KONINKL. NEDERL. AKADEMIE VAN WETENSCHAPPEN – AMSTERDAM
Reprinted from Proceedings, Series B, 63, No. 3, 1960

BIOCHEMISTRY

POSSIBLE INTERMEDIATES IN THE BIOSYNTHESIS OF PROTEINS: THE OCCURRENCE OF NUCLEOTIDE-BOUND CARBOXYL ACTIVATED PEPTIDES IN PREPARATIONS OF RIBONUCLEIC ACIDS FROM SOLUBLE AND PARTICULATE FRACTIONS OF YEAST CELLS

A PRELIMINARY NOTE [1] [2]

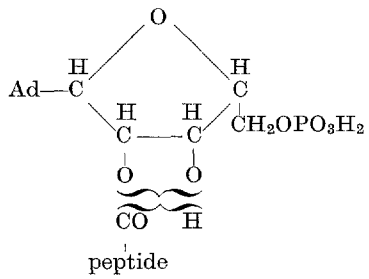
BY

S. R. DE KLOET, A. H. W. M. SCHUURS,
V. V. KONINGSBERGER AND J. TH. G. OVERBEEK

(Communicated at the meeting of March 26, 1960)

1. *Introduction*

The occurrence of dialysable carboxyl-activated peptide-nucleotide compounds in extracts of ether-CO_2 frozen baker's yeast was reported by a group of investigators from this laboratory about three years ago (1). During a study on the occurrence and the composition of these activated compounds, it was found (2–6) that they can be isolated from both the 100.000 g supernatant- and the microsomal RNP particulate fractions of yeast cells. Their probable chemical structure can be indicated as follows (4):



The isolation of similar peptide-nucleotide compounds from various tissues and microorganisms was described by a number of other authors (6–16, 19).

Regarding the reported rather general occurrence of peptide-nucleotide compounds in cells synthesizing proteins, it was considered of interest to

[1] This work was supported by the Netherlands Organization for Pure Research (Z.W.O.).

[2] The following abbreviations will be used: RNA, ribonucleic acid; RNP, ribonucleoprotein; Ad, adenine; AMP, adenosine monophosphate; ATP, adenosine triphosphate; PP, inorganic pyrophosphate.

obtain more experimental data on the origin of these compounds in the cell. In this paper, preliminary data are presented indicating the occurrence of carboxyl-activated nucleotide-bound peptides in preparations of ribonucleic acids (RNA) from both the 100.000 g supernatant (s-RNA)- and the microsomal RNP-particulate (m-RNA) fractions from cells of baker's yeast. Attention should be given to the fact, that the activated peptides occurring in preparations of RNA are not dialysable, in contradistinction to the activated peptides in yeast dialysate (1–6). This may be seen as an indication that they are bound to more than one nucleotide.

2. *Material and methods of preparation*

In most experiments, freshly obtained commercial baker's yeast ("koningsgist", Gist- en Spiritusfabriek, Delft) was used. The cells were broken up by grinding with carborundum; they were extracted with a 0.005 molar phosphate buffer pH 6.8 containing 3 μ mol Mg^{++} per ml. Whole cells, cellwalls and larger particles were spun off at 12.000 g in a refrigerated centrifuge: 100.000 g supernatant-and microsomal RNP-particulate fractions were obtained from the remaining extracts according to the method of FU-CHUAN CHAO and SCHACHMAN [16]. RNA was isolated from these fractions by the phenol extraction method as described by KIRBY [17]. As it has been stated [18] that the active RNA from yeast 100.000 g supernatant does not precipitate at pH 5, the extraction was performed without any preceding precipitations. The s- and m-RNA preparations were dialysed against water for 24 hours in the cold and concentrated by freeze-drying.

An instantaneous and very distinct ferric hydroxamate colour was observed when s-RNA preparations were incubated for a few minutes or less with 2 m salt-free hydroxylamine at 30° C, followed by the addition of an acidified (0.67 N HCl, 5 % TCA) $FeCl_3$ solution. With m–RNA preparations, the same procedure would yield hardly any distinguishable colour. Better results were obtained when the freeze-dried reaction mixture of m–RNA and hydroxylamine was subjected to paperelectrophoresis, the hydroxamate colour being developed by spraying the paper with the $FeCl_3$ solution.

The applied analytical reagents and procedures were essentially the same as have been described before (2, 4, 5); s–RNA and m–RNA preparations and the products of their reaction with salt-free hydroxylamine were subjected to paperelectrophoresis and paperchromatography.

3. *Paperelectrophoresis*

Preparations of s–RNA and of m–RNA were subjected to paperelectrophoresis on Whatman No. 3 paper in 0.02 m citrate buffer pH 3 and pH 4.1 and in 0.02 m phosphate buffer pH 6.2. The results of some representative experiments are shown in figs. 1 and 2. Measurements of the 260 mμ extinction indicated the presence of at least three nucleotide components in the s–RNA preparations. A distinct peak was found in the 260 mμ absorption in the direction of the cathode, at the spot where ninhydrin positive carboxyl activated compounds could be located by

S. R. DE KLOET, A. H. W. M. SCHUURS, V. V. KONINGSBERGER AND J. TH. G. OVERBEEK: *Possible intermediates in the biosynthesis of proteins: the occurrence of nucleotide-bound carboxyl activated peptides in preparations of ribonucleic acids from soluble and particulate fractions of yeast cells.*

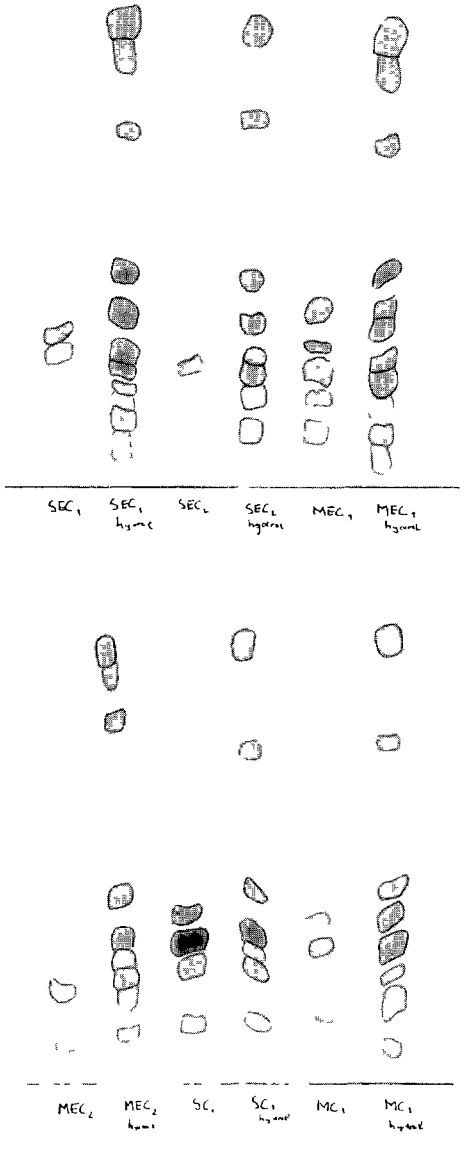


Fig. 3. Ninhydrin-sprayed chromatograms of hydrolysed and un-hydrolysed hydroxamic acids prepared from s-RNA and m-RNA preparations: see text and table I.

spraying with 2 m salt-free hydroxylamine, heating to dryness (80° C) and subsequent spraying with $FeCl_3$ solution. Furthermore, a striking similarity was noticed between our present results with undialysable s–RNA and those with the carboxyl activated nucleotide-bound peptides from freeze-dried yeast dialysate (2–6), the main difference being the larger amount of nucleotide material in s–RNA.

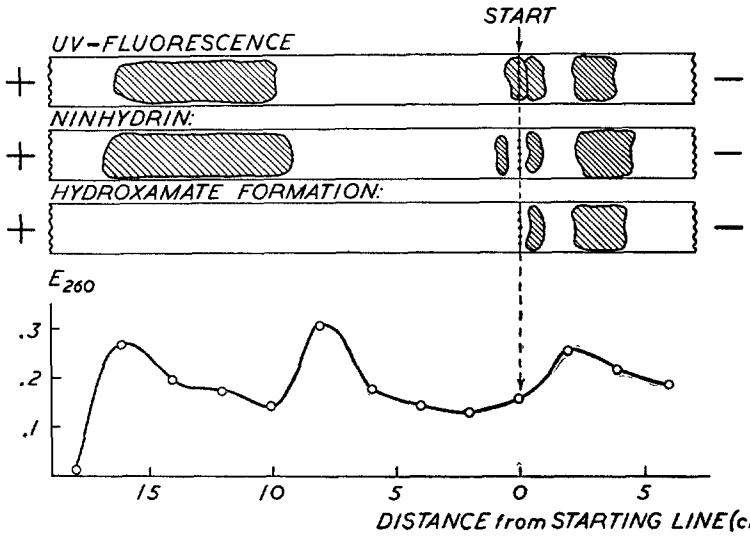


Fig. 1. Schematic outline of a paperelectrophoresis experiment with s-RNA, showing the spots with blue and violet fluorescence in U.V. light, a positive ninhydrin reaction, hydroxamate forming material and the course of the absorption at 260 mμ. Electrophoresis at 8 V cm^{-1} for 17 hours in 0.02 m phosphate buffer pH 6.2.

Hardly any fluorescence and no distinct hydroxamate colour could be observed when m–RNA preparations were subjected to paperelectrophoresis (fig. 2).

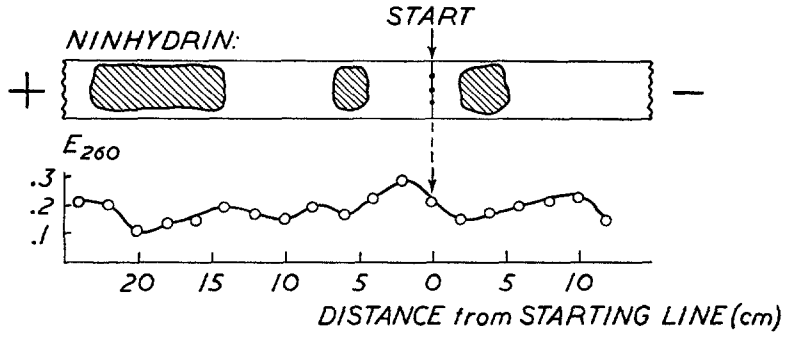


Fig. 2. Outline of a paperelectrophoresis experiment with m-RNA, showing ninhydrin positive spots and the course of the absorption at 260 mμ. Hardly any fluorescence was observed and the carboxyl-activated compounds could not be located. Electrophoresis at 8 V cm^{-1} for 17 hours in 0.02 m phosphate buffer pH 6.2.

At least four peaks in the 260 mμ extinction indicated that the m–RNA preparation was also rather heterogeneous.

However, a distinct ferric hydroxamate colour and some blue-violet fluorescence was observed when the reaction mixture of m–RNA and 2 m salt-free hydroxylamine was subjected to electrophoresis after being kept at 30° C for 5 minutes and subsequent freeze-drying.

Hydroxamic acids from s–RNA and m–RNA preparations moved with the same velocity into the direction of the cathode and showed a similar blue-violet fluorescence in U.V. light. They were eluated and chromatographed in order to study their composition.

4. *Paperchromatography*

The following mixtures and compounds were subjected to paperchromatography on Whatman No. 1 filterpaper with n–butanol–acetic acid-water (9:1:1) as the solvent.

1. Preparations of s–RNA and of m–RNA were incubated for 5 minutes at 30° C with 2 m salt-free hydroxylamine, freeze-dried and extracted with a mixture of 50 % ethanol-50 % H_2O. Paperchromatograms of these extracts showed 3 (m–RNA) – 5 (s–RNA) spots with blue and violet fluorescence in U.V. light. Both preparations yielded only one spot showing a distinct ferric hydroxamate colour: this spot was eluated, one half being kept as a blank, the other half being hydrolysed with 6 N HCl. The hydrolysates and their blanks were run in separate chromatograms.
2. Hydroxamic acids from s–RNA and from m–RNA were subjected to paperelectrophoresis in 0.02 m citrate buffer pH 3. The zones containing the hydroxamic acids were eluated and chromatographed after freeze-drying. With both preparations, two spots showing a distinct ferric hydroxamate colour were obtained. These spots were eluated, treated as has been described above and re-chromatographed.

The results of our experiments are summarized in table I and illustrated in fig. 3.

TABLE 1

R_F values of hydroxamic acids from s-RNA and m-RNA in *n*-butanol-acetic acid-H_2O (9:1:1)

Spot	Hydroxamic acid from	Purified by	R_F
SC_1	s-RNA	single chromatography of 50 % EtOH-50 % H_2O extract	.23
MC_1	m-RNA	same as SC_1	.24
SEC_1	s-RNA	paperelectrophoresis at pH 3, followed by chromatography	.23
MEC_1	m-RNA	same as SEC_1	.24
SEC_2	s-RNA	same as SEC_1	.54
MEC_2	m-RNA	same as SEC_1	.55

The results with hydroxamic acids showing a low R_F are not conclusive for the presence of activated peptides (see fig. 3); both amino acyl- and peptide hydroxamates may be involved. The results obtained with the hydroxamic acids having a rather high R_F (SEC_2 and MEC_2) strongly suggest the presence of carboxyl-activated peptides in our preparations of both s- and m-RNA.

The sedimentation of both s- and m-RNA preparations has been studied in a SPINCO analytical ultracentrifuge. At least three peaks were obtained with our s-RNA preparations, showing sedimentation constants of 5, 2 and <1 Svedberg units. The m-RNA preparation showed four peaks with sedimentation constants of 5, 14, 20 and 30 Svedberg units. It is rather striking to notice the qualitative similarity of the hydroxamic acids that were isolated from these apparently different RNA preparations.

Our present knowledge about the biosynthesis of cytoplasmic proteins may be briefly outlined as follows:

1. Individual amino acids are activated (e.g. 20) according to:
   $R\text{–}HCNH_2\text{–}COOH + ATP + E(nzyme) \leftrightarrows E\text{–}AMP\text{–}CO\text{–}HCNH_2R + PP.$
2. Enzyme-bound carboxyl-activated amino acids are transferred to ribonucleic acids from the soluble fraction of the cell (e.g. 21, 22):
   $E\text{–}AMP\text{–}CO\text{–}HCNH_2\text{–}R + s\text{–}RNA \leftrightarrows s\text{–}RNA\text{–}CO\text{–}HCNH_2R + E + AMP$
3. The final condensation of carboxyl-activated protein precursors to proteins occurs in microsomal and other particulate fractions.

With regard to this knowledge, attention may be drawn to:

1. The probable structure of the carboxyl-activated peptides, which seem to be bound to one or more nucleotides.
2. The reported general occurrence of peptide-nucleotide compounds in cells synthesizing proteins.
3. The presence of carboxyl-activated peptide-nucleotide compounds in preparations of both s- and m-RNA, as well as in isolated microsomal RNP particles.

In our opinion, this experimental evidence suggests the occurrence of a "pre-particulate" condensation of s-RNA bound carboxyl-activated amino acids to specific nucleotide-bound activated peptides.

5. *Summary*

Ribonucleic acids have been isolated from 100.000 g supernatant- and microsomal ribonucleoprotein particulate fractions of baker's yeast. Experimental evidence is presented, indicating the presence of carboxyl-activated peptide compounds in both ribonucleic acid preparations. The possible bearing of these activated compounds on the biosynthesis of proteins is briefly discussed.

REFERENCES

1. KONINGSBERGER, V. V., CHR. O. VAN DER GRINTEN and J. TH. G. OVERBEEK, Proc. Kon. Ned. Akad. Wetensch. **B 60**, 144 (1957).
2. ———, ——— and ———, Biochim. Biophys. Acta **26**, 483 (1957).
3. GRINTEN, CHR. O. VAN DER, A. H. W. M. SCHUURS and V. V. KONINGSBERGER, IVth Int. Congr. Biochem., Abstracts **30** (1958).
4. ———, Thesis, Utrecht (1959).
5. KONINGSBERGER, V. V., 10 Coll. d. Gesellschaft f. physiol. Chemie, Mosbach, 50 (Springer Verlag, Berlin, 1959).
6. WEIL, J. H., G. DIRHEIMER and J. P. EBEL, IVth Int. Congr. Biochem., Abstracts **21** (1958).
7. BROWN, A. D., Biochim. Biophys. Acta **30**, 447 (1958).
8. HARRIS, G., J. W. DAVIES and R. PARSONS, Nature **182**, 1565 (1958).
9. GILBERT, D. A. and E. W. YEMM, Nature **182**, 1745 (1958).
10. BERGKVIST, R., Acta Chem. Scand. **12**, 364 (1958).
11. EBEL, J. P., 10 Coll. d. Gesellschaft f. physiol. Chemie, Mosbach, 70 (Springer Verlag, Berlin, 1959).
12. MANDEL, P., ibidem 71 (1959).
13. HARRIS, G., Nature **184**, 788 (1959).
14. HASE, E. and S. MIHARA, Arch. Biochem. Biophys. **83**, 170 (1959).
15. ——— and ———, Biochim. Biophys. Acta **32**, 298 (1959).
16. FU-CHUAN CHAO and K. H. SCHACHMAN, Arch. Biochem. Biophys. **61**, 220 (1956).
17. KIRBY, K. S., Biochem. J. **64**, 405 (1956).
18. OSAWA, S. and E. OTAKA, Biochim. Biophys. Acta **36**, 549 (1959).
19. HABERMANN, V., Biochim. Biophys. Acta **32**, 297 (1959).
20. DAVIE, E. W., V. V. KONINGSBERGER and F. LIPMANN, Arch. Biochem. Biophys. **65**, 21 (1956).
21. HOAGLAND, M. B., P. C. ZAMECNIK and M. L. STEPHENSON, Biochim. Biophys. Acta **24**, 215 (1957).
22. ———, ——— and ———, Symp. Mol. Biol. at Chicago, 104 (1959).