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ANALYTIK

INVESTIGATING THE INTERACTION OF α-AMYLASE WITH MALTOOLIGOSACCHARIDES AND THE EFFECT OF FLAVAN-3-OLS ON THE α-AMYLASE ACTIVITY BY SURFACE PLASMON RESONANCE

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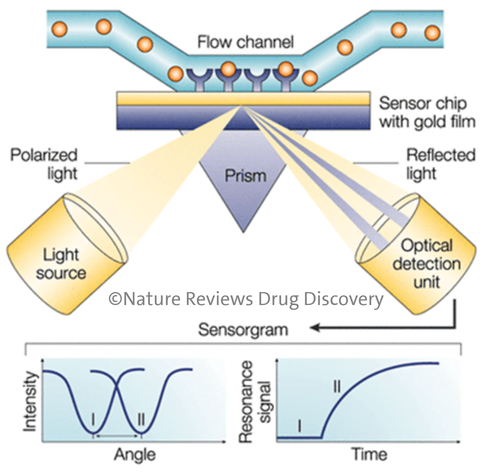
INTRODUCTION

A plant-based diet with high contents of fruits and vegetables is recommended to reduce the risk of type 2 diabetes mellitus and its comorbidities.[1] For secondary plant metabolites, e.g., flavanols and in particular proanthocyanidins, an inhibitory effect on α-amylase activity is proposed helping to control postprandial hyperglycemia.[2] Previous studies by isothermal titration calorimetry (ITC) revealed that a reduction of α-amylase activity was highly dependent on the molecular weight.[3] To verify these results and to gain more knowledge about α-amylase inhibition by flavanols, the inhibitory effects of flavanols on α-amylase activity was studied by surface plasmon resonance (SPR) focusing on maltooligosaccharides.

METHODS

Surface Plasmon Resonance[4]

- Measures changes in mass at the surface
- Label-Free, Real-Time Detection



(1) Surface preparation

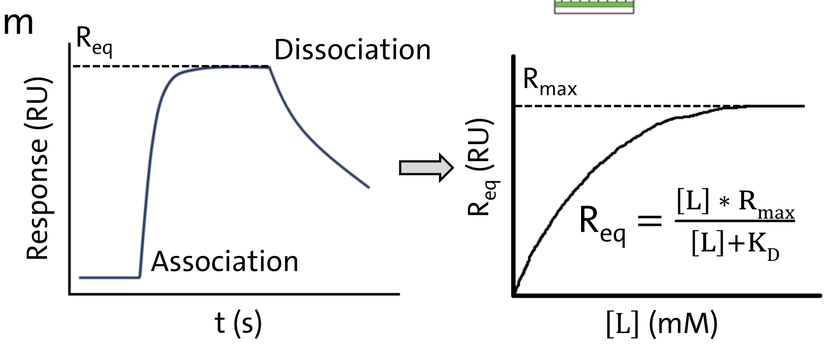
- Immobilization of α-amylase on a sensor chip surface

(2) Ligand injection

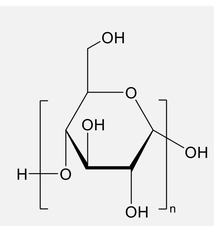
- Sample 1: Maltooligosaccharides
- Sample 2: *O*-MAL-7 + flavanols
- Sample 3: Flavanols

(3) Wash-off with running buffer

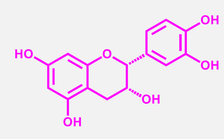
(4) Sensorgram



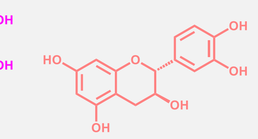
L: Ligand
R: Response
K_D: Dissociation constant



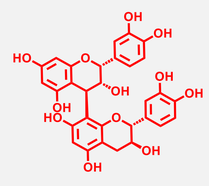
n = 3, maltotriose *O*-MAL-3
n = 4, maltotetraose *O*-MAL-4
n = 5, maltopentaose *O*-MAL-5
n = 6, maltohexaose *O*-MAL-6
n = 7, maltoheptaose *O*-MAL-7
n = 8, maltooctaose *O*-MAL-8



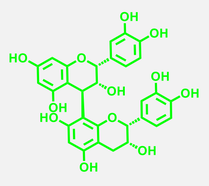
(-)-epicatechin (EC)



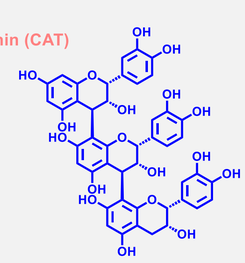
(+)-catechin (CAT)



procyanidin B1 (PC B1)



procyanidin B2 (PC B2)



procyanidin C1 (PC C1)

RESULTS

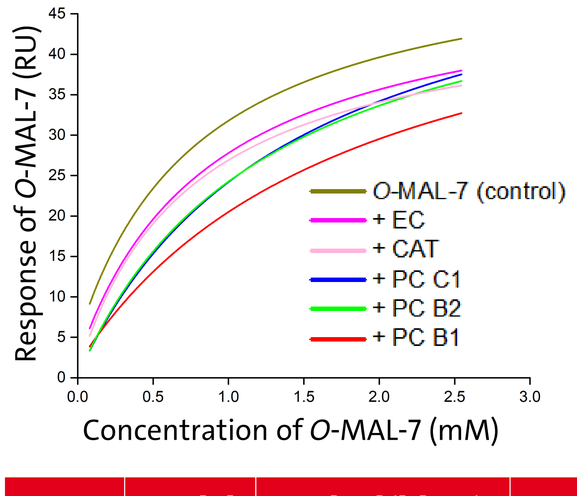
Interaction of α-amylase with maltooligosaccharides

Analyte	K_D [mM]
O-MAL-3	1.36 ± 0.21[a]
O-MAL-4	1.17 ± 0.22[ab]
O-MAL-5	2.12 ± 0.21[c]
O-MAL-6	1.39 ± 0.25[a]
O-MAL-7	0.83 ± 0.09[b*]
O-MAL-8	0.75 ± 0.13[b*]

n = 4 or 5; c = 0.08 – 14 mM, *c = 0.08 – 2.55 mM. Different letters indicate significant differences between mean values ($p < 0.05$).

Binding affinity:
O-MAL-5 < *O*-MAL-3, *O*-MAL-4, *O*-MAL-6 < *O*-MAL-7, *O*-MAL-8

Inhibition represented by reduced substrate affinity



Substrate affinity decreases

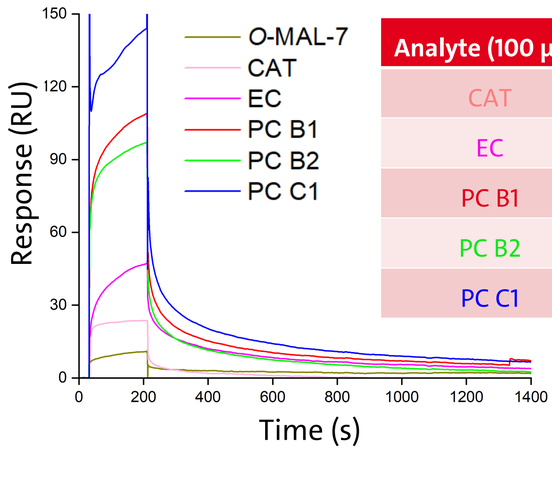
⇩

Stronger inhibition of α-amylase by flavanols

Analyte	V_{max} [%]	IC_{50} [µM] ([S]=1M)	K_D [mM]
CAT	79 ± 7	440 ± 250	0.70 ± 0.09[a]
EC	75 ± 10	430 ± 222	0.88 ± 0.06[a]
PC B1	57 ± 6	147 ± 32	1.58 ± 0.07[b]
PC B2	54 ± 7	127 ± 31	1.38 ± 0.10[b]
PC C1	50 ± 4	103 ± 16	1.44 ± 0.07[b]

n = 2; c = 100 µM; V_{max} [%] and IC_{50} data were published previously.[3] Different letters show significant differences between mean values ($p < 0.05$).

Interaction of α-amylase with flavanols



Analyte (100 µM)	Binding [RU]
CAT	9.4
EC	33.4
PC B1	51.7
PC B2	41.6
PC C1	82.7

Inhibition strength:
PC C1, PC B1, PC B2 > EC, CAT
Interaction strength:
EC > PC C1 > PC B1 > PC B2 > > CAT

CONCLUSION

The affinity of α-amylase to maltooligosaccharides increased with the number of glucose units, except for *O*-MAL-5. Monomeric flavanols did not affect the binding of α-amylase with *O*-MAL-7, whereas the affinity was reduced significantly by dimeric and trimeric flavanols. Notably, the inhibition experiments carried out with SPR were consistent with analogous experiments performed by ITC. However, data from SPR showed a correlation between the interaction of flavanols with α-amylase and enzyme inhibition, except for EC. Although a strong interaction between EC and α-amylase is detected by ITC and SPR, EC does not show an inhibitiory effect on α-amylase, which is currently under investigation.

References: [1] Deshpande et al., *Phys Ther.* **2008,** *88(11),* 1254–1264. [2] Dai et al., *International Journal of Biological Macromolecules.* **2018,** *113,* 427–433. [3] Xiong et al., *Untersuchungen zur Interaktion ernährungsrelevanter Polyphenole mit Verdauungsenzymen mittels isothermer Titrationskalorimetrie, 50. Lebensmittel-chemietagung.* **2022**. [4] Biacore, *An introduction to Biacore's SPR technology,* **2002**.

This work was financially supported by Dr. Leni Schöninger Stiftung and Fonds der chemischen Industrie

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