Propositions of PhD theses

Summary of PhD thesis

INVESTIGATION OF THE FUNCTION OF EXO GLYCOSIDE HYDROLASE ENZYMES

STUDY OF SWEET POTATO β-AMYLASE AND DISPERSINB ENZYMES

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I. INTRODUCTION AND THE AIMS OF THE WORK

Investigation of the carbohydrate degrading enzymes is a border area between chemical and biological researches, because of the chemical techniques applied to answer the biological questions. Glycoside hydrolases (EC 3.2.1.-) are a widespread group of enzymes which hydrolyse the glycosidic bond between two carbohydrates or between a carbohydrate and a non-carbohydrate moiety. Based on earlier work dealing with α-amylases the aim of my work was to investigate some *exo* acting glycoside hydrolases. An inverting and a retaining enzyme were chosen for examination of the mechanism and the active site structure.

Sweet potato β-amylase (βAMY) was used as inverting model enzyme. The prognosis, that more than 2 billion people feeding will depend from tuberous and rooty plants for 2020, shows the importance of sweet potato. *Ipomoea batatas* is one of the main source of β-amylase, accounting for approximately 5% of the total soluble proteins. Another tuberous plants contain only traces of βAMY. βAMY (EC 3.2.1.2.) catalyses the hydrolysis of the second glycosidic bond from the nonreducing end of the starch, inverting the anomer configuration and liberating β-maltose. Its three-dimensional structure was solved (PDB identification code is 1FA2) and found to be very similar to other plant origin GH14 family glycoside hydrolases (CAZy database). 2-chloro-4-nitrophenol maltooligomers (CNPG3-15) were used as substrates for our researches. Substrates were detected at 302 nm, the absorption maximum of the chromophore group.

DispersinB (DspB) (EC 3.2.1.52 or 3.2.1.-) is an N-acetyl-glucosaminidase, with unique specificity toward β(1,6) glycosidic linkages from the nonreducing end of the substrate. DspB, produced by the Gram-negative periodontopathogen *Aggregatibacter actinomycetemcomitans,* is able to hydrolyse the poly-N-acetyl-glucoseamine based biofilms. Biomaterial-associated infections are caused mostly by biofilm producing microorganisms and are affecting up to millions of patients worldwide, who use implanted biomedical devices. The coordinates for the structure of DspB have been deposited in the Protein Data Bank (PDB ID: 1YHT), and the enzyme has been classified as a member of the Glycoside Hydrolase Family 20 (CAZy: Carbohydrate Active Enzymes database http://www.cazy.org). DspB has a $(\beta/\alpha)_8$ barrel fold, the active site glutamic acid and the substrate N-acetyl group take part in the retaining cleavage mechanism. We used chromophore aglycone containing oligomer substrates, β(1,6)-linked N-acetylglucosamine thiophenyl glycosides (Ph-S-NAG_n) with degree of polymerisation (DP) of 2, 3, 4 and 5, to investigate the cleavage mode of DspB in detail.

In combination with high performance liquid chromatography (HPLC) analysis, we used MALDI-TOF MS in order to identify reducing and nonreducing end products in both enzyme reactions. We wanted to know how the oligomer substrate binding and cleaving occurred at the active sites of the model enzymes. Kinetic measurements and action pattern studies was carried out with additional ^{1}H-NMR measurements. The substrate series which are used in the measurements are not commercially available, thus their synthesis had to be resolved.

II.EXPERIMENTAL METHODES

HP1090 liquid chromatograph equipped with diode array detector, autosampler and ChemStation was used in analytical HPLC measurements. Zorbax Eclipse XDC C18 (15 cm x 4,6 mm, 5 μm), Kinetex XB C18 (10 cm x 4,6 mm, 2,6 μm), YMC Polyamine II (25 cm x 4,6 mm, 5 μm) were used to separate the substrate and hydrolysis products, which were detected at the absorption maximum of the CNP group (302 nm) and thiophenyl group (254 nm). Younglin semi-preparative liquid chromatograph equipped with degasser, manual sampler and UV-Vis and CoronaCad detector were used for purification on Supelcosil^{TM} LC-18 (25 cm x 10 mm, 5 μm) and YMC Polyamine II (25 cm x 10 mm, 5 μm) columns.

Data evaluation was made using method of initial rates and integrated rate equations. Initial rate constants were calculated at low substrate concentration, until 10% conversion rate. The product concentrations were calculated from the product peak area ratio of HPLC chromatogram and the initial substrate concentration. The amount of product formed at different time intervals was plotted as a function of time and linear curves were fitted with Grafit 2.1. programe (Erithacus Software LTD, Harley, UK). Concentration-time data pairs which were calculated from the total hydrolysis reactions were evaluated with Scientist® program (Micromath St. Luis, USA). Scientist® software offers an opportunity to fit curves to experimental points using model equations. The mathematical model in this case is a complex system of differential equations obtained from the assumed reaction scheme. The software allows the numerical solution of differential equations. Kinetic constants (k) were determined. MALDI-TOF MS analysis of the compounds was performed in positive-ion mode using a Bruker Biflex MALDI-TOF mass spectrometer equipped with delayed-ion extraction. Desorption/ionisation of the sample molecules was effected with a 337 nm nitrogen laser. Samples with 2,4,6-trihydroxyacetophenon (THAP) matrix were spotted to the sample holder and allowed to dry. Spectra from multiple laser shots (at least 100) were summarized using 19 kV accelerating and 20 kV reflectron voltage. [M+Na]+ peaks of cyclodextrins with DP 6-8 (m/z 995.31, 1157.36 1319.41 Da, respectively) were used for external calibration. ^{1}H-NMR spectroscopy was used to follow enzyme reactions and observe transition state products. Products identification were made by Dr. Gyula Batta and his coworkers with Bruker Avance II on 500 MHz.

III. NEW SCIENTIFIC ACHIEVEMENTS

1. Results of the investigation of sweet potato βAMY

A) Model for total hydrolysis reactions

Prolongated hydrolysis reactions of sweet potato β-amylase on chromophor containing oligomer substrates were followed by HPLC method. The chromophor containing reducing end products from βAMY catalysed hydrolysis were quantitatively determined. Concentration-time data points were evaluated with the Scientist® program. If n-1 sequential reactions occur, then n differential equations can be written for the temporal variations of the concentrations of the n-1 substrates and the final product. Paralel and consecutive reaction steps were assumed during the model development. The best fitting model for the CNPG11 substrate hydrolysis is presented in Figure 1.

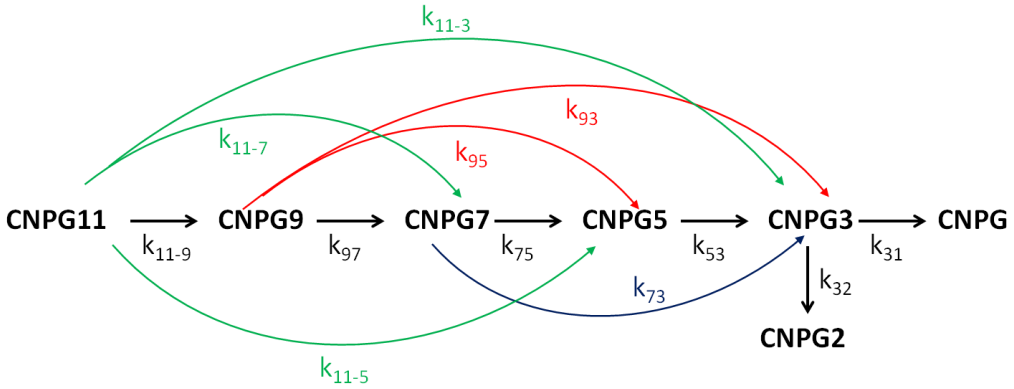


Fig. 1: Reaction model of sweet potato βAMY catalysed total hydrolysis of CNPG11, where paralel and consecutive reaction steps were presented during the hydrolysis (kxy- rate constant of one reaction step, where x the number of the initial substrate, y the number of the formed product)

Similar assumptions were used for developing the model of other substrates. Index of the „k" kinetic constant of each reaction step consist of the DP of the initial substrate and the formed product. Transglycosylation and processive cleavage were observed on all substrates except the shortest CNPG5. Curve fittings were successful when the initial quick hydrolysis and transglycosidic periodes were neglected and model was fitted only the third part of the reaction curves.

Our substrates contain aromatic rings as chromophore aglycon and beta glycosidic bonds, which are caused favourable binding to the active centre. In contrast with earlier results, we found glucose cleavage from maltotriose resulted in CNPG2 product in case of odd numbered substrates (DP 5, 7 and 11). The reaction rate of trimer was much lower than rate of tetramer or pentamer hydrolysis indicating that +2 and +3 aglycon binding subsites are important in substrate fixation. However, the higher amount of dimer reducing end product in case of

trimer substrate suggests favourable binding of a glucose unit at subsite +2 aglycon than at subsite -2 glycon binding site.

B) Substrate specificity and *exo* character of sweet potato βAMY

Catalytic efficiences were determined using low substrate concentration with HPLC methode. From the initial rates the catalytic efficiency k_{cat}/K_M values were calculated on the basis of the next basic equation (1):

$$v_o \approx \left(\frac{v_{max}}{K_M} \right) [S] \qquad \text{if } [S] << K_M \qquad (1)$$

where v_{max} reveals the turnover number (k_{cat}) of an enzyme if [Et] is known because $v_{max} = k_{cat}[Et]$. The k_{cat}/K_M term is a measure of the enzyme's catalytic efficiency.

Catalytic efficiency increased with increasing DP of the substrates, indicating favorable binding and higher reaction rate of longer substrates (Figure 2).

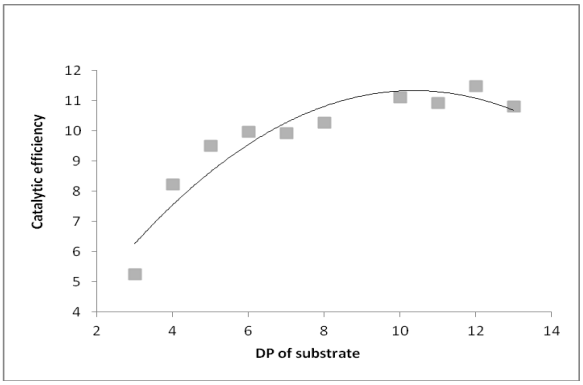


Fig. 2: Calculated catalytic efficiencies determined on sweet potato βAMY catalysed hydrolysis reactions of CNP maltooligomers as a function of DP of the substrates

Exclusiveness of maltose cleavage from the nonreducing end was checked by MALDI-TOF MS analysis of reaction mixture containing CNPG7 substrate (Figure 3). After one day reaction time, in addition to the remaining substrate, two reducing end products, namely pentamer and trimer appeared, while dimer maltose was present as the corresponding non-reducing end product. It refers to processivity, when the substrate does not dissociate from the surface of the enzyme but slids onward and a newer bond cleavage occurs. The rate was not determined exclusively by the binding but the number of processive steps also plays a role, the rate was smaller since the substrate occupy the enzyme active site longer time. Thus calculation the subsite map was not possible.

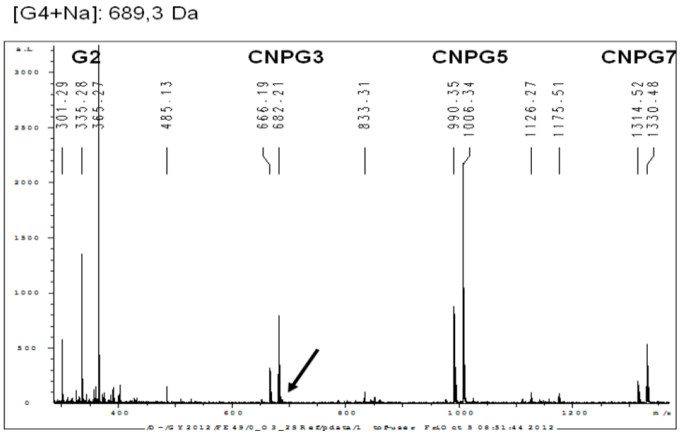


Fig. 3: MALDI-TOF MS spectra of sweet potato βAMY catalysed hydrolysis reactions of CNPG7 after 1 day reaction time. Calculated m/z values: CNPG7= 1330,348; CNPG5= 1006,242; CNPG3= 682,132; G2= 365,106, G4= 689,3, which not appear on the spectra.

C) Transglycosylation during hydrolysis reactions

Our observations show that spontaneous maltose transfer happened in the early stage of the hydrolysis of each substrate, where the processive cleavage is possible. During CNPG11 hydrolysis the amount of transglycosylated products were remarkable (Figure 4). We compare the concentration of compounds in the reaction mixture at 65 minute and at 155 minute, values of CNPG11 was twofold: 0,24 mM and 0,54 mM, respectively. Changing the concentration of CNPG9 and CNPG7 shows the similar twofold increasing. CNPG9 and CNPG7 might be hydrolysis products, but increase of CNPG11 concentration was only due to the transglycosylation. Similar tendency was observed in CNPG8 as well as CNPG7 and CNPG6 hydrolysis reaction, although the ratio was smaller. Transglycosylation reactions of α-amylase and other retaining glycosidases are well known and studied in details. This activity are not characteristics for hydrolysis reactions of inverting glycosidases. This was the first time that during an inverting glycosidase catalysed hydrolysis of maltooligomers transglycosylation was observed. This confirm the expanded glycosyl transfer concept, shows that glycosylation and deglycosylation are similar processes and the transition state structure may be identical of some retaining and inverting enzymes. The processive reaction assumes longer staying of the reducing end product in the active center, and in the presence of the accumulating „nascent" β-maltose the microscopic reversibility manifested in the longer transfer product.

Using maltose donor and chromophore containing acceptor we did not observed transglycosylation. The reason has not been clarified yet. Possible explanation, that β-maltose formed during the hydrolysis does not dissociate from the surface of the enzyme, thus the

reverse reaction is happen immediately, which is not the case if α/β anomer mixture is present in the reaction mixture.

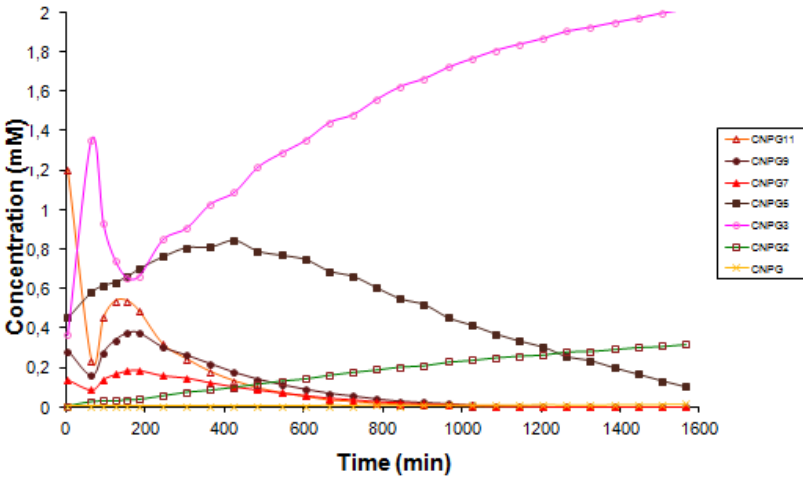


Fig. 4: Sweet potato βAMY (3,2 x 10^{-8} M) catalysed CNPG11 (2,45 mM) total hydrolysis followed by HPLC

D) Confirmation of processive cleavage mode

Processivity or multiple attack is a characteristic property of several enzymes acting on polymer substrates which often hydrolyse non-soluble substrates for example chitin or cellulose. For the calculation of probability number n, which give the average number of processive steps in an ES complex, we used the product pattern determined by HPLC and equation (2).

$$n = \frac{P}{1-P}\left(1-P^{N}\right) \qquad (2)$$

where P is the probability of the secound cleavage during the hydrolysis without the dissociation of the enzyme-product complex, 1-P is probability of the dissociation of the product and N is the maximum number of sliding.

The mean number of steps increase with DP of substrate, the relationship is *quasi*-linear and the x-axis intercept showed the size of substrate without processivity. The highest value was 3.34 measured on substrate of DP 11, which is in a good agreement with values 3.3 found earlier for amylose with average DP 44 (Figure 5).

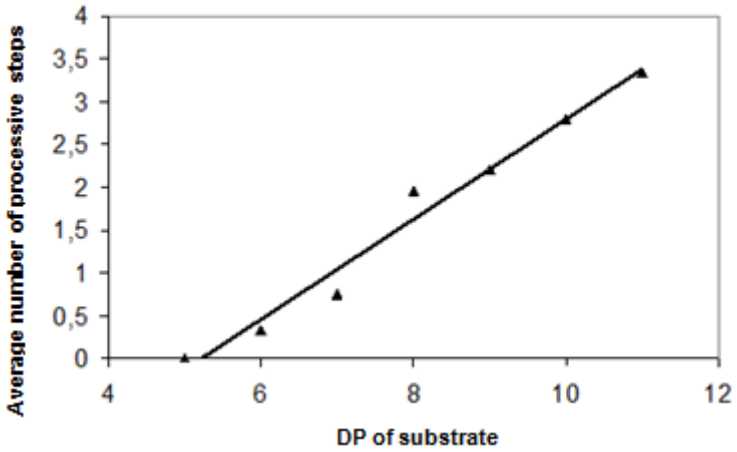


Fig. 5: Number of processive steps of sweet potato βAMY catalysed hydrolysis on CNP maltooligomers (DP5-11)

2. Results of the investigation of DispersinB enzyme

A) Effect of aromatic amino acid mutations at the active centre

First the effect of the substrate chain lenght to the reaction rate of hydrolysis was examined. We concluded that the hydrolysis rate increased with the increasing chain lenght. Reaction rate increased with degree of polymerisation until tetramer, but the fifth GlcNAc unit in case of pentamer did not result in further increase of velocity. These results indicate that effective binding requires at least four GlcNAc residues to fill the subsites.

Hydrolytic activity of Tyr187Ala, Tyr278Ala and Trp237Ala mutant enzymes were also studied on thiphenyl glcosides as substrates and compared to hydrolytic activity of wild type enzyme (Figure 6). We have found that wild type enzyme was much more active on pentamer substrate than Tyr187Ala mutant, Tyr278Ala mutant showed low activity, while Trp237Ala mutant was completely inactive. Our results confirm the conclusion of Manuel and coworkers, who suggested that Trp237 is an important participant of hydrophobic substrate binding pocket of DspB.

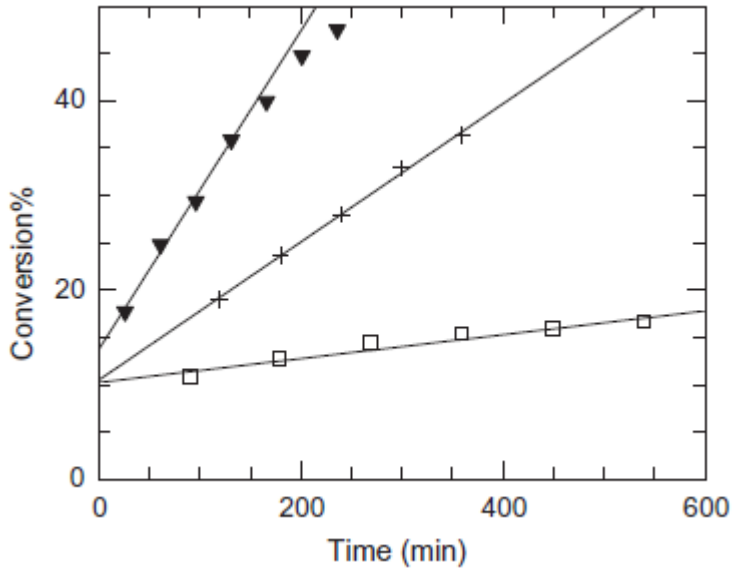


Fig. 6: Conversation of pentamer substrate hydrolysis catalysed by ▼DspB, + Tyr187Ala and □ Tyr278Ala mutants.

B) Confirmation of the stereochemistry of the enzyme reaction by ^{1}H-NMR

^{1}H-NMR technique was used to confirm retention cleavage mode.

On-line monitoring of the para-nitrophenyl-β-D-N-acetylglucosamine hydrolysis showed the appearance of new doublets in the NMR spectra corresponding to free aromatic and anomer protons. Signal of α-anomer protons at the later stage of the reaction was observed (Figure 7) showing formation of α-GlcNAc due to the mutarotation from the primer hydrolysis product β-GlcNAc. The anomer proton signal of β-GlcNAc was also present in ^{1}H-NMR spectrum,

but it was overlapped with water signal. The obtained results prove clearly that the DspB enzyme works with retention mechanism.

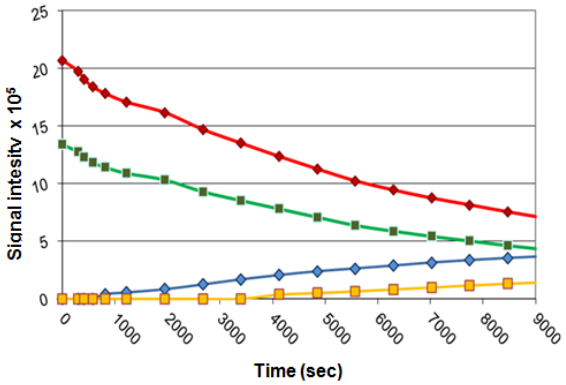


Fig.7: Changes of different proton signal intensities during ^{1}H-NMR measurement (◊-aromatic proton in PNP- glycoside ◊-aromatic proton in free PNP, □-β-anomer proton in glycoside □-α-anomer proton in free monosaccharide)

C) Demonstration of the presence of more productive binding modes

Using MALDI-TOF MS, we analysed the composition of the reaction mixture in order to detect the nonreducing end products. Figure 8 illustrated the recorded spectra at different time intervals during the pentamer hydrolysis reaction. After 10 minutes (Figure 8A), two reducing end products, tetramer (S_4) and trimer (S_3) appeared, while monomer (P) and dimer (P_2) were present as the corresponding nonreducing end products. A peak with m/z 204.3 Da corresponding to $[M\text{-}H_2O+H]^+$ ion also appeared showing the presence of oxazoline, the intermediate product at the substrate assisted hydrolysis mechanism in case of DspB, as earlier suggested. Later, at 20 minutes (Figure 8B) all reducing end—nonreducing end pair of products (S_4+P, S_3+P_2, S_2+P_3) appeared in mass spectrum. At one hour reaction time (Figure 8C) the monomer glycoside, as an end product of the consecutive reaction steps, appeared. After one day (Figure 8D) the products of consecutive steps S, S_2 and P, P_2 became the main products. We verified the presence of more alternative productive binding modes.

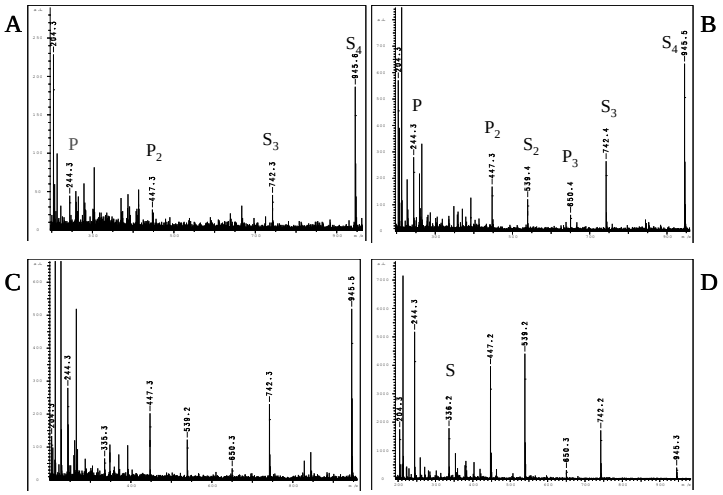


Fig.8: MALDI-TOF spectra of pentamer hydrolysis reaction catalysed by DspB at different time intervals. S_x-nonreducing end products, P_x-reducing end products (A: 10 miin, B: 20 min, C: 60 min, D: one day)

D) Determination of catalytic efficiency on an oligomer substrate series

The calculation of catalytic efficiencies relies on three independent experiments. The initial rate of reaction was measured at low substrate concentration (0.2 mM) using discontinuous direct measurement of product concentration by HPLC method. Substrate concentration-time data were extracted from the total hydrolysis reactions. Curve fitting to data points was made on the basis of Michaelis-Menten equation using Scientist® programe. The k_{cat}/K_M was calculated from the obtained v_{max} and K_M values and the total enzyme concentration $[E]_0$. Finally, initial rate measurements were made over a range of substrate concentration. The initial rate increased linearly at low substrate concentration, the slope of the linear curve was the corresponding rate constant k_2/K_M from which the catalytic afficiency could be calculated. The obtained catalytic efficiency were averaged and summarised in Figure 9. Catalytic efficiency increased with increasing DP of the substrates, indicating favourable binding and higher reaction rate of longer substrates. The calculated pseudo first order kinetic constants show similar values and tendency.

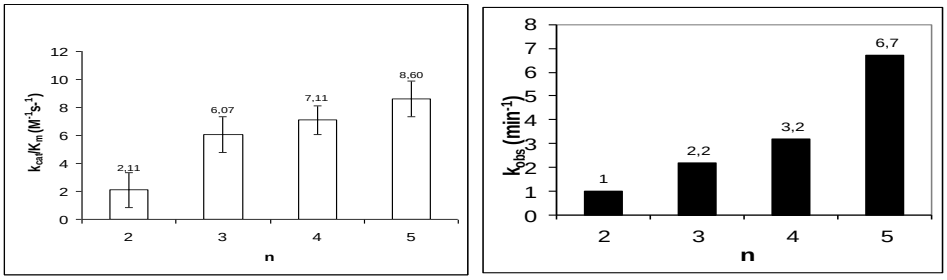
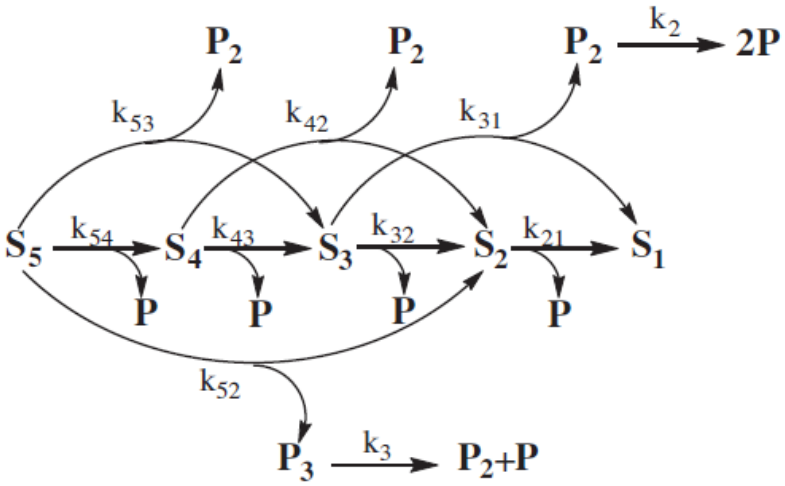


Fig. 9: Calculated catalytic efficiency average value and the summa of the first order kinetic constants as a function of the chain lenght of the substrates. DspB catalysed total hydrolysis reactions on thiophenyl glycoside substrates (S_2-S_5) followed by HPLC

E) Evaluation of kinetic data

Systematic action pattern and kinetic study were made on oligomer substrate series to explore the active site structure and bond cleavage mode of DspB enzyme. The process curves were fitted using Scientist® program and a model assuming paralel and consecutive reaction steps presented on Scheme 1.



Scheme 1: Pentamer substrate hydrolysis with DspB.

The process (total hydrolysis) curve analysis revealed that parallel hydrolysis occured as a result of more productive binding in the case of longer (tetramer and pentamer) substrates. We observed a monotonus increase in the pseudo first order kinetic constants obtained from the kinetic evaluation (Table 1).

The binding of new monomer unit improved the positioning of substrate in the active site for catalytic turnover. These data clearly indicated that five GlcNAc moieties in the substrate were not enough for the total subsite occupancy of the enzyme. Sythesis of longer substrates is in progress for successful subsite mapping of DspB.

Table 1. Calculated kinetic constants for DspB catalysed total hydrolysis reactions

Parameters	Kinetic constans ($perc^{-1}x10^3$)			
	pentamer	tetramer	trimer	dimer
k_{54}	3.82	-	-	-
k_{53}	2.36	-	-	-
k_{52}	0.66	-	-	-
k_{51}	0	-	-	-
k_{43}	2.11	1.85	-	-
k_{42}	2.66	1.46	-	-
k_{41}	0	0	-	-
k_{32}	2.2	2.06	1.76	-
k_{31}	0.76	0	0.28	-
k_{21}	1.26	1.46	0.68	0.95
Σ^a	6.84	3.33	2.04	0.95
k' (PFO)	6.7	3.2	2.2	1.0
k_{cat}/K_m[b]	8.60±1.27	7.11±1.03	6.07±1.27	2.11±1.25

[a] summarised kinetic constants for the given substrates

[b] $M^{-1}s^{-1}$

- data cannot be calculated

0 calculated but value is very low

F) Verification of strictly nonreducing end specificity

The product pattern of hydrolysis was calculated from HPLC measurements. Comparison of kinetic constants was made for the parallel reactions on each substrate. The bond cleavage frequency (BCF) data and the calculated ratios were summarised in Figure 10. BCF values were in good agreement with the ratio of the kinetic constants. It confirmed the validity of our suggested model (Scheme 1).

DspB showes a strictly nonreducing end preference, but more productive binding modes are also possible.

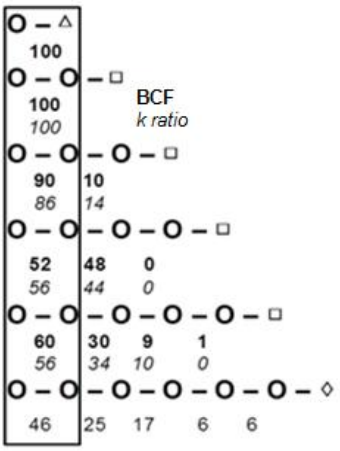


Fig. 10: Action pattern of DspB on thiophenyl glycosides; first line: calculated area ratios of product peaks measured by HPLC; second line: ratios of kinetic constants from the same reaction calculated by Scientist® program

(**O**-GlcNAc unit, - β(1-6) glycosidic bond, □- thiophenyl aglycon, Δ- 4-nitrophenyl aglycon, ◊- 4-metoxiphenyl aglycon)

G) Suggestion to the right classification of DspB

The classification of DspB as EC 3.2.1.52 enzyme and/or Family GH20 enzyme and EC 3.2.1.- (CAZy) happened earlier based on specificity, sequence homology and mechanism similarity. The reaction catalysed by EC 3.2.1.52 group enzymes is defined as the „hydrolysis of terminal nonreducing N-acetyl-D-hexosamine residues in N-acetyl-β-D-hexosaminides. Acts on N-acetylglucosides and N-acetylgalactosides." DspB acts only on PNP-β-GlcNAc and not on PNP-β-GalNAc, probably that is why CAZy database uses the EC 3.2.1.- typology. EC 3.2.1.30. group of β-D-acetylglucosaminidase was deleted from EC number system and now included with EC 3.2.1.52 β-D-N-acetylhexosaminidase. However, other databases, like BRENDA (BRENDA: The comprehensive enzyme information system (http://www.brenda-enzymes.info/) and PDB (Protein Data Bank (http://www.rcsb.org/pdb/home/home.do), still use EC 3.2.1.52 classification for DspB enzyme.

We suggested the general use of EC 3.2.1.- classification, since DspB does not hydrolyse N-acetylgalactosides, produces more productive binding modes and is not a real *exo* enzyme.

IV.SUMMARY

Results of the research on sweet potato β-amylase:

1. We studied the action pattern of sweet potato β-amylase on a chromophore-labelled substrate series. Our substrates contain aromatic ring as chromophore aglycon which caused favourable binding to the active centre. It could be differentiate maltose released from the nonreducing end and maltoside from the reducing end of the even numbered oligomers.
2. In contrast with earlier results, we found glucose cleavage from trimer substrate resulted in CNPG2 product in case of odd numbered substrates.
3. Transglycosylation was observed on each substrate except CNPG5 and smaller. Spontaneous maltose transfer happened in the early stage of the hydrolysis reactions, where processive cleavage is possible.
4. Processive cleavage mechanism was verified. The mean number of steps increased with the chain lenght of the substrate.

Results of the research on DispersinB:

1. We verified with ^{1}H-NMR technique that the enzyme belongs to the retaining glycosidases hydrolysing β-glycosidic bond in the substrate chain and forming β-anomer product.
2. Using MALDI-TOF MS measurements all the reducing and nonreducing end products were detected and strictly nonreducing end preference was verified. The enzyme forms more productive enzyme-substrate complex with oligomer substrates.
3. Determined catalytic efficiencies increased with increasing DP of the substrates. Favourable binding and higher reaction rate were observed in case of longer substrates. The calculated pseudo first order kinetic constants show similar values and tendency.
4. We concluded that five GlcNAc moieties in the substrate were not enough for the total subsite occupancy of the enzyme. Synthesis of longer substrates is planned for successful subsite mapping of DspB.
5. We suggested the exact classification of the enzyme in different databases.

V.PUBLICATIONS

Articles connected to the theses

1. Anikó Fekete, Gyöngyi Gyémánt, Anikó Borbás, Lili Kandra, Erika Fazekas, Narayanan Ramasubbu, Sándor Antus
Synthesis of β-(1,6)-linked N-acetyl-D-glucosamine oligosaccharide substrates and their hydrolysis by DispersinB
Carbohydrate Research (2011) 346, 1445-1453. Impact factor: 2,332

2. Erika Fazekas, Lili Kandra, Gyöngyi Gyémánt
Model for β-1,6-N-acetylglucosamine oligomer hydrolysis catalysed by DispersinB, a biofilm degreding enzyme
Carbohydrate Research (2012) 363, 7-13. Impact factor: 2,332

3.Erika Fazekas, Katalin Szabó, Lili Kandra, Gyöngyi Gyémánt
Unexpected mode of action of sweet potato beta-amylase on maltooligomer substrates
BBA- Protein and Proteomics (2013) 1834, 10, 1976-1981. Impact factor: 3,73

Articles not detailed in the theses

1. János A. Mótyán, Erika Fazekas, Haruhide Mori, Birte Svensson, Péter Bagossi, Lili Kandra, Gyöngyi Gyémánt
Transglycosylation by barley α-amylase 1
Journal of Molecular Catalysis B – Enzymatic (2011) 72, 229-237. Impact factor: 2,4

2.Zs. M. Szigeti, Sz. Szaniszló, E. Fazekas, Gy. Gyémánt, J. Szabon, K. Antal, T. Emri, J. Balla, Gy. Balla, L. Csernoch and I. Pócsi
Optimization of triacetylfusarinine C and ferricrocin productions in *Aspergillus fumigatus*
Acta Microbiologica et Immunologica Hungarica-submitted

Lectures

1.Lili Kandra, János Mótyán, Erika Fazekas, Birte Svensson, Gyöngyi Gyémánt
Mapping of barley amylases. effect of mutation on subsite maps and activities
Annual Meeting of the Carbohydrate Working Group of Hungarian Academy of Sciences, Mátrafüred, Hungary, 29-30. May 2008.

2.Mótyán,J.A., Fazekas,E., Sevensson,B., Kandra,L., Gyémánt,G.
Transglycosylations by modified barley amylase 1 enzymes-Enzymatic synthesis of 4-methylumbelliferyl-β-D-maltoologosaccharides
Annual Meeting of the Carbohydrate Working Group of Hungarian Academy of Science Mátrafüred, Hungary, 28-29. May 2009.

3.Erika Fazekas, Gyöngyi Gyémánt
Study of subsite stucture of exo enzymes
Annual Meeting of the Carbohydrate Working Group of Hungarian Academy of Sciences, Mátrafüred, Hungary, 27-28. May 2010.

4.János A. Mótyán, Erika Fazekas, János Harangi, Péter Bagossi, Birte Svensson, Lili Kandra, Gyöngyi Gyémánt
Enzymatic synthesis 4-methylembelliferyl group-containing maltooligosaccharides as new substrates for α-amylases

Annual Meeting of the Carbohydrate Working Group of Hungarian Academy of Sciences, Mártafüred, Hungary, 27-28. May 2010.

5.Fazekas Erika, Marinó Sándor, Harangi János, Gyémánt Gyöngyi
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1.Mótyán János András, Fazekas Erika, Harangi János, Bagossi Péter, Birte Svensson, Kandra Lili, Gyémánt Gyöngyi
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2.Erika Fazekas, Lili Kandra, Gyöngyi Gyémánt
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5.Erika Fazekas, Lili Kandra, Gyöngyi Gyémánt
Glycan analysis as a tool for enzymatic studies
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Investigation of active site structure and mechanism of a β(1,6)-N-acetyl-glucosaminidase enzyme
13th International Symposium and Summer School on Bioanalysis, Debrecen, Hungary, 27 June- 27 July 2013.

DECLARATION

I bear to testimony to Erika Fazekas predoctor fellowship student for the displayed below article contributed 50%.

Scientific article:

Anikó Fekete, Gyöngyi Gyémánt, Anikó Borbás, Lili Kandra, Erika Fazekas, Narayanan Ramasubbu, Sándor Antus

Synthesis of β-(1,6)-linked N-acetyl-D-glucosamine oligosaccharide substrates and their hydrolysis by DispersinB

Carbohydrate Research (2011) 346, 1445-1453.

Debrecen, 31th October 2013.

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Dr. Gyöngyi Gyémánt

supervisor