

hemidiaphragm with, however, two notable exceptions. WOOL *et al.*⁷ have reported an effect of insulin upon histidine incorporation into protein in hemidiaphragm from fasted (not from fed) rats, in the total absence of glucose, while MANCHESTER⁸ observed this effect in tissues from both fasted and fed animals. Also, cortisol added *in vitro* was without effect upon glycine incorporation into diaphragm protein⁹. In rat epididymal adipose tissue insulin accelerates the incorporation of pyruvate into tissue protein even in the absence of glucose¹⁰, a discrepancy which might derive from better ability of this substrate to support oxidation and energy production than is the case for glycine itself. Indeed, KRAHL (personal communication) has observed that pyruvate, as well as glucose, restores the insulin response for glycine incorporation into protein by this tissue.

These studies clearly suggest that the metabolism of glycine in adipose tissue is both hormonally controlled and modified by the availability of metabolic substrates such as glucose.

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Isolation of cytidine 5'-monophosphate-bound carboxyl-activated peptides from *Saccharomyces cerevisiae*

After the first reports from this laboratory about the occurrence of nucleotide-bound carboxyl-activated peptides in yeast^{1,2} a number of these compounds have been isolated from different organisms, and been identified in some detail³⁻¹⁴. Some authors reported the occurrence of nucleotide-peptide compounds without giving sufficient details to conclude if those compounds were carboxyl activated¹⁵⁻¹⁷.

During further investigations with the aid of ion-exchange chromatography, a new carboxyl-activated nucleotide-peptide compound has been isolated from yeast.

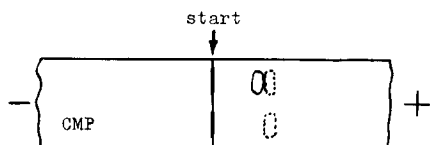
Abbreviations: CMP, cytidine monophosphate; AMP, adenosine monophosphate.

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Pressed baker's yeast was frozen in ether-CO₂, thawed, and dialysed at 3° against distilled water for about 40 h (2 replacements of the water). The pH of the dialysate was brought to about 7 by adding NH₄OH, and the solution was applied to a column of Dowex-2-Cl'-X4, 20-50 mesh, 20 cm × 4.5 cm². The elution was started with 0.003 *N* HCl after washing with water until the absorbancy at 260 mμ was low. The eluates were analysed by measuring the absorbancy at 260 and 280 mμ. The first component eluted was freeze-dried, and subjected twice to paper electrophoresis (0.05 *M* ammonium formate, pH 3.5, 25 V/cm, 16-20 h, 3°, Whatman No. 3 MM). A spot moving slowly in the direction of the cathode showed blue fluorescence in u.v. light (360 mμ) and a significant absorption at 260 mμ; it yielded a ferric hydroxamate colour after spraying with NH₂OH and FeCl₃ (see ref. 2). It was eluted, the eluate concentrated by freeze-drying, incubated with 2 *M* salt-free NH₂OH (5 min, 60°; see ref. 2) and freeze-dried. The reaction products were subjected to another fractionation by paper electrophoresis. The result of this purification is shown in Fig. 1.

A similar result was obtained after hydrolysis of the activated compound at pH 10.5 (NH₄OH, 10-15 min at 100°). After this treatment, however, spot No. 1 contained peptides instead of peptide hydroxamates.

The hydroxamates from spot No. 1 were eluted and separated by descending paper chromatography in *n*-butanol-acetic acid-water (9:1:1) at 21°. Five spots could be detected by spraying with FeCl₃, the approximate *R_F* values being 0.11, 0.16 (very weak), 0.37 (weak), 0.50 (strong), 0.60 (very weak). The spot with *R_F* 0.50 (ninhydrin-negative) was eluted, one half being kept as a blank, the other half being



Spot No. 1 (full lines): blue fluorescence in u.v.; hydroxamate colour after spraying with FeCl₃. Spot No. 2 (dotted lines): absorption in u.v.

Fig. 1. Paper electrophoresis of the reaction product of the purified carboxyl-activated compound with NH₂OH (0.02 *M* ammonium formate, pH 3.5, 15 V/cm, 20 h, Whatman No. 1).

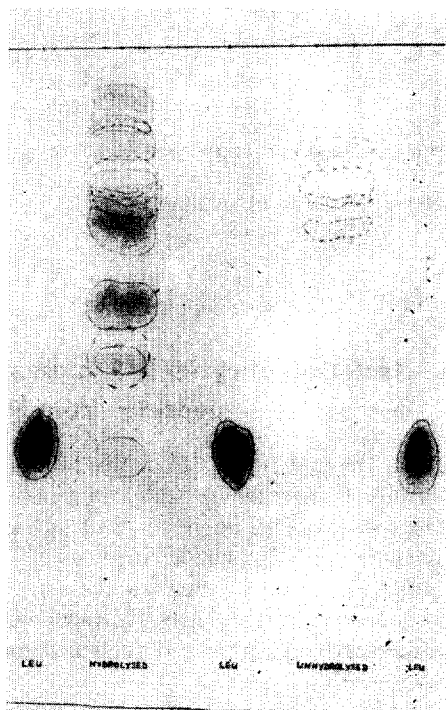


Fig. 2. Ninhydrin-sprayed chromatogram of hydrolysed and non-hydrolysed peptide hydroxamates. —, ninhydrin positive; ---, weak fluorescence in u.v. light.

hydrolysed with 6 N HCl. Hydrolysate and blank were rechromatographed in the same solvent system. The result is shown in Fig. 2.

Spot No. 2 was analysed by measuring the u.v.-absorption spectra at pH 2.5 and 7.0, by paper chromatography in the systems isobutyric acid-conc. NH_4OH - H_2O (66:1:33; see ref. 18) and ethanol-ammonium acetate pH 7.5, (75:30; see ref. 18), and by paper electrophoresis. It appeared to be cytidine 5'-monophosphate.

Using a comparable isolation procedure, but starting from extracts of *Polyporus squamosus*, BERGKVIST¹⁷ demonstrated the occurrence of a CMP-peptide, which, however, on hydrolysis would yield cytidine 2'- and 3'-monophosphates. He did not mention results of any test for hydroxamate formation, but compared his compound with those detected by POTTER AND DOUNCE¹⁹ in alkaline digests of ribonucleic acids.

A phosphoamide bond was proposed to account for the alkaline stability of the compounds. The cytidine-5'-monophosphate-bound peptide, however, is alkali-labile and hydroxamate positive.

The latter compound is a peptide bound through its carboxyl to CMP; the carboxyl is activated (e.g. it reacts easily with NH_2OH); the position of the carboxyl on the CMP moiety has not yet been established.

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