A New Acidic Ultraviolet Absorbing Substance Containing Amino Acids in Bacteria, Yeasts and Spore Preparations

J. JONSEN, S. LALAND,
I. SMITH-KIELLAND and R. SÖMME

Department of Biochemistry The University of Oslo, Blindern, Oslo, Norway

In recent years nucleotides containing amino acids have been isolated from extracts of mammalian tissues [1] and bacterial cells [2]. In a previous paper [3] we have described such substances from *E. coli* and *B. subtilis* harvested in the logarithmic growth phase. We now wish to report the occurrence and properties of an unidentified substance containing amino acids and with a characteristic ultraviolet absorption spectrum.

The substance has been isolated by gradient elution of cell extracts adsorbed on a Dowex 1 × 8 column in the formate form. Fig. 1 shows a typical elution of an extract

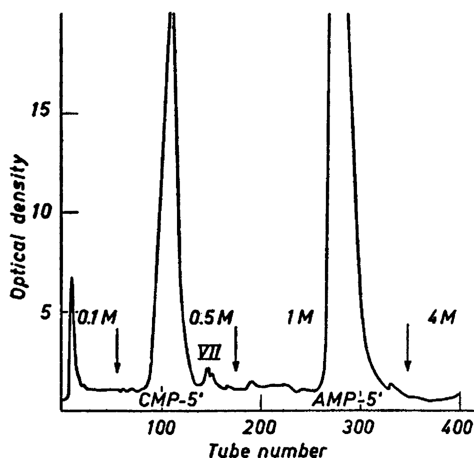


Fig. 1. Part of the gradient elution diagram of *B. cereus* extract. Volume of mixing flask 750 ml. Dowex 1 × 8 in the formate form (3 cm × 18 cm). Elution was started with 0.001 M formic acid in the reservoir flask followed by 0.002 M, 0.02 M, 0.1 M. The arrows indicate concentrations of 0.5 M, 1.0 M and 4 M formic acid. Volume of individual fractions. 29 600 optical units put on column.

from *B. cereus*. The unknown substance was eluted in fraction VII close to CMP-5' when using 0.5 M formic acid in the reservoir. It was purified by successive paper chromatography and paper electrophoresis.

The substance has been found in *E.coli*, *B. cereus* spores and vegetative cells and in *Candida tropicalis* var. *lambica*. It was present in acid-soluble extracts from cells disrupted in the Hughes bacterial press and was also released by boiling the cells in distilled water for 10 min. The substance could be adsorbed on activated charcoal and again eluted at 0°C with 50 % ethanol containing 2 % conc. ammonia. The amount varied from 0.3—0.6 % of the total U.V. absorption at 260 mμ fo the crude extracts.

The most conspicuous feature of the ultraviolet absorption spectrum of the highly purified material (Figs. 2 and 3) is the ratio 280/260 in 0.1 N HCl. This is on an average 0.1 and is therefore lower than that of known nucleotides. The ratio 250/260 is usually 0.60. While the point of maximum absorption has constantly been found at 261—262 mμ, the inflection points at about 258 mμ and 268 mμ (Fig. 2) have been more or less pronounced and may be almost missing as shown in Fig. 3. Figs. 2 and 3 represent the extremes of the variation in the absorption spectrum and the variation is not caused by U.V. absorbing impurities since it can be experienced in eluates from two identical chromatograms.

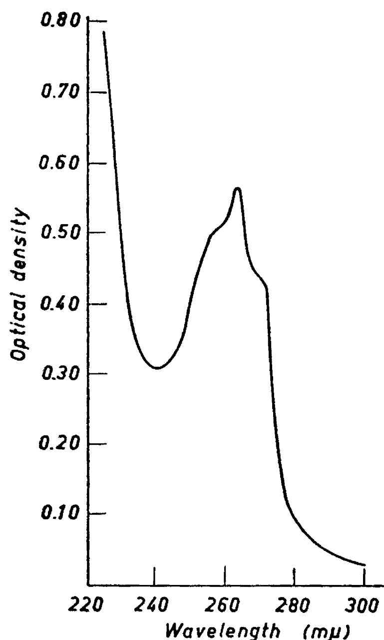
No significant change in the U.V. absorption was found after hydrolysis with formic acid at 175°C for 2 h or with 6 N HCl at 120°C for 18 h.

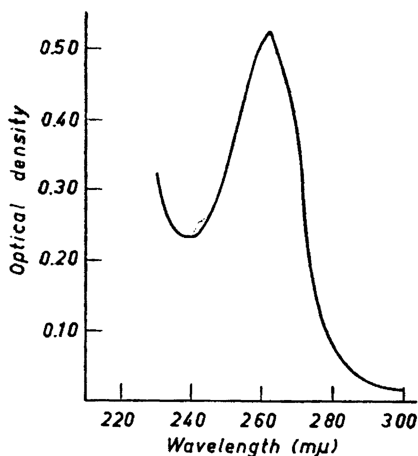
In paper electrophoresis the unidentified substance moved towards the anode at pH values above pH 4.0. In acetate buffer at pH 5.9 it had approximately the same mobility as orotic acid. In phosphate buffer of pH 7.4 the mobility was about 20 % greater than that of AMP-5'.

The R_F values in paper chromatography was greater than those of the usual nucleotides. For instance in *iso*butyric acid-N-ammonia-EDTA (100:60:1.6) it had R_F 0.78 whereas the R_F for AMP-5' is 0.52. The mobility was unchanged after hydrolysis. The U.V. absorption of the substance and its hydrolysate could not be reduced by treatment with sodium amalgam [4].

No ribose was found by the orcinol test using 20 min of heating at 100°C. Tests on phosphorus on material hydrolyzed with 6 N HCl were negative.

The purified U.V. absorbing substance gave a negative ninhydrine test while





Figs. 2 and 3. Ultra violet absorption spectra in 0.1 N HCl of eluates of spots of the unknown substance from two identical chromatograms developed in ethanol-conc. ammonia-water (80:5:15)

several ninhydrine positive spots split off with varying rates when chromatographed. This seemed to occur particularly when using *n*-butanol-acetic acid-water (40:10:50) as a solvent. After hydrolysis for 18 h at 120°C in 6 N HCl, two dimensional chromatography in *n*-butanol-acetic acid-water (40:10:50) followed by phenol-*m*-cresol-borate buffer pH 8.3 [5] showed ninhydrine positive spots corresponding to the position of serine, glutamic acid, aspartic acid, glycine and alanine together with an unidentified spot.

The stability of the substance to hydrolysis makes the possibility of it being a purine unlikely and the failure of reducing it with sodium amalgam seems to exclude the possibility of a pyrimidine.

The unidentified substance is interesting for several reasons. So far it seems to be distributed widely in the microbial world. The fact that it seems to be a constituent of a bacterial spore may suggest an important function in essential processes of cell development.

The liberation of amino acids by paper chromatography in an acidic solvent suggests a very labile form of amino acid linkages not commonly found in peptides.

Large scale preparation of this substance, together with structural studies and its possible participation in the biosynthesis of proteins or cell wall substances are being carried out.

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