5. Doi, N., Kumadaki, S., Oishi, Y., Matsumura, N., and Yanagawa, H. (2004). Nucleic Acids Res. *32*, e95.
6. Nakano, M., Nakai, N., Kurita, H., Komatsu, J., Takashima, K., Katsura, S., and Mizuno, A. (2005). J. Biosci. Bioeng. *99*, 293–295.
7. Nakano, M., Komatsu, J., Matuura, S., Takashima, K., Katsura, A., and Mizuno, A. (2003). J. Biotechnol. *102*, 117–124.
8. Kojima, T., Takei, Y., Ohtsuka, M., Kawarasaki, Y., Yamane, T., and Nakano, H. (2005). Nucleic Acids Res. *33*, e150.
9. Samatov, T.R., Chetverina, H.V., and Chetverin, A.B. (2005). Nucleic Acids Res. *33*, e145.
10. Sepp, A., Tawfik, D.S., and Griffiths, A.D. (2002). FEBS Lett. *18*, 455–458.
11. Dittrich, P.S., Jahnz, M., and Schwille, P. (2005). ChemBioChem *6*, 811–814.
12. Shendure, J., Porreca, G.J., Reppas, N.B., Lin, X., McCutcheon, J.P., Rosenbaum, A.M., Wang, M.D., Zhang, K., Mitra, R.D., and Church, G.M. (2005). Science *309*, 1728–1732.
13. Margulies, M., Egholm, M., Altman, W.E., Attiya, S., Bader, J.S., Bemben, L.A., Berka, J., Braverman, M.S., Chen, Y.J., Chen, Z., et al. (2005). Nature *437*, 376–380.
14. Boder, E.T., Midelfort, K.S., and Wittrup, K.D. (2000). Proc. Natl. Acad. Sci. USA *97*, 10679–10681.
15. Varadarajan, N., Gam, J., Olsen, M.J., Georgiou, G., and Iverson, B.L. (2005). Proc. Natl. Acad. Sci. USA *102*, 6855–6860.
16. Griffiths, A.D., and Tawfik, D.S. (2003). EMBO J. *22*, 24–35.

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Acyclic Peptide Inhibitors of Amylases

In this issue of *Chemistry & Biology*, a library screening approach reveals a linear octapeptide inhibitor of α-amylases reached by de novo design [1]. The selected molecule shares characteristics with naturally occurring protein inhibitors—a result that suggests general rules for the design of peptide-based amylase inhibitors may be achievable.

Amylase proteins, part of the broader class of hydrolytic enzymes called glycosidases, cleave the glycosidic linkages of starch into disaccharide fragments that are subsequently broken down into glucose (Figure 1). Inhibitors of amylases have already demonstrated their utility in aiding diabetics [2]. The glucose levels of diabetics can be controlled after meals by administration of an amylase inhibitor such as acarbose. Acarbose is a natural product obtained by fermentation and is structurally related to the amylase oligosaccharide substrate, as up to five glucose residues are known to be accommodated in the amylase active site [3].

Interestingly, some plants and microorganisms produce amylase inhibitors that are based on protein rather than carbohydrate motifs. These inhibitory proteins, which range in size from 32 amino acids with 3 disulfide bonds to over 19 kDa, serve to regulate endogenous amylase activity, for example in plant seeds, as well as to defend against digestive amylases from other organisms such as insects [4].

Although X-ray structures for five of the seven proteinaceous inhibitor family members are known [5–9], the complex nature of the interaction has made rational design of smaller versions of these inhibitors challenging. Phage display methods have been used to produce altered proteins to serve as amylase inhibitors [10, 11]. However, in the last decade, the use of peptides rather than whole proteins to mimic carbohydrates has emerged as a strategy for vaccine design as well as for the design of glycosidase inhibitors [12]. Although metabolic stability is an issue, smaller peptides usually are easier to synthesize than carbohydrates and less likely to be immunogenic than large proteins. Smaller peptides are also more amenable to computational modeling to correlate properties such as charge distribution with activity [13]. The 74 amino acid protein Tendamistat has only 15 amino acids that actually interact with amylase and therefore has served as a good model for the rational design of a variety of linear and cyclic peptide inhibitors [13–16]. However, the discovery of unrelated peptides has been a challenge. Unrelated peptides have the potential for improved properties such as solubility, stability, and selectivity.

In this issue of *Chemistry & Biology*, the Mares group reports the generation of a random combinatorial peptide library for the discovery of an octapeptide inhibitor of the reaction catalyzed by porcine pancreatic α-amylase [1]. Structure/function relationship studies of the resulting octapeptide found that addition of a tosylate group by chemical means to one arginine residue generated an even tighter binding inhibitor (Figure 1). The new peptide inhibits the porcine α-amylase more strongly than the clinical drug acarbose. Interestingly, the modified octapeptide appears to use some of the same binding motifs as the natural protein-based inhibitors, namely aromatic and arginine moieties, but arranges these motifs in a simple linear scaffold. In fact, the flexibility of the linear scaffold is crucial for effective binding, as the cyclic version of the same peptide is completely inactive [1].

Selectivity among varies glycosidase families [17] is the next issue that has to be addressed in the discovery of inhibitors. Although other α-amylases and α-glucosidases are also inhibited by the octapeptide, the new compound does appear to be selective for glycosidases found in family 13. No evidence for inhibition of enzymes from seven other families was seen. Therefore, the octapeptide likely will inhibit amylases without fear of also shutting down other structurally unrelated glycosidases with important cellular functions.

The work by Mares and coworkers is an important first step in the de novo design of amylase inhibitors, but

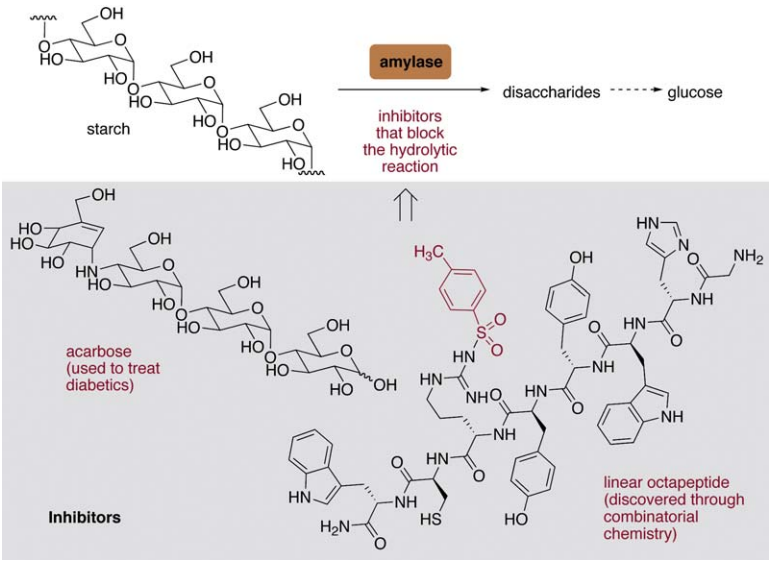

Figure 1. Carbohydrate- and Peptide-Based Inhibitors of the Reaction Catalyzed by Amylase to Break Down Starch

many questions remain. Screening against a wider panel of glycosidases as they become available will clarify the extent of the family-selectivity of this new octapeptide. Structural data characterizing binding of the octapeptide to α-amylase is also needed to visualize the features important for binding and selectivity to serve as a platform for rational design.

In addition, the application of this selection approach to other glycosidases and carbohydrate binding proteins with and without natural proteinaceous inhibitors is important to establish if new binding determinants can be derived using a combinatorial approach. Because the same structural determinants for amylase inhibition emerged from a de novo design approach as are found for larger proteinaceous inhibitors, general rules for the design of peptides that mimic natural carbohydrates may actually be a possibility.

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Selected Reading

1. Dolečková-Marešová, L., Pavlík, M., Horn, M., and Mareš, M. (2005). Chem. Biol. *12*, this issue, 1349–1357.
2. Franco, O.L., Rigden, D.J., Melo, F.R., and Grossi-De-Sá, M.F. (2002). Eur. J. Biochem. *269*, 397–412.
3. Machius, M., Vértesy, L., Huber, R., and Wieland, G. (1996). J. Mol. Biol. *260*, 409–421.
4. Breuer, H.W. (2003). Int. J. Clin. Pharmacol. Ther. *41*, 421–440.
5. Bompard-Gilles, C., Rousseau, P., Rougé, P., and Payan, F. (1996). Structure *4*, 1441–1452.
6. Wiegand, G., Epp, O., and Huber, R. (1995). J. Mol. Biol. *247*, 99–110.
7. Pereira, P.J., Lozanov, V., Patthy, A., Huber, R., Bode, W., Pongor, S., and Strobl, S. (1999). Structure *7*, 1079–1088.
8. Vallée, F., Kadziola, A., Bourne, Y., Juy, M., Rodenburg, K.W., Svensson, B., and Haser, R. (1998). Structure *6*, 649–659.
9. Strobl, S., Maskos, K., Wiegand, G., Huber, R., Gomis-Rüth, F.X., and Glockshuber, R. (1998). Structure *6*, 911–921.
10. Lehtiö, J., Teeri, T.T., and Nygren, P.Å. (2000). Proteins *41*, 316–322.
11. Desmyter, A., Spinelli, S., Payan, F., Lauwereys, M., Wyns, L., Muyldermans, S., and Cambillau, C. (2002). J. Biol. Chem. *277*, 23645–23650.
12. Beauvais, A., and Latgé, J.-P. (2005). Chem. Biol. *12*, 7–8.
13. Heyl, D.L., Fernandes, S., Khullar, L., Stephens, J., Blaney, E., Opang-Owusu, H., Stahelin, B., Pasko, T., Jacobs, J., Bailey, D., et al. (2005). Bioorg. Med. Chem. *13*, 4262–4268.
14. Etzkorn, F., Guo, T., Lipton, M., Goldberg, S., and Bartlett, P. (1994). J. Am. Chem. Soc. *116*, 10412–10425.
15. Matter, H., and Kessler, H. (1995). J. Am. Chem. Soc. *117*, 3347–3359.
16. Ono, S., Umezaki, M., Tojo, N., Hashimoto, S., Hiroko, T., Kaneko, T., Morita, H., Fujii, T., Yamazaki, I., Shimasaki, C., et al. (2001). J. Biochem. (Tokyo) *129*, 783–790.
17. Davies, G., and Henrissat, B. (1995). Structure *3*, 853–859.