

OXIDATION OF VALINE, LEUCINE AND PHENYL ALANINE BY CHLORAMINE-T

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ABSTRACT

Chloramine-T oxidises valine, leucine and phenyl alanine in various solvent media and the reaction is stoichiometric with a four electron change in buffer medium of pH 4-6. Back titration procedures for the estimation of these essential amino-acids in a buffer medium of pH 5 have been described. The products are the corresponding nitriles and *p*-toluene sulphonamide. The effect of foreign ions and other amino-acids on the oxidation has been investigated.

SEVERAL applications of chloramine-T (CAT) as an oxidant in the estimation of compounds of biochemical origin have been made recently^{1,2}. In the present investigations, we have examined the behaviour of CAT as an oxidimetric reagent for the estimation of valine, leucine and phenyl alanine (PAN). It was found that these essential amino-acids are oxidized by CAT at room temperature in several solvent media. However, a stoichiometric oxidation with a 4-electron change is noticed at pH 5. Back titration procedures had to be adopted as the reaction was not fast enough for a direct titration with a visual or potentiometric end-points.

MATERIALS AND METHODS

DL-valine (BDH), leucine (Diamalt—West Germany) and DL- β -phenyl alanine (BDH), were recrystallized from aqueous medium and the purity was checked by the standard acetous perchloric acid method³. Chloramine-T (E-Merck) was purified by the method

of Morris *et al.*⁴ and the solution was standardised by the iodometric method. Reagent grade chemicals were used in preparing other solutions.

Preliminary experiments indicated that one m. mole of the amino-acid could consume 2 m. moles of CAT at pH 4-6 only. At other pH values, lower values were obtained. Even in presence of mineral acids (HCl, H₂SO₄ and HClO₄, 0.25-1.00 M), this ratio was considerably lower.

RECOMMENDED PROCEDURE

Solutions of the amino-acid (0.1-3.0 m. moles) in acetate buffer of pH 5.0 were pipetted to a measured excess (10-20%) of 0.1 N CAT. The reaction mixture was set aside for 30-40 minutes and the unconsumed CAT was estimated iodometrically. A blank titration was carried out with the same volume of CAT.

Some results of analyses are given in Table I. The results are accurate within 0.5%.

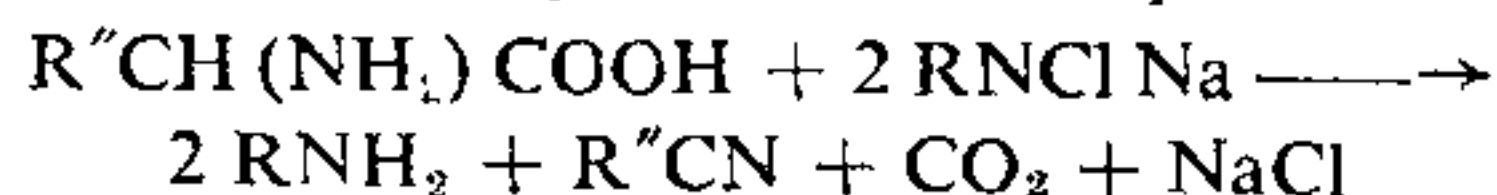
TABLE I

Estimation of valine, leucine and phenyl alanine by chloramine-T in pH 5 buffer medium
Amounts are expressed in mg.

Valine			Leucine			Phenyl alanine		
Taken	Found	% Error	Taken	Found	% Error	Taken	Found	% Error
2.00	2.01	+0.50	4.00	3.99	-0.25	4.00	4.01	+0.25
4.00	4.01	+0.25	6.00	6.01	-0.17	6.00	6.01	+0.17
6.00	5.99	-0.17	8.00	8.02	+0.25	8.00	8.02	+0.25
8.00	8.00	0.00	10.00	10.00	0.00	10.00	10.02	+0.20
10.00	10.01	+0.10	12.00	12.02	-0.17	20.00	19.98	-0.10
12.00	12.00	0.00	15.00	14.97	-0.20	25.00	24.95	-0.20
15.00	15.01	+0.07	20.00	19.94	-0.30	30.00	30.06	+0.20
20.00	20.01	+0.05	25.00	24.95	-0.20	35.00	34.92	-0.23
40.00	40.00	+0.05	30.00	29.94	-0.20	40.00	39.92	-0.20

RESULTS AND DISCUSSION

The 1:2 stoichiometry observed for the oxidation of amino-acids by CAT can be represented as:



where $R = p\text{-CH}_3\text{C}_6\text{H}_4\text{SO}_2$ and R'' is $(\text{CH}_3)_2\text{CH}$ for valine, $(\text{CH}_3)_2\text{CHCH}_2$ for leucine and $\text{C}_6\text{H}_5\text{CH}_2$ for phenyl alanine.

Paper chromatography² was used to identify the sulphonamide ($R_f = 0.905$). Benzyl alcohol saturated with water was used as the solvent with 0.5% vanillin in 1% HCl solution in ethanol as the spray reagent. The nitriles formed, namely, 2-methyl propionitrile, 2-methyl butyronitrile and phenyl acetone nitrile were detected by their colour reactions⁵ with hydroxylamine and ferric chloride.

The rate of oxidation of the amino-acids by CAT is highly retarded in sulphuric and perchloric acid media. The same trend was observed in the oxidation of unsaturated alcohols by CAT^{6,7}. This could probably be attributed to the combined specific inhibitory effect of H^+ , SO_4^{2-} and H^+ , ClO_4^- ions. In contrast, presence of hydrochloric acid accelerated the reaction between CAT and unsaturated alcohols, while a retardation effect is noticed in the present case. It is likely that protonated HOCl is not the species⁸ responsible for the oxidation of amino-acids.

The presence of foreign ions such as Ba^{2+} , Zn^{2+} , NO_3^- , SO_4^{2-} and PO_4^{3-} and sodium chloride (up to

0.2 mole) had no effect on the rate or stoichiometry of oxidation of amino-acids by CAT. Further, the stoichiometry is unaffected by a reversal of the order of addition of oxidant and the amino-acid.

The rapid rate of oxidation at pH 4–6 can probably be attributed to the rapid disproportionation of monochloramine-T present in acidified CAT solutions to dichloramine-T and *p*-toluene sulphonamide in this pH range, as suggested by Higuchi *et al.*⁹. It is also to be noted that the isoelectric points of these amino acids lie within this pH range.

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ISOLATION AND CHARACTERIZATION OF SOME NEW FORMATE UTILIZING BACTERIA

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ABSTRACT

Five isolates degrading and assimilating formate were isolated from chicken dung. Characterization indicated two different types. One of these belonged to the genus *Alcaligenes* and assimilated formate autotrophically. The other four isolates were identical, belonged to the genus *Protaminobacter* and assimilated formate heterotrophically by the serine pathway.

INTRODUCTION

FORMATE, the simplest of organic compounds is oxidized by a number of bacteria, plants and animals¹⁻². However, only a few bacteria utilize it as a sole carbon source and its assimilation involves two different pathways. Organisms like *Pseudomonas* AM1 assimilate it by the serine pathway³, while *Ps. oxalaticus*, *Bacterium formoxidans*, *Hydrogenomonas eutropha* Z-1 and *Thiobacillus novellus* (see discussion) assimilate it autotrophically. The fact that only a few cases of autotrophic growth on formate have been reported indicates that this may be a rare type of metabolism as considerations of economy may favour

the selection of organisms such as *Ps. AM1* which conserve the reduction level of formate⁴. The only other C_1 organic compound on which autotrophic growth has been recorded is on methanol by *Micrococcus denitrificans*⁵. As these C_1 compounds are at the borderline between organic and inorganic, a wider study of autotrophic growth on such compounds in a number of genera can be expected to throw light on the biochemical basis of facultative autotrophy.

We report here the isolation and characterization of an autotrophic formate-utilizing bacterium *Alcaligenes* FOR₁ and heterotrophic *Protaminobacter* FOR₂ from chicken dung by enrichment culture with formate