NUCLEOSIDE TRIPHOSPHATE DEPENDENT PEPTIDE ACTIVATION BY A CRUDE SOLUBLE PROTEIN FRACTION OF BAKER'S YEAST: A NEW TYPE OF ACTIVATING MECHANISM

A.H.W.M. Schuurs, S.R. de Kloet and V.V. Koningsberger

van 't Hoff Laboratory, State University of Utrecht
Utrecht, The Netherlands

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The occurrence of carboxyl activated peptides in extracts of baker's yeast was reported by a group of workers from this laboratory about three years ago (Koningsberger et al., 1957 a, b). Adenosine-5'-monophosphate (v.d.Grinten, 1959; Koningsberger, 1959) and cytidine-5'-monophosphate (Schuurs et al., 1960) bound carboxyl activated peptides have been isolated and identified, as well as phosphate bound peptides without nucleotide material (de Kloet et al., 1960 b). Furthermore, it was shown that ribonucleic acid preparations from both soluble (s-RNA) and particulate ribonucleoprotein (m-RNA) fractions of yeast contain carboxyl activated peptides (de Kloet et al., 1960 a). Moreover, a number of similar nucleotide bound peptides has been isolated from various tissues and microorganisms synthesizing protein (e.g. Dirheimer et al., 1958; Harris et al., 1959; Hase et al., 1959).

During a trial to study peptide activation (Tuboi et al., 1960) by a crude fraction of yeast soluble (100.000 g supernatant) protein a new type of energy-dependent hydroxamate formation was found to occur. In this paper, preliminary data

are presented on the mechanism involved in this activating reaction.

Materials and methods

The yeast was a freshly obtained commercial baker's yeast (''koningsgist'', Koninklijke Gist- en Spiritusfabriek at Delft). The crude protein fraction used in our experiments was prepared by repeated precipitation of the protein from a 100.000 g yeast supernatant extract prepared according to Chao et al (1956). The precipitate collected between 0.2 and 0.7 ammonium sulphate saturation was redissolved in water and dialysed overnight in the cold against a 0.002 M buffer pH 7, containing 2 μmol Mg^{++} per ml. Before use, the dialysed protein solution was treated with Norit (0.2 g/ml) in order to remove contaminating nucleotide material.

The methods used to locate and/or determine hydroxamic acids have been described previously (Koningsberger et al., 1957 b). Inorganic phosphate was determined according to Fiske and Subbarow (1925) with the modification of Sumner (1944). Nucleoside triphosphates were products from Pabst. For paperchromatography and paperelectrophoresis experiments Whatman no. 1 filterpaper was used.

Experimental results

a. Hydroxamate formation. A remarkable feature of the hydroxamate formation catalysed by the crude yeast protein fraction is that the amount formed is proportional to time for at least 90 minutes without the addition of any substrate but ATP, Mg^{++} and NH_2OH (Fig. 1).

Furthermore, hydroxamate formation requires the presence of either ATP, UTP, CTP or GTP (Table I).

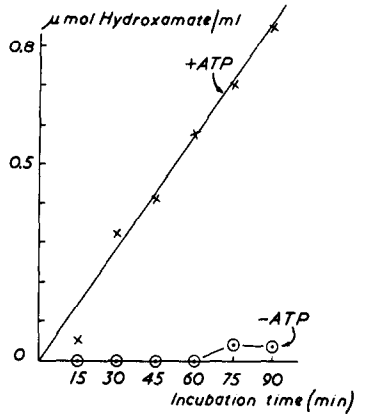

Fig. 1. Hydroxamate formation at 25° C and pH 7 by a crude yeast protein fraction. The reaction mixture contained 15 mg protein, 10 μmol ATP, 10 μmol Mg^{++}, 100 μmol TRIS buffer pH 7 and 1 mmol NH_2OH per ml. Tyrosine hydroxamate was used as a standard for the determination of hydroxamate (v.d.Ven, 1958).

Table I

nucleoside triphosphate	μmol Hx/ml/2 hrs
no	-
ATP	2.0
UTP	1.3
CTP	0.7
GTP	0.6

Hydroxamate (Hx) formation in the presence of equal (10 μmol/ml) concentrations of different nucleoside triphosphates. Experimental conditions were the same as described in Fig. 1.

b. Stoichiometry of the hydroxamate formation. Analytical data obtained so far indicate that one μmol of inorganic phosphate (P_i) is released per μmol hydroxamic acid (Hx) formed (Table II).

Table II

time of incubation (min.)	μmol P_i/ml	μmol Hx/ml
30	1.5	1.3
60	2.4	2.3
90	3.3	3.3

Release at 25° C of inorganic phosphate (P_i) and hydroxamate (Hx) formation by a mixture containing 20 mg protein, 10 μmol ATP, 10 μmol $MgCl_2$, 10 μmol NaF and 1 mmol NH_2OH. As a blank for P_i formation the same mixture was used with the exception of NH_2OH.

This finding seems to be confirmed by the fact that the same protein fraction catalyses the exchange of P^{32} labelled inorganic phosphate with ATP, UTP, CTP and GTP:

this could be shown by radioautography of a paperelectropherogram of the nucleotides isolated from a reaction mixture containing 32 mg protein, 7.5 μmol $MgCl_2$, 10 μmol NaF, 30 μmol (20 μC) $Na_2HP^{32}O_4$ and 3 μmol nucleoside triphosphate, which was incubated in a total volume of 1.3 ml for 45 min. at 25° C (Fig. 2).

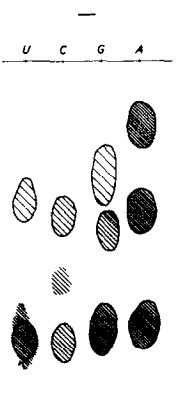

Fig. 2 Radioautogram showing the incorporation of P^{32} labelled inorganic phosphate in ATP, UTP, CTP and GTP. Experimental conditions are mentioned in the text. The circled areas represent U.V. quenching, the shaded areas radioactivity. Electrophoresis at 5 $V.cm^{-1}$ for 16 hrs in 0.02 M citrate buffer, pH 3.9.

It may be assumed that small amounts of contaminating nucleoside di- and monophosphates were labelled with P^{32} by the action of nucleoside phosphokinases, which are known to occur in yeast (Dixon and Webb, 1958).

c. Identification of the products of the activation reaction. The hydroxamates formed in the reaction with ATP tend to stick together; we could separate them quite well by repeated chromatography with n-butanol-acetic acid-water (4 : 1 : 5) as the solvent. After 2 chromatographic separations, 9 distinct spots were obtained, showing a ferric hydroxamate colour. So far, 4 of these spots have been eluted and hydrolysed with 6 N HCl, one half of each eluate being kept as a blank. From re-chromatography of the blanks and the HCl-hydrolysed hydroxamates and spraying with ninhydrin, it appeared that the products formed in the reaction with ATP as an energy source were peptide hydroxamates, each peptide containing 7-9 different amino acids. This finding suggests that the activating

reaction proceeds with the products of some proteolytic process, as no peptide or amino acid substrate was added to the dialysed protein.

Discussion

Recently, nucleotide-bound peptides have been shown to occur as a product of an energy-dependent breakdown of serum albumin by liver- and brain mitochondria. As a consequence of this finding and with regard to the rather general occurrence of peptide nucleotide compounds, Penn (1960) has suggested a coupling between certain energy-dependent breakdown and biosynthetic processes.

It is not yet possible to draw any conclusion about the mechanism of the peptide activating reaction described in this paper or about its possible role in the process of protein biosynthesis. But it is hardly speculative to conclude that this reaction is not the same as that described for amino acid activation (Hoagland, 1955) and that its occurrence indicates an energy-dependent coupling between some protein breakdown and -synthetic pathways as suggested by Penn (1960).

There may also be a relation with the finding of Beljanski (1960 a, b) who synthesized peptides from amino acids with the aid of the amino acid incorporation enzyme in the presence of nucleoside triphosphates.

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