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Unusual differences in the reactivity of glutamic and aspartic acid in oxidative decarboxylation reactions

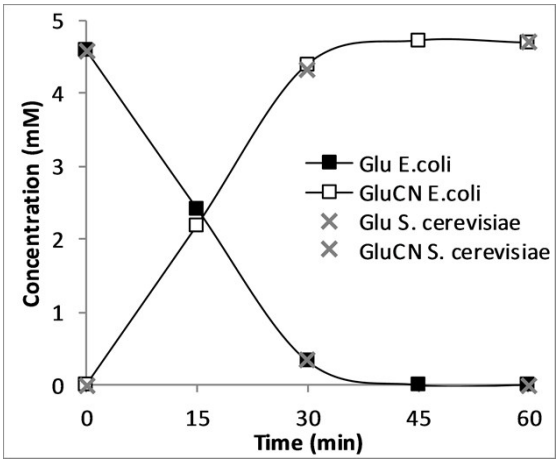
Andrada But[a], Evie van der Wijst[a], Jérôme Le Nôtre[a], Ron Wever[b], Johan P. M. Sanders[a], Johannes H. Bitter[a], Elinor L. Scott[a]*

[a] Chair of Biobased Chemistry and Technology, Wageningen University, PO Box 17, 6700AA Wageningen; [b] Van't Hoff Institute for Molecular Sciences, University of Amsterdam, PO Box 94157, 1090 GD Amsterdam; the Netherlands; *corresponding author

Supplementary Information

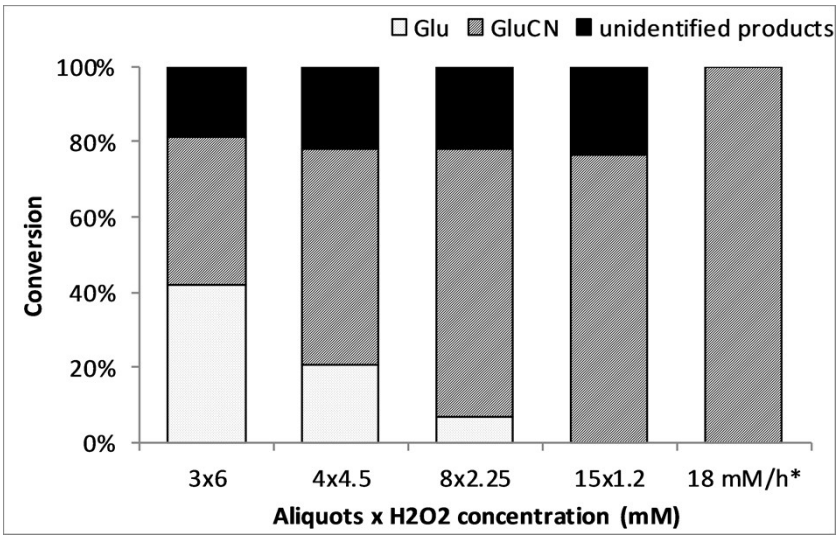
Based on previously reported research[1] the reaction conditions were optimised in order to obtain shorter reaction times (see Experimental).

In this research the enzyme used, vanadium chloroperoxidase (VCPO), was expressed in *E. coli*[2] ($VCPO_{E. Coli}$). A validation was performed to investigate whether the partially purified $VCPO_{E. Coli}$ has the same reactivity towards the amino acids as the highly purified VCPO produced in *S. cerevisiae* ($VCPO_{S. cerevisiae}$), which was used in previous research.[1] Validation was performed for the conversion of glutamic acid (Glu) into 3-cyanopropanoic acid (GluCN) (Scheme 2, n=2). For this validation the same units of VCPO from the two organisms was used. The units of VCPO were determined based on the monochlorodimedone activity assay (see Experimental). The validation test confirmed that the changes performed did not affect the conversion of Glu to GluCN (SI-Fig. 1).

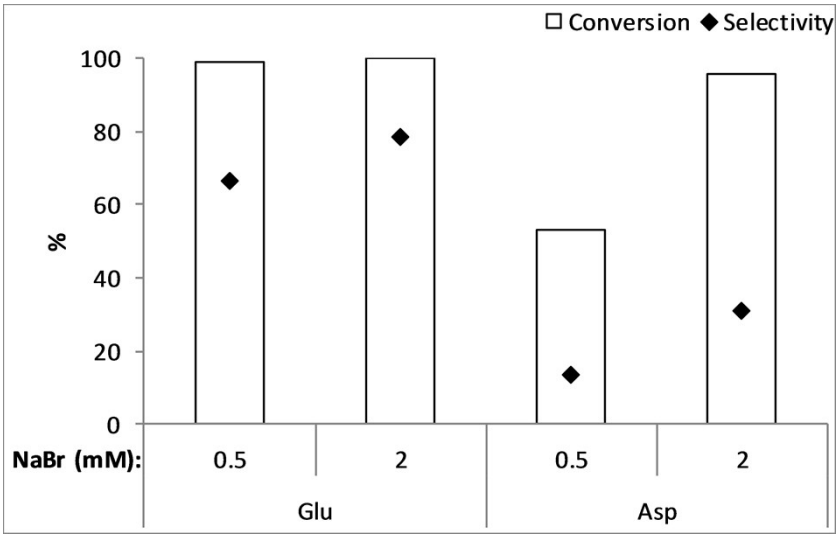


SI-Figure 1. Glutamic acid (Glu) conversion into 3-cyanopropanoic acid (GluCN) by vanadium chloroperoxidase (VCPO) at 0.5 mM NaBr for the validation of $VCPO_{E. coli}$.

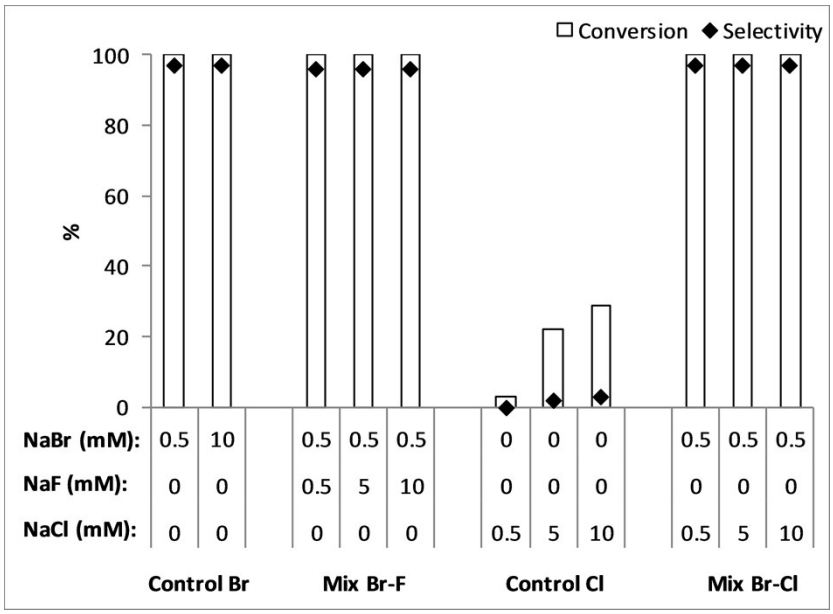
The addition of H_2O_2 was tested on the conversion of Glu to GluCN (SI-Fig. 2). The addition of H_2O_2 was performed using different amounts of aliquots of H_2O_2. It can be observed that the more concentrated the aliquot of H_2O_2 the less the conversion of Glu. Only when 1.2 mM aliquots were used full conversion was achieved. The unidentified products corresponding to about 20% of the starting Glu could be due to the use of a crude extract of the VCPO which could have other enzymatic activities beside the VCPO. A continuous addition of H_2O_2 and a purified VCPO ensured full conversion and no side reactions.



SI-Figure 2. Conversion of Glu to GluCN after 1 h as a function of addition of H_2O_2 at 2 mM NaBr. The unidentified products were calculated based on the mol balance of each reaction. *H_2O_2 was added continuously using a syringe pump.

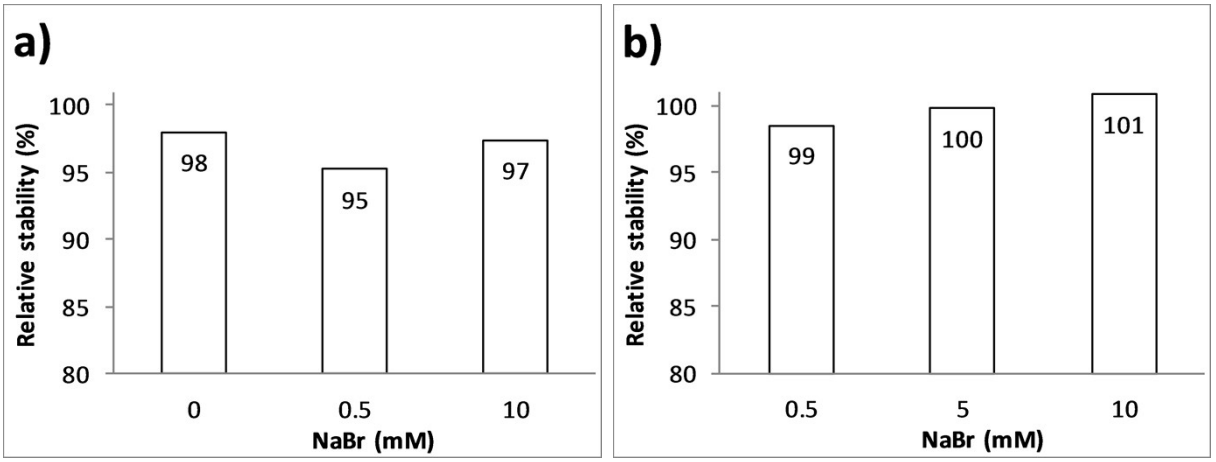


SI-Figure 3. Conversion of Glu and Asp and the selectivity to GluCN and AspCN after 1 h as a function of NaBr concentration using NaOCl as oxidant at a constant addition rate (18 mM/h), 5 mM amino acid was used as starting concentration.



SI-Figure 4. Conversion of glutamic acid (Glu) and selectivity towards 3-cyanopropanoic acid (GluCN) as function of halides concentration. The data represent results from single experiment.

It can be observed (SI-Fig. 4) that the reactivity of Glu is not influenced by the concentration of NaBr nor by the additional presence and concentration of NaF and NaCl. Even when only chloride was present the conversion of Glu and selectivity to GluCN is low. From these results it can be concluded that the ionic strength does not influence the reactivity of Glu.



SI-Figure 5. Stability test of **a)** AspCN and **b)** Malonic acid after 1 h under oxidative decarboxylation reaction conditions at different concentrations of NaBr. 5 mM of AspCN or Malonic acid were used as initial concentrations and 18 mM/h of H_2O_2 were added continuously.

References

1. A. But , J. Le Nôtre, E. L. Scott, R. Wever and J. P. M. Sanders, *ChemSusChem*, 2012, 5, 1199-1202.
2. Z. Hasan, R. Renirie, R. Kerkman, H. J. Ruijssenaars, A. F. Hartog and R. Wever, *Journal of Biological Chemistry*, 2006, 281, 9738-9744.