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Note

Single-column analysis of amino acids in hydrolysates of samples containing chromic oxide

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Analysts are frequently presented with samples of feed and faeces which contain chromic oxide (Cr_2O_3), added as a quantitative marker in digestibility trials[2].

During the hydrolysis of samples of feed, gut contents or faeces with boiling 6 *N* HCl for the amino acid determination, chromic ions are liberated as chromium(III) cations. When the hydrolysate is analysed on a cation-exchange resin column, the active sites on the resin will gradually be bound by the Cr(III) ions and the separation efficiency will decrease. This effect is irreversible when all the common protein amino acids are analysed using a single-column procedure on a high-pressure small-bore column. A column of this type is used in our fully automatic Dionex D-500 amino acid analyzer.

It has therefore been necessary to develop a special chromatographic procedure for hydrolysates from samples containing chromic oxide. When the column is eluted with a buffer of low pH and high sodium ion concentration before the normal third buffer is applied, there is no adverse effect of the chromic ions. This flush is performed after the acid and neutral amino acids are eluted. To separate the basic amino acids, two additional buffers are necessary. Table I shows a comparison of our normal procedure[2] and the one used for hydrolysates containing chromic ions.

The retention times for the acid and neutral amino acids are identical for the two procedures. The times for phenylalanine, histidine, lysine, ammonia and arginine are, for the normal procedure, 56.50, 62.67, 69.67, 74.17 and 87.50 min, respectively, and for the special one 56.50, 105.17, 110.33, 80.00 and 131.83 min, respectively. At this stage of analys is the elutionof ammonia is preliminarily determined by the sodium ion concentration rather than by temperature and pH.

It is normal practice to determine cyst(e)ine and methionine in a sample oxidized prior to hydrolysis as cysteic acid and methionine sulphone, respectively. In this case only one elution buffer is used. The elution is stopped after glycine is eluted, the latter being used for double determination. Before regeneration with sodium hydroxide solution the chromic ions must be washed out of the column by means of the same flushing buffer as mentioned before. The details for the normal and the special runs for the hydrolysates of the oxidized samples are also shown in Table I.

TABLE I

CHROMATOGRAPHIC PROCEDURE DETAILS

All buffers are sodium citrate buffers containing 0.1% phenol. Regeneration is in all cases done with 0.3 *N* NaOH containing 0.025% EDTA for 5 min at 50°. Column: steel 480 × 1.75 mm I.D. factory-filled with Durrum DC-4A resin, 8 ± 2 μm bead diameter. Standard size: 30 μl containing 10 nmol of each amino acid. Sample size: 30 μl containing *ca.* 3 μg sample-N.

	Buffer no.	*Buffer pH*	*Sodium conc. (N)*	*Col. temp. (°C)*	*Buffer change time (min)* *	*Elution stop (min)*
Normal hydrolysate run for unoxidized samples	1	3.25	0.2	50	−14	
	2	4.25	0.2	65	+33	
	3	7.60	1.1	65	+50	+90
Normal hydrolysate run for oxidized samples	1	3.25	0.2	50	−14	+38
Special run for hydrolysates of unoxidized samples containing Cr_2O_3	1	3.25	0.2	50	−14	
	2	4.25	0.2	65	+33	
	3	2.30	1.1	65	+57	
	4	3.25	1.1	65	+75	
	5	7.60	1.1	65	+112	+135
Special run for hydrolysates of oxidized samples containing Cr_2O_3	1	3.25	0.2	50	−14	
	2	2.30	1.1	65	+33	+55

* Injection time = 0.

The maximum concentration of chromium in the hydrolysates analysed was 24 mg/150 ml, calculated as Cr_2O_3, equal to 5 μg on the column. A comparison of recoveries of amino acids from feed with and without added Cr_2O_3 in about the same concentration gave differences within the range of normal analytical error. A similar comparison with pig faeces showed the same result for the oxidized hydrolysate, but for the unoxidized hydrolysate, with added chromic oxide, the recovery was somewhat higher, probably due to a catalytic effect.

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