Derivatization of amino acids to corresponding α-hydroxy acids and their analysis in fermentation broths

Daniel Pleissner[1, 2] (danielp@biology.sdu.dk), Reinhard Wimmer[2], Niels Thomas Eriksen[2]

[1]University of Southern Denmark, Institute of Biology, Campusvej 55, 5230 Odense M, Denmark
[2]Aalborg University, Section of Biotechnology, Sohngaardsholmsvej 49, 9000 Aalborg, Denmark

Introduction

We describe a method for quantitative analysis of amino acid derivatives, which are created using the van Slyke reaction. The van Slyke reaction is based on the transformation of amino acids into their corresponding α-hydroxy acids (Fig. 1) by a reaction between amino groups and dinitrogen trioxide, formed from nitrite under acidic conditions, and the formation (nitrosation) of a labile diazonium compound. The diazonium group is released by an intramolecular lactone formation with a carboxyl group as molecular nitrogen. Hydrolysis of the lactone by an increase of pH results in the formation of different α-hydroxy acids depending on the original amino acid.

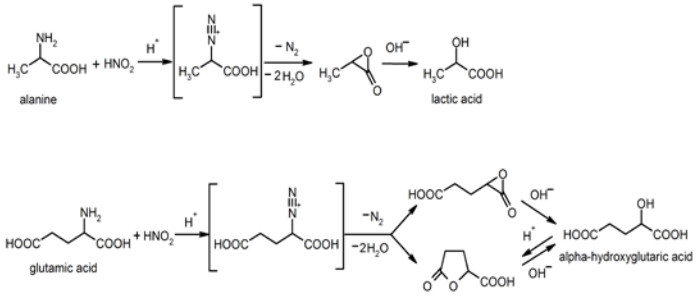

Figure 1: Derivatization of alanine and glutamic acid into their corresponding α-hydroxy acids.

Methods

Derivatizations of amino acids were carried out in 2.5 mL polypropylene tubes at 45°C for 90 min, containing 1 mL of amino acid solution (0-3 g L^{-1}) mixed with 0.2 mL of 1 M potassium nitrite. The reaction was started by decreasing pH to 1-2 by addition of 0.04 mL 12 M HCL and stopped by addition of 0.2 mL 2 M NaOH.

Quantification α-hydroxy acids, glucose, and phosphoric acid were carried out using an Aminex HPX-87H column (Bio-Rad), isocratically eluted with 0.4 mL min^{-1} of 5 mM H_2SO_4 and detection was performed using a RI detector.

Results

The reaction between dinitrogen trioxide and 13 of the 20 classical amino acids resulted in products, which could be separated by the Aminex HPX-87H column, and detected and quantified by RI detection. Linear relationships were observed between peak areas of end-products and initial concentrations of all 13 amino acids (Table 1 and Fig. 2).

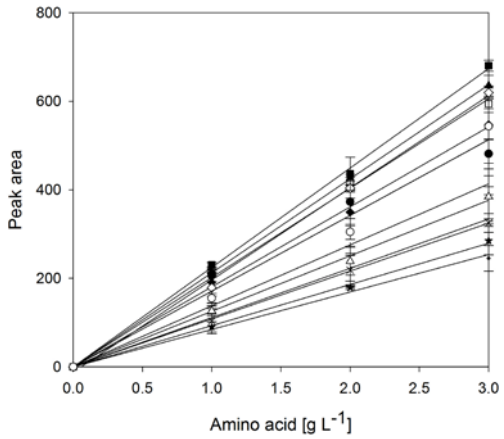

Figure 2: Peak areas from HPLC analysis of products versus initial concentrations of amino acids (L-aspartic acid ◆, L-serine □, L-threonine ▲, L-valine ×, L-alanine ✳, L-glutamine ●, glycine +, L-methionine ▬, L-isoleucine ▬, L-asparagine ◇, L-glutamic acid ■, L-leucine △, L-proline ○).

Table 1: Retention times (RT) of derivatives of the 13 amino acids, that could be quantified after reaction with dinitrogen trioxide.

Amino acid	RT	Slope	r^2
	min	L g^{-1}	
Glycine	10.9	85 ± 8	0.99
L-alanine	10.5	93 ± 3	0.99
L-valine	16.1	108 ± 4	0.99
L-leucine	26.9	126 ± 3	0.99
L-isoleucine	24.1	138 ± 2	0.99
L-methionine	26.9	111 ± 3	0.99
L-serine	7.9	202 ± 3	0.99
L-threonine	8.5	212 ± 2	0.99
L-asparagine	10.7	203 ± 4	0.99
L-glutamine	8.7	171 ± 7	0.98
L-aspartic acid	6.4	181 ± 8	0.99
L-glutamic acid	8.7	224 ± 4	0.99
L-proline	21.9	171 ± 6	0.98

In Figure 3, amino acid concentrations and additional data from *Crypthecodinium cohnii* cultures grown on glycine, L-alanine, or L-glutamic acid as nitrogen source are shown. Inset shows phosphoric acid peaks, analysed prior to reactions with nitrite on expanded scale.

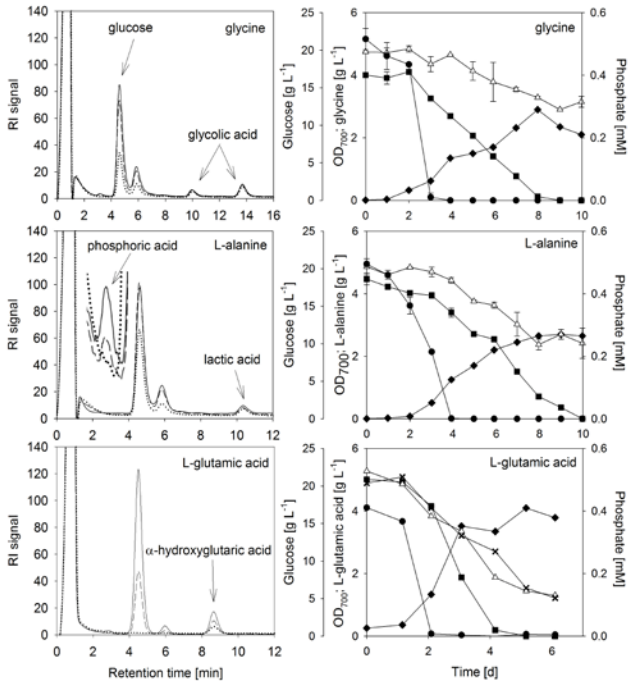

Figure 3: Optical density ◆, phosphate ●, glucose ■, and amino acids △ in supernatants of cultures of the heterotrophic dinoflagellate *Crypthecodinium cohnii* and RI chromatograms of culture supernatants reacted with 160 mM nitrite at day 0 (▬), day 3 (▪ ▪ ▪), and day 6 (''''''''') of cultivation. For comparison L-glutamic acid was also quantified enzymatically (×).

Conclusion

- Method is suitable for analysis of 13 of the 20 classical amino acids
- Linear relationships between peak areas and initial amino acid concentrations
- Derivatization is not only restricted to pure solutions of amino acids but worked also for fermentation broths
- Glucose measurement is not affected and was carried out in parallel
- Method can also be used for amino acid analysis in food, feed, and diagnostics, and for analysis of other amines

Pleissner D, Wimmer R, Eriksen NT (2011) Quantification of amino acids in fermentation media by isocratic HPLC analysis of their α-hydroxy acid derivatives. *Anal Chem* 83: 175-181