KONINKL. NEDERL. AKADEMIE VAN WETENSCHAPPEN – AMSTERDAM
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POSSIBLE INTERMEDIATES IN THE BIOSYNTHESIS OF PROTEINS: EVIDENCE FOR THE PRESENCE OF DIALYSABLE CARBOXYL ACTIVATED PEPTIDE NUCLEOTIDE COMPOUNDS IN EXTRACTS OF BAKER'S YEAST.

A PRELIMINARY NOTE [1])

BY

V. V. KONINGSBERGER, CHR. O. VAN DER GRINTEN AND J. TH. G. OVERBEEK

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During the course of a study on the enzymatic carboxyl group activation of tyrosine [1] it was found that the dialysis liquid of crude extracts of ether-CO_2 frozen baker's yeast yields a fairly high ferric hydroxamate colour after a short incubation with saltfree hydroxylamine followed by the addition of an acidified $FeCl_3$ solution. As this finding looked rather interesting, a concentrated solution was prepared by freeze-drying the dialysis liquid of saltfree extracts of ether-CO_2 frozen freshly obtained pressed baker's yeast. The experiments performed with this solution and the preliminary results obtained can be summarized as follows:

Paperchromatography: An almost instantaneous ferric hydroxamate colour was obtained upon incubation of the concentrated dialysis solution with saltfree neutralized hydroxylamine for 2–10 minutes at room temperature. However, the intensity of this colour would increase considerably when the reaction was allowed to take place for a few minutes at 100° C.

Consequently, a reaction mixture containing the concentrated dialysis solution and saltfree hydroxylamine (pH 7) was kept at 100° C for 5 minutes, freeze-dried and extracted with a 60 % ethanol–40 % H_2O mixture. These extracts were subjected to paperchromatography on Whatman Nr 1 filter paper with *n*-butanol-acetic acid-water as the solvent; after development with $FeCl_3$ solution usually 5–7 distinct ferric hydroxamate spots were obtained. Moreover, before adding $FeCl_3$ three of these spots could be detected rather easily by their bright blue and violet fluorescence in the U.V. The hydroxamic acid containing spots were

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eluted, hydrolyzed for 4–6 hours with 6 N HCl, freeze-dried and run in separate paperchromatograms with the same (But OH–HAc–H_2O) solvent. The chromatograms showed 5–10 ninhydrine positive spots, indicating that the isolated hydroxamates were peptide – rather than amino acyl hydroxamates. No fluorescence was observed in chromatograms of the hydrolyzed compounds.

Paper-electrophoresis: 50 % ethanol–50 % H_2O extracts of the freeze-dried dialysis solution were subjected to paper-electrophoresis on Whatman Nr 3 filterpaper in 0.02 m citrate buffer solution (pH 3.9). The starting line was taken in the middle of the paper. After 16 hours of electrophoresis at 10 Volt/cm, most of the nucleotide material had migrated into the direction of the anode, as was determined by extinction measurements in the U.V. However, a very high and distinct increase in the 260 mμ absorption was found at the other side of the starting line, right at the spot that showed the ferric hydroxamate colour after spraying with saltfree hydroxylamine and ferric chloride solution. This phenomenon was not observed in a similar experiment with an extract that had been prae-incubated with hydroxylamine; a distinct increase in the 260 mμ absorption was found on the starting line or a little into the direction of the anode. Moreover, the typical U.V. absorption spectrum of nucleotides and nucleic acids was found in the eluate of the same spot, indicating that the hydroxamate forming compound may contain nucleotide material.

Finally, the blue and violet fluorescence was also observed at this spot, indicating that the hydroxamic acid forming peptides are present as such before the incubation with hydroxylamine.

These experiments suggest strongly that the concentrated dialysis liquid contains peptides that are bound to nucleotides by a high energy bond involving the carboxyl-group.

Any further conclusion from the preliminary results described above should be drawn very cautiously. The peptide material may have been formed by a condensation reaction between individual nucleotide bound activated amino acids during the isolation procedure.

Moreover, as the hydroxamate reaction is not a very sensitive one, it is quite possible that apart from the 5–7 peptide-hydroxamates found by paperchromatography, more compounds of the same nature are present in the dialysis liquid.

Our findings seem to be in good agreement with those published quite recently by HOAGLAND c.s. [2]. These authors found that in rat liver extracts "a low molecular weight ribonucleic acid" would function as an acceptor for enzymatically activated amino acids, forming an intermediate (ferric hydroxamate yielding) complex. Their finding and the preliminary results described above may indicate the actual occurrence of carboxyl-group activated amino acid nucleic acid compounds of the

type assumed about four years ago [3] in a hypothesis concerning the biosynthesis of proteins.

Van 't Hoff Laboratory,
University of Utrecht

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