1. Smith, L. and Baltscheffsky, M. *Federation Proc.* **15** (1956) 357.
2. Geller, D.M. *VIIth International Congress for Microbiology, Abstracts*, Stockholm 1958, p. 73.
3. Baltscheffsky, H. *Biochim. et Biophys. Acta.* **40** (1960) 1.
4. Baltscheffsky, H. and Baltscheffsky, M. *To be published.*

Diphosphothiamine Disulfide in Baker's Yeast

Heikki Suomalainen, Sakari Rihtniemi and Erkki Oura

Research Laboratories of the State Alcohol Monopoly, Helsinki, Finland

About 15 years ago Myrbäck and coworkers [1] indicated that baker's yeast, produced in vigorously aerated conditions, contains nearly all its thiamine as diphosphothiamine disulfide. His findings were met with criticism and his viewpoint was not generally accepted, also owing to the fact that the physiological function of cocarboxylase disulfide has not been conclusively verified.

We have now, in contrary to our earlier attempts [2] been able to confirm the appearance of diphosphothiamine disulfide in aerobically cultivated baker's yeast. We first repeated the experiments of Myrbäck and found a thiamine derivative in baker's yeast, which after cystein reduction acted like cocarboxylase in the thiochrome reaction. With ionexchange chromatography, using Dowex 1-X resin in formiate form, a thiamine derivative was separated from the perchloric acid extract of the commercial stage of baker's yeast, which was eluted by gradient eluation with formic acid or with ammonium formiate considerably later than thiamine, thiamine disulfide or thiamine monophosphate and cocarboxylase, the speed corresponding to that of synthetic diphosphothiamine disulfide (Merck). When oxidized after cystein reduction it gives a thiochrome reaction corresponding to that of cocarboxylase.

1. Myrbäck, K., Wallin, I. and Magnell, I. *Svensk Kem. Tidskr.* **57** (1945) 124.
2. Forsander, O. and Suomalainen, H. *Acta Chem. Scand.* **11** (1957) 1094.

Changes in the Microbial Amino Acid Metabolism Induced by 4-Deoxypyridoxine

J. Jännes and T. Lahikainen

Aurora Hospital, Helsinki, Finland

Normal strains of *E.coli* were grown in media containing 4-deoxypyridoxine in amounts of 10, 20, 30 and 40 mg per 10 ml of the culture media. Asparagine was the only source of nitrogen, and the incubation time was 7 to 8 days at 37 C°. At the end of the incubation time the media were centrifuged. The clear supernatants were treated with Amberlite IR 120 resins in acid form and eluated with 1 N ammonium hydroxide. The analyses of the amino acids were performed with paper chromatography. The most characteristic finding was the disappearance of the spots of both glutamic and γ-amino-butyric acids when 4-deoxypyridoxine was present in the culture media. The effect of 4-deoxypyridoxine on the growth was also noticed.

Two Unusual U.V. Absorbing Substances Associated with Amino Acids in Bacterial Extracts

Jon Jonsen, S. Laland and I. Smith-Kielland

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

During an investigation of the nucleotides in various extracts from different bacteria we have detected unusual ultraviolet absorbing acidic substances associated with amino acids.

Crude acid-soluble extracts were made by cold perchloric acid treatment of bacteria disrupted in a bacterial press.

Crude water-soluble extracts were obtained by heating living cells in boiling water for 10 min.

The bacteria were harvested during rapid growth. In some cases the extracted material was adsorbed on Norite and again eluted with 50 % ethanol containing 2 % of conc. ammonia. The extracts were fractionated on a Dowex

Table 1.

	Spectra in acid max	min	250/260	280/260	Spectra in alkali max	min	250/260	280/260
Substance I	266	237	0.76	0.82	275	244	0.79	1.16
Substance II	261	237	0.63	0.13	261		0.76	0.17

1 × 8 column in the formate form by gradient elution with the formic acid-ammonium formate system of Hurlbert *et al.* A slight modification was introduced as the elution prodecure was started with very dilute formic acid (0.001 M) in the reservoir.

In addition to amino acid complexes containing adenine nucleotides, two other complexes with amino acids were isolated which had U.V. spectra different from any of the usual purines and pyrimidines. Both were eluted early in the fractionation procedure. One (substance I) appeared earlier than any of the known nucleotides, the other (substance II) together with CMP-5′ or in a fraction following immediately after CMP-5′.

In paper chromatography both substances moved as single spots in five different solvents. In most solvents the R_F values exceeded those of the known nucleotides. No change in the mobility of the U.V. absorbing substances was observed after hydrolysis for 18 h in N HCl at 120°C. The two substances were homogeneous and negatively charged at pH 7.0 as indicated by electrophoresis in phosphate buffer.

Whereas the unhydrolyzed material was ninhydrine negative, hydrolysis in 6 N HCl gave for both substances ninhydrine positive spots corresponding to the position of alanine, serine, aspartic acid, glutamic acid, glycine and lysine in two dimensional paper chromatography. Small amounts of these amino acids also split off when the unhydrolyzed material was subjected to paper chromatography. None of the substances contained phosphate. One of them (substance I) could be reduced with sodium amalgam and the reduced material gave positive test for ribose.

The two substances exhibited a slight difference in the electrophoretic mobility at acid pH values and pronounced differences in the spectra values as shown in Table 1.

We wish to thank *Norges Almenvitenskapelige Forskningsråd* for financial support.

1. Jonsen, J., Laland, S., Smith-Kielland, I. and Sömme, R. *Acta Chem. Scand.* **13** (1959) 838.

Biologically Active Fractions from *Haemophilus Pertussis*

H. Billaudelle, L. Edebo, E. Hammarsten, C.-G. Hedén, B. Malmgren and H. Palmstierna

Department of Bacteriology, Karolinska Institutet, Stockholm, Sweden

Cells from *H. pertussis*, cultivated in the 500 litres scale, were disintegrated in the frozen state in the Hughes press. The disintegrate was extracted by a series of aqueous salt solutions. The supernatant was highly neurotoxic and dermatonecrotic. The neurotoxin was purified by column electrophoresis on a stabilized medium (320 cm columns). Due to the presence of acid polyelectrolytes the conditions for a purification of the neurotoxin on cellulose ion exchangers are unfavourable. They were consequently removed by electrophoresis before chromatography. The neurotoxin occurs in the same p*K* region (around 7.4) as a blue coloured protein. There exist dermatonecrotic proteins in other p*K* regions. The neurotoxin is reasonably stable only in its isoelectric region.

The neurotoxin has been purified to a MLD per 10 g mouse of 10 μg. Further purification is tried.

The residue after extractions forms very stable suspensions after the salts have been removed. It can be spun down at 100 000 *g*. It is salted out by monovalents salts. It can be solubilised again by either removing the salts or by making it 2 M with regard to urea. The residue contains two lipid fractions, protein and (amino-)polysaccharide. It is nontoxic up to at least 2 mg per 10 g mouse, and protects mice against 1 000 MLD of *H. pertussis* down to at least 50 μg per 10 g mouse. Electron microscopy shows that it consists primarily of bacterial cell walls. A *Haemophilus pertussis* immunogen has thus been found that shows no toxic activity on mice even in high dosages.

The work has been performed under a grant from the *U.S. Army European Research Office.*