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Note

Presence of sodium azide during acid hydrolysis of protein samples causes the destruction of tyrosine, phenylalanine and histidine

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We have observed that certain protein samples presented to us for amino acid analysis did not appear to contain the amino acids tyrosine, phenylalanine and histidine, although the samples were expected to contain these amino acids. We have shown that destruction of these amino acids has been occurring during sample hydrolysis in 6 *N* HCl due to the presence of small amounts of sodium azide which was present (as an antibacterial agent) in the phosphate buffered saline in which the proteins were originally presented.

MATERIALS AND METHODS

The chromosomal protein HMG2 (mol. wt. 26,000) which has been extensively studied and well characterised in our laboratories in recent years[1,2] was used as our test protein in the following experiment. Protein was dissolved in water containing 0.1 % (w/v) sodium azide, at a protein concentration of 1 mg/ml. A 50-μl aliquot was taken and dried down under vacuum in a Pyrex tube. 6 *N* HCl (0.5 ml) was added, the tube sealed, and the sample hydrolysed at 110° for 24 h. The concentration of sodium azide in the hydrolysing acid was, therefore, 0.01 % (w/v). The protein hydrolysate was then dried under vacuum and amino acid analysis carried out using a Rank-Hilger Chromaspek amino acid analyser. An identical control experiment was carried out but in the absence of sodium azide.

RESULTS AND DISCUSSION

Table I presents the results of the amino acid analyses, where sample hydrolysis was carried out both in the presence (analysis 1) and absence (analysis 2) of sodium azide. The most obvious features are the complete loss of tyrosine and phenylalanine in the azide containing sample (analysis 2). Additionally, histidine is almost completely destroyed and the peak due to the methionine sulphone has disappeared. An extremely high ammonia peak also prevented the resolution of arginine in this analysis. Additional unknown peaks were observed in the second analysis. Two unknown peaks eluted between histidine and lysine. These are presumably degradation products from the destroyed amino acids. The only other difference between the 2 analyses is an

TABLE I

AMINO ACID ANALYSIS OF TEST PROTEIN IN THE PRESENCE AND ABSENCE OF SODIUM AZIDE

Amino acid	*Recovery (nmoles)*	
	Analysis 1 (6 N HCl)	*Analysis 2 (6 N HCl + sodium azide)*
Mes	13.4	—
Asp	72.9	81.7
Thr	16.9	16.9
Ser	61.1	58.2
Glu	138.5	136.6
Pro	50.0	49.4
Gly	53.3	51.6
Ala	61.3	58.8
Cys	—	—
Val	15.1	14.1
Gln	12.7	12.2
Leu	15.7	14.9
Tyr	21.1	—
Phe	30.0	—
His	14.4	2.7
Lys	146.4	144.5
Arg	34.1	*
NH_3	60.0	$>10^3$

* Not resolved from large ammonia peak.

increase in the aspartic acid peak by about 15% in analysis 2, which suggests that a further degradation product is running in this position.

The amount of sodium azide present in our hydrolysing medium (0.01% w/v), is, of course, extremely small, but even lesser amounts are likely to cause at least partial destruction of some amino acids. We have shown with previous samples that dialysing the protein agains distilled water overnight is sufficient to remove the sodium azide, resulting in a normal amino acid analyses.

REFERENCES

1 J. M. Walker, G. H. Goodwin and E. W. Johns, *Eur. J. Biochem.*, 62 (1976) 461.
2 G. H. Goodwin, J. M. Walker and E. W. Johns, in H. Busch (Editor), *The Cell Nucleus*, Vol. VI, Academic Press, New York, London, 1978, p. 181.