules sequestered by micelles. The enzyme molecules soluble in supernatant were used to hydrolyze the quenched fluorogenic substrate and the activities were measured. The experiments were repeated 4 times. The enzyme activities at no detergent are normalized to 1.0 arbitrary fluorescent unit per min (a.u./min). The enzyme activity is significantly lower at 0.1% (0.83 mM) and at higher concentrations of Brij-35 than that at 0.01% (0.08 mM, near CMC of 0.09 mM) ($P < 0.01$). At extreme high concentration of 1% (8.34 mM) and 3% (25.01 mM) of Brij-35, all the enzyme molecules were sequestered or denatured by the detergent and no enzyme activity was detected.

Figure 9.

Test of detergent-substrate interaction. Quenched fluorescence peptide substrate, Mca-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH₂, was incubated with different concentrations (0, 0.08, 0.42, 0.83, 8.34, and 25.01 mM) of Brij-35. Then, the reaction mixtures were centrifuged to precipitate micelles and substrate molecules sequestered by micelles. The substrate molecules soluble in supernatant were used for MMP-26 hydrolysis assay and the product formation was monitored. The experiments were repeated 3 times. The MMP-26 hydrolytic activities at no detergent are normalized to 1.0 arbitrary fluorescent unit per min (a.u./min). The substrate hydrolysis is not significantly lower at 0.05% (0.42 mM) and 0.1% (0.83 mM) of Brij-35 than that at 0.01% (0.08 mM, near CMC of 0.09 mM). At extreme high concentration of 1% (8.34 mM) and 3% (25.01 mM) of Brij-35, the substrate molecules were sequestered or denatured by the detergent and little or no substrate hydrolysis was detected.

Table 1Critical Micelle Concentration and Endometase Catalysis^a

Detergent	CMC (μM)	Optimum concentration (μM)	IC ₅₀ (μM)
Brij-35 (C ₁₂ E _{<23>})	90 (91) ^b	56	560
Tween-20 (C ₁₂ sorbitanE _{<20>})	54 (60) ^c	79	640
Triton X-100 (p-tertC ₈ ØE _{<9.5>})	220 (250) ^c	140	1300
SDS	(7000) ^d	9.0	110
TTAB	(3510) ^e	7.5	79

^aSDS = sodium dodecyl sulfate; TTAB = tetradecyltrimethylammonium bromide; CMC = critical micelle concentration; ND = not determined. Nomenclature: C_xE_y: x refers to the number of carbons in the alkyl chain and y to the average number of polyoxyethyleneglycol units; Ø denotes a phenyl group. All assays were performed in pH 7.5 and 50 mM HEPES buffer containing 0.2 M NaCl and 10 mM CaCl₂. The final substrate and active endometase concentrations in the assay buffer were 1 μM and 1 nM, respectively.

^b parenthetical value from reference (21)

^c parenthetical values from reference (22)

^d parenthetical values from reference (29)

^e parenthetical values from reference (30)

Table 2Optimum Concentrations for Maximum MMP Activity and IC₅₀ Values of Brij-35

Enzymes	Optimum Concentration (μ M)	IC ₅₀ (μ M)
MMP-26	56	560
MMP-7	100	4600
MMP-9	100	2200
MT-1 MMP	100	1200

Table 3

Conversion Factor under Different Detergent Concentrations

[Brij-35], mM	0	0.08	0.42	0.83	8.34	25.01
Conversion Factor (F.U./nM)	4.50	4.56	4.32	4.43	0.37	0.06

F.U./nM: Fluorescence intensity unit/nM of substrate hydrolyzed.