Degradation Of Progoitrin And Its Breakdown Product VOT By Microorganisms Of Intestine Of Rats In Vitro

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Summary

The degradation of progoitrin (2-hydroxy-3-butenyl-glucosinolate) and its toxic splitting product VOT (5-vinyloxazolidin-2-thion, goitrin) by the microorganisms of the intestine of rats in vitro was investigated. The experiments were carried out with faeces, caecum and colon contents of conventional and germfree rats in vitro under aerobic and anaerobic conditions.

After adding of progoitrin to gut contents we found a degradation rate after an incubation of 24 hours under aerobic conditions of 29, 59 and 47 per cent, respectively. It was important that the splitting product VOT was not detectable.
Under anaerobic conditions similar results were obtain (47 to 55 per cent decrease of progoitrin) but small amounts of VOT were formed.
After adding of VOT in all experiments no degradation took place. Degradation effects could not be observed in experiments with material from germ-free rats.
So we concluded that the intestinal microflora was responsible for progoitrin degradation.

Introduction

The relative hight amounts of the glucosinolate progoitrin (2-hydroxy-3-butenylglucosinolate) and its toxic splitting product VOT (5-vinyl-oxazolidin-2-tnion, goitrin) in rapeseed limited the use of this protein for human consumption.
The general knowledge of metabolism of progoitrin and VOT in mammalian animals in verry slight. In this connection the role of the microorganisms of the intestine of rats was important to investigate. Only some reports (1, 2, 3) give hints that the microflora of intestine of rats and men could degrade progoitrin.
In connection with investigation of metabolic pathways of glucosinolates and there breakdown products in mammalian organisms the role of the intestinal microflora in very important. Therefore we investigate the influence of the microflora of faeces, caecum and colon contents of conventional rats in vitro.

Material and methods

Pure cristalline glucosinolate (free of VOT) and VOT (m. p. 48 - 50 °C) were isolated from rapeseed (4, 5). The identity and quantity of VOT were prooved by HPLC (AS GDR, detection UV_{254}, Lichrosorb Si 60, n-heptan/isopropanol/ethanol = 8/1/1, column 4,6 x 250 mm). Progoitrin was determined after enzymatic splitting to VOT with a crude myrosinase preparation (4).
The experiments were carried out with faeces, caecum and colon contents of conventional (Shoe:Wist) and germfree rats (Wistar, germfree strain WAG/Rij Rehbrücke) in vitro under aerobic and anaerobic (plexiglass isolators with pure nitrogen atmosphere) conditions.

The incubation conditions were: 24 hours at 37 °C, 10 ml of a sucrose solution in 100 ml Erlenmeyerflaks, 6 mg of progoitrin or 1 mg VOT from a stock solution were added. Progoitrin and VOT solutions without gut content and germfree material were the controls.
At the end of the experiments the test solutions were neutraliziesed with NaOH and VOT was extracted with chloroform.

Results

The alterations of pH, resulting in the growth of micro-organisms was measured, because the acid conditions could be a reason for progoitrin reductions. In Fig. 1 we can see the decrease of pH after 24 hours observed in all experiments, but not in controls and by using of germfree material.

During incubation of progoitrin with faeces, caecum and colon content of conventional rats under aerobic conditions the degradation rate was 29, 59 and 47 per cent, respectively (Fig. 2 - 4). After 5 hours the degradation was significant ($p < 0,05$) and after 15 hours ($p < 0,01$).

It was important, that the toxic breakdown product VOT was not formed.
No degradation was observeable during incubation of progoitrin and VOT separately with gut contents of germfree animals and in controls (without gut content).
The same results were also observed under anaerobic conditions.
The microflora of caecum and colon of conventional rats reduced the progoitrin content after 24 hours under anaerobic conditions about 47 and 55 per cent (Fig. 5, 6).
Under this regime small amounts of VOT were formed.

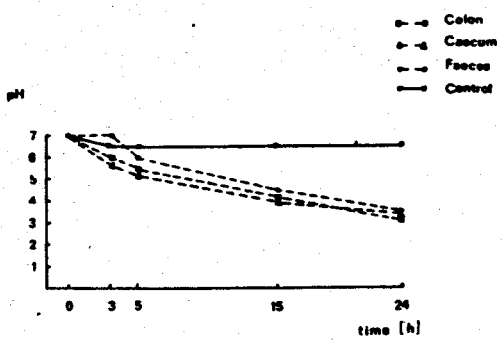

Figure 1: pH-alteration by microorganism of intestine of conventional rats in vitro

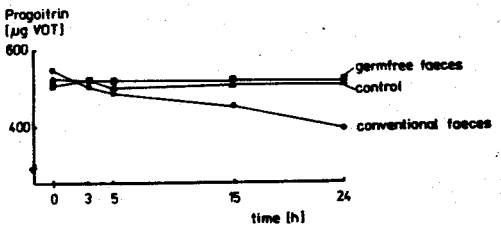

Figure 2: Progoitrin degradation by microorganism of faeces in vitro under aerobic conditions

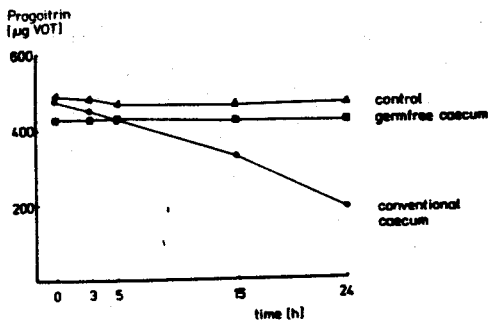

Figure 3: Progoitrin degradation by microorganism of caecum in vitro under aerobic conditions

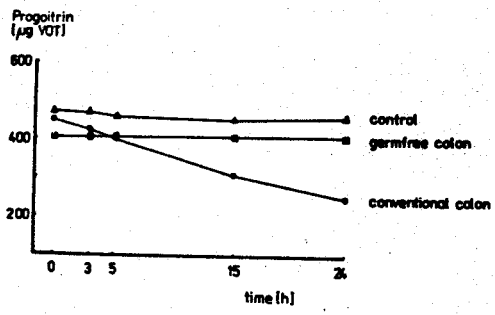

Figure 4: Progoitrin degradation by microorganism of colon in vitro under aerobic conditions

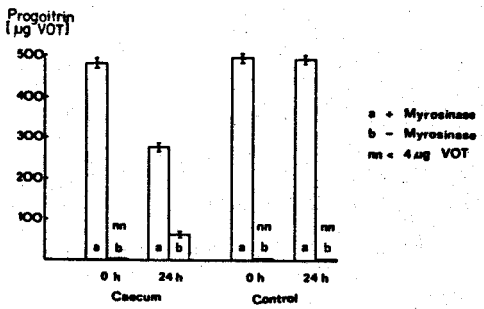

Figure 5: Progoitrin degradation by microorganism of caecum in vitro under anaerobic conditions

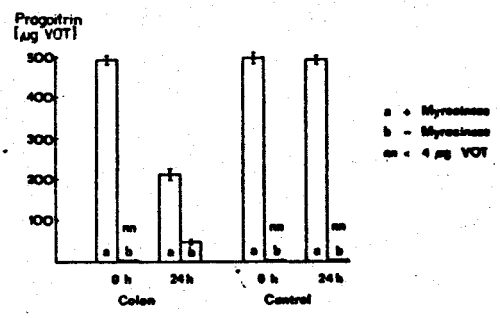

Figure 6: Progoitrin degradation by microorganism of colon in vitro under anaerobic conditions

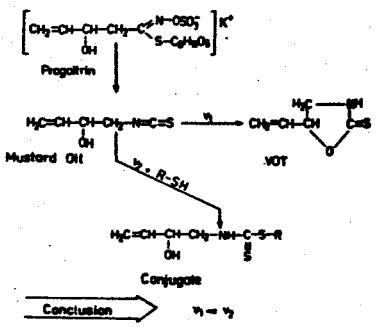

Figure 7: Competetive reactions to VOT and/or conjugates

Discussion

It should be proofed that the microflora of rat intestine is responcable for the degradation of progoitrin in vitro without forming the toxic splitting product VOT.
The change of pH resulting in the growth of microorganism is not the reason of progoitrin degradation (1). It seems that the cyclization of hydroxybutenyl ITC to VOT and reactions with activ groups of proteins etc. are competetive reactions. In the presence of nucleophile groups(f.i. SH-, NH_2-) it could be possible that no significant amount of VOT are formed (Fig. 7). Now we try to identify secondary reaction products of hydroxybutenyl ITC in the organism.

References

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