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Organic Reactions Promoted by Mucin Glycoproteins

Natalie Shraga, Bogdan Belgorodsky, and Michael Gozin*

School of Chemistry, Raymond and Beverly Sackler Faculty of Exact Sciences, Tel-Aviv University, Ramat Aviv, Tel-Aviv 69978, Israel

Received May 19, 2009; E-mail: cogozin@mgchem.tau.ac.il

Mucins are glycoproteins that constitute 80% of the organic components of mucus,[1] which coats many organs, including the respiratory, digestive, and reproductive tracts and, in some amphibia, the skin.[2] It is believed that the main function of these glycoproteins is to protect epithelial cells from infection and dehydration as well as from physical and chemical injuries.[1−3] Although mucins exhibit a broad range of adhesive interactions with various hydrophobic materials,[1a,2,4] including polycyclic aromatic hydrocarbons,[2c] there has been no clear evidence that mucins are capable of promoting chemical reactions. Here we demonstrate for the first time that under physiological conditions, two representative mucins, bovine submaxillary mucin type I (BSM) and porcine gastric mucin type III (PGM), accelerate the rate of fatty acid ester hydrolysis up to 337 times relative to the mucin-free reference reaction. Moreover, under the same reaction conditions, a Diels−Alder (DA) reaction between *N*-propylmaleimide and anthracene is promoted by these glycoproteins. The latter reaction does not occur in aqueous media without mucins, and the rate was accelerated up 200 times in the presence of a mucin relative to the rate of the reference process performed in chloroform. Mucins consist of branched oligosaccharide chains attached to a protein backbone.[1,4a,5] This unique structure was discovered to be critically important to the rate acceleration, as various cyclic and noncyclic oligosaccharides were far less efficient in promoting the same reactions.

The first reaction we investigated was the hydrolysis of carboxylic esters[6] (Figure 1a). A series of *p*-nitrophenol (*p*NP) esters of octanoic, decanoic, dodecanoic, and tetradecanoic acids were used as substrates. The reaction was evaluated under physiological conditions using a mucin concentration of 1 mg/mL. For all of the evaluated substrates, both the BSM and PGM glycoproteins dramatically increased the hydrolysis rate relative to that for the reaction in the absence of mucin. The rates fitted typical pseudo-first-order kinetic curves,[7] with longer alkyl chain esters reacting at lower rates (Figure 1b). The evaluated mucins were capable of accelerating the rate of fatty acid ester hydrolysis up to 337 times relative to the mucin-free reference reaction performed in sodium phosphate buffer (Figure 1b). We found that in the case of both BSM and PGM, complete conversion of *p*NP esters to the corresponding products was achieved. Interestingly, PGM exhibited a much greater difference in hydrolysis rates (k_{obs}) than BSM for *p*NP octanoate and *p*NP tetradecanoate (16-fold). Also, BSM demonstrated less selectivity: there was only a 4-fold difference between the highest and lowest rates for the evaluated esters. These rate variations probably result from differences in the spatial arrangements of these amorphous glycoproteins.

To assess the influence of the glycosidic moieties on the capability of mucins to promote hydrolytic reactions, we compared the rate of ester hydrolysis in the presence of BSM and PGM glycoproteins with those observed in the presence of various oligosaccharides. A series of oligosaccharides, including lactose,

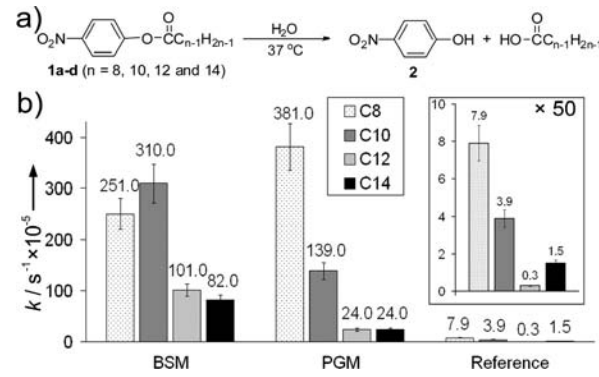

Figure 1. (a) Hydrolysis reaction of *p*NP esters **1a**−**d**. (b) Kinetic data for hydrolysis of **1a**−**d** by BSM and PGM vs the mucin-free reference reaction.

γ-cyclodextrin,[8] amylose,[9] and arabic acid, were evaluated as promoters of *p*NP octanoate and *p*NP dodecanoate ester hydrolysis.

We found that the smaller lactose and γ-cyclodextrin sugars had practically no effect on the rate of hydrolysis of either *p*NP ester. In contrast, a clearly detectable acceleration in the reaction rate was observed when the larger amylose and arabic acid oligosaccharides were present in the solution. More specifically, amylose promoted hydrolysis of *p*NP octanoate and dodecanoate with 5- and 24-fold rate increases, respectively, relative to the reference oligosaccharide-free reactions, whereas arabic acid exhibited 9- and 21-fold accelerations, respectively. However, the rate accelerations by amylose and arabic acid oligosaccharides were significantly less than those observed with BSM and PGM glycoproteins (Figure S1 in the Supporting Information). Our findings strongly suggest that the spatial arrangement of glycosidic moieties resulting from their attachment to mucin protein backbone[1,4a,5] plays an important role in the hydrolytic activity of these glycoproteins. To support this hypothesis, a deglycosilated BSM (dBSM) was prepared and evaluated as a hydrolysis promoter. For the *p*NP octanoate and dodecanoate ester hydrolyses, the dBSM gave 26-fold and 13-fold slower reactions, respectively, than the corresponding parent BSM (Figure S1). For comparison, a control mixture of dBSM and arabic acid (mimicking the parent BSM) was capable of promoting the hydrolysis of these esters at rates very similar to that for arabic acid only.

The second chemical transformation that we studied was a DA cycloaddition. To demonstrate the remarkable and unprecedented properties of mucins to promote the DA reaction in phosphate buffer solution, highly hydrophobic unsubstituted anthracene and *N*-propylmaleimide were selected as the diene and dienophile, respectively (Figure 2a).[10] Both the BSM and PGM glycoproteins not only were capable of solubilizing these substrates[2c] (*anthracene was added to the reaction mixture in its solid form and not as a nanosuspension*) but also markedly promoted the DA process

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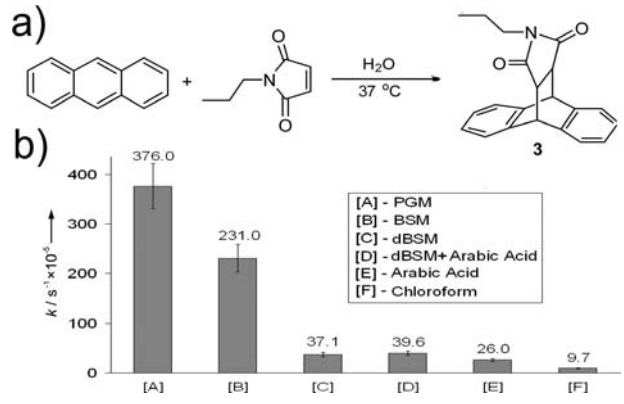

Figure 2. (a) DA reaction for the preparation of compound **3** catalyzed by mucins. (b) Kinetic data for DA reactions catalyzed by BSM, PGM, and arabic acid vs the reference reaction performed in chloroform.

between them; in contrast, *without* the presence of mucins, no product **3** could be detected, eliminating possibility of an "on-water"-type[10d,h] chemical process involving these reactants. For kinetic studies, solutions of either BSM or PGM were preincubated with solid anthracene for 48 h. Next, mucin-bound anthracene complexes were separated from nonbound solid anthracene by filtration and reacted with excess *N*-propylmaleimide. After workup, the amount of extracted product **3** was determined by HPLC using separately synthesized **3** as a reference standard. We found that in case of BSM, a typical conversion of anthracene to **3** was 65%, whereas for PGM, 47% conversion was observed. Under the tested conditions, the reaction was accelerated 39- and 24-fold by BSM and PGM, respectively, relative to the rate of the same reaction in chloroform (Figure 2b).

To date, several investigators have reported a rate enhancement for certain DA reactions performed in aqueous solutions.[10] This phenomenon has generally been explained by the hydrophobic "on-water" effect. However, in those cases, starting materials with better water solubility were used, such as anthracene-9-carbinol and *N*-ethylmaleimide.[10e] It should be stressed that numerous thermal DA reactions between unsubstituted anthracene and various dienophiles are known.[10f] All of these thermal reactions require organic solvents and high temperatures. In contrast, *our mucin-promoted DA process was successfully performed in phosphate buffer at 37 °C*.

The importance of the mucin glycosidic moieties was evaluated by analysis of the same cycloaddition reaction in the presence of lactose, γ-cyclodextrin, amylose, or arabic acid oligosaccharides. Only arabic acid accelerated the rate of the DA reaction (Figure 2b). However, in the presence of the latter oligosaccharide, the measured acceleration in the rate was an order of magnitude less than in the presence of either the BSM or PGM glycoprotein. We hypothesize that the lack of activity of lactose, γ-cyclodextrin, and amylose is due to the inability of these oligosaccharides to solubilize anthracene. When the aforementioned dBSM protein was tested as a promoter of the DA reaction, we found that for the evaluated substrates the DA reaction was 6-fold slower than in the presence of the parent BSM, while the dBSM−arabic acid mixture performed at the level of arabic acid only (Figure 2b).

The discovered property of mucins to accelerate organic chemical reactions provides a new and unique example of natural nonenzymatic proteins capable of promoting reactions of hydrophobic materials in aqueous solution. As heavily glycosylated mucins showed unique properties in comparison with various oligosaccharides, dBSM, and their mixtures, we propose that mucins perform the task of dissolving the hydrophobic compound (with subsequent promotion of organic reactions) by folding of their amorphous oligomeric structure in order to create local hydrophobic environments in which such reactions can take place. Furthermore, our results may lead to a better understanding of how various highly reactive therapeutic agents undergo metabolic processes in a mucus layer. Studies of mucin chemical reactivity are also relevant to development of biocompatible materials for implantable devices, such as birth control devices, orthodontic devices, digestive-tract-implantable devices, and contact lenses, all of which have surfaces that come into direct, long-term interaction with mucus.[1a,4c,5,11,12]

In this report, we have demonstrated mucin-based acceleration of carboxylic ester hydrolysis and Diels−Alder carbon−carbon bond-forming reactions. Further studies will determine whether these glycoproteins accelerate other organic transformations in aqueous solution, such as hetero-DA reactions and dipolar cycloadditions.

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Supporting Information Available: Detailed procedures for kinetic analysis of hydrolysis and Diels−Alder reactions. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) For general references, see: (a) Bansil, R.; Turner, B. S. *Curr. Opin. Colloid Interface Sci.* **2006**, *11*, 164. (b) Perez-Vilar, J.; Mabolo, R. *Histol. Histopathol.* **2007**, *22*, 455. (c) Strous, G. J.; Dekker, J. *Crit. Rev. Biochem. Mol. Biol.* **1992**, *27*, 57. (d) Lichtenberger, L. M. *Annu. Rev. Physiol.* **1995**, *57*, 565.
(2) (a) Perez-Vilar, J.; Robert, L. H. *J. Biol. Chem.* **1999**, *274*, 31751. (b) Roussel, P.; Delmotte, P. *Curr. Org. Chem.* **2004**, *8*, 413. (c) Belgorodsky, B.; Drug, E.; Fadeev, L.; Gozin, M. Submitted.
(3) Rose, M. C. *Am. J. Physiol.* **1992**, *263*, 413.
(4) For additional references, see: (a) Harding, S. E. *Biochem. Soc. Trans.* **2003**, *31*, 1036. (b) Holzer, P. *Curr. Opin. Gastroenterol.* **2000**, *16*, 469. (c) *Nanotechnology in Therapeutics*; Huang, Y., Peppas, N. A., Eds.; Horizon Bioscience: Wymondham, U.K., 2007; p 109.
(5) (a) Fendler, E. J.; Fendler, J. H. *Adv. Phys. Org. Chem.* **1970**, *8*, 271. (b) Ma, Z.; Taylor, J. S. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 11159. (c) *Hydrolysis in Drug and Prodrug Metabolism*; Testa, B. J., Mayer, M., Eds.; Wiley-VCH: Weinheim, Germany, 2003.
(6) For reports on *p*NP ester hydrolysis, see: (a) Rodulfo, T.; Cordes, E. H. *J. Org. Chem.* **1974**, *39*, 2281. (b) Broo, K. S.; Brive, L.; Ahlberg, P.; Baltzer, L. *J. Am. Chem. Soc.* **1997**, *119*, 11362. (c) Sakurai, Y.; Ma, S.; Watanabe, H.; Yamaotsu, N.; Hirono, S.; Kurono, Y.; Kragh-Hansen, U.; Otagiri, M. *Pharm. Res.* **2004**, *21*, 285. (d) Varma, M. N.; Madras, G. *Appl. Biochem. Biotechnol.* **2008**, *144*, 213. (e) Kokubo, T.; Uchida, T.; Tanimoto, S.; Okano, M.; Sugimoto, T. *Tetrahedron Lett.* **1982**, *23*, 1593. (f) Hollfelder, F.; Kirby, A. J.; Tawfik, D. S. *Nature* **1996**, *383*, 60.
(7) For references reporting pseudo-first-order kinetic curves for hydrolysis of *p*NP esters, see: (a) Lawin, L. R.; Fife, W. K.; Tian, C. X. *Langmuir* **2000**, *16*, 3583. (b) Baltzer, L.; Broo, K. S.; Nilsson, H.; Nilsson, J. *Bioorg. Med. Chem.* **1999**, *7*, 83. (c) Zuev, Y. F.; Mirgorodskaya, A. B.; Kudryavtseva, L. A.; Idiyatullin, B. Z.; Khamidullin, R. N. *Russ. J. Gen. Chem.* **2004**, *74*, 1051.
(8) For a general review of the reactions catalyzed by cyclodextrins in water, see: Breslow, R.; Dong, S. D. *Chem. Rev.* **1998**, *98*, 1997, and references within.
(9) For an example of *p*NP ester hydrolysis bound by amylose, see: Hui, Y.; Wang, S.; Jiang, K. *J. Am. Chem. Soc.* **1982**, *104*, 347.
(10) For examples of similar Diels−Alder reactions in water, see: (a) Seelig, B.; Jaschke, A. *Chem. Biol.* **1999**, *66*, 167. (b) Seelig, B.; Keiper, S.; Stuhlmann, F.; Jaschke, A. *Angew. Chem., Int. Ed.* **2000**, *39*, 4576. (c) Yoshizawa, M.; Tamura, M.; Fujita, M. *Science* **2006**, *312*, 251. (d) Narayan, S.; Muldoon, J.; Finn, M. G.; Fokin, V. V.; Kolb, H. C.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2005**, *44*, 3275. (e) Breslow, R.; Rideout, D. C. *J. Am. Chem. Soc.* **1980**, *102*, 7816. (f) Atherton, J. C. C.; Jones, S. *Tetrahedron* **2003**, *59*, 9039. (g) Kumar, A.; Pawar, S. S. *Tetrahedron* **2002**, *58*, 1745. (h) Chanda, A.; Fokin, V. V. *Chem. Rev.* **2009**, *109*, 725. (i) Li, C. *Chem. Rev.* **2005**, *105*, 3095.
(11) (a) Sandberg, T.; Carlsson, J.; Ott, M. K. *Microsc. Res. Tech.* **2007**, *70*, 864. (b) Shi, L. *Trends Glycosci. Glycotechnol.* **2000**, *12*, 229.
(12) Harding, S. E. *Trends Food Sci. Technol.* **2006**, *17*, 255.

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