This hypothesis is now under test, using oxygen-18 as a tracer*.

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Intracellular peptides of *Pseudomonas hydrophila**

Although intracellular peptides have been isolated from a wide variety of cells and tissues[1], there is no assurance in many of these reports that the peptides were not formed by proteolysis. Numerous metabolically active peptides were obtained by TURBA AND ESSER[2] from extracts of *Torula utilis* grown on 1-^{14}C-acetate as the sole carbon source. The same authors were unable to isolate measurable quantities of peptides from extracts of *Leuconostoc mesenteroides*[3]. If peptides do represent intermediates in protein synthesis[4] they should be detectable in rapidly growing bacteria. The present communication reports the separation of intracellular peptides from log-phase cultures of *Pseudomonas hydrophila* grown on glucose as the sole carbon source.

A large number of such peptides has been obtained from these cultures. Alcoholic extracts of washed cells were concentrated, extracted with ether and dialyzed. After fractionation of the permeates by high voltage paper electrophoresis[5], five to seven ninhydrin-positive acidic bands appeared, one of which corresponded to the position of glutamic acid. A band remaining near the origin contained most of the neutral amino acids, and at least one neutral peptide. As many as seven ninhydrin-positive bands have appeared on the basic side, although their number and position have varied in different preparations. Photometric analyses[6] on eluates before and after acid hydrolysis indicated the presence of numerous basic peptides.

The peptides and amino acids on the acidic side were further separated by a combination of paper chromatography and repeated high-voltage paper electrophoresis. Three of the original ninhydrin-positive bands have so far been resolved into a total of eleven peptides, containing from five to eleven constituent amino acids. Whether these peptides have been separated as individual chemical species, or as groups of related compounds remains to be determined.

Evidence for the metabolic activity of the peptides was obtained from measurements of total and specific radioactivity. Dialyzates from ether-extracted alcoholic extracts of samples, taken at 1, 5, 12, 30, 60 and 120 min after the addition of uniformly-labelled ^{14}C-glucose to a log-phase culture, were radio-autographed after fractionation by high-voltage paper electrophoresis. All ninhydrin-positive bands were radioactive in less than 1 min, and no new bands appeared in the 2 h period. Elution of acidic, neutral and basic fractions as three separate groups was followed by estimation of the weight[7] and radioactivity[8,9] of carboxyl-C before and after acid hydrolysis. Residual cell protein had the highest total radioactivity even in the 1 min sample, with lower activity in the combined amino acid *plus* peptide fraction. Specific radioactivities plotted against time gave parallel hyperbolic curves, with peptides below the curve for amino acids, and with cell protein still lower and almost linear. This evidence suggests rapid synthesis of peptides from amino acids. Work is in progress on the structure and function of these compounds.

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Non- enzymic reactions of acyl adenylate and imidazole

Mixed anhydrides of adenylic and carboxylic acids (acyl AMP) have recently been shown to behave like intermediates in the enzymic activation of carboxylic acids, but have not been detected as reaction products in such systems[1,2]. During studies on a fatty acid activating enzyme[3] it was observed that these compounds transfer their acyl group non-enzymically to imidazole. The demonstration that acyl imidazole is easily formed from these anhydrides indicates that they are more "energy-rich" than had been anticipated, and provides a partial explanation for their failure to accumulate in enzymic reactions. Furthermore, imidazole at low concentrations acts as a catalyst for the transfer of acyl groups from acyl AMP to mercaptans, phosphate, arsenate and sugars, presumably via the highly reactive acyl imidazole[4].

Incubation of 90% pure acetyl AMP[5] with imidazole results in the formation of acetyl imidazole, identified by its difference spectrum (λ_{max} 247 mμ) and its rate of spontaneous hydrolysis[4], as well as in the formation of AMP (identified by paper electrophoresis) and acid (measured by titration with NaOH). An unexpected product of the reaction is ribose acetylated AMP. This compound was identified by its electrophoretic mobility on paper, which was identical to that of AMP in acetate and citrate buffers but slower than AMP in borate buffer, by its negative periodate reaction, and by its half-time for hydroxamic acid formation in 1.1 *M* hydroxylamine at 37° C of 5 min, compared to 0.6 min for acetyl AMP. In the absence of imidazole the disappearance of acetyl AMP and the formation of ribose acetylated AMP are negligible during short incubation periods.

Acetyl transfer from acetyl AMP to imidazole proceeds readily even in the presence of an approximately equimolar amount of added acetyl imidazole (Fig. 1). If NaOH is added during the reaction to maintain the pH above 7 the reaction proceeds at least 90% to completion. The reverse reaction occurs only to a very small extent at neutral pH. At pH 5.8–6.2, a 10–20% yield of acetyl AMP was obtained from acetyl imidazole, estimated by paper electrophoresis of the reaction products in pH 6.58 citrate buffer at 0° and elution of 260 mμ absorbing material. Although, owing to hydrolysis of acetyl imidazole and the formation of ribose acetylated AMP, an accurate equilibrium constant for the reaction could not be obtained, these results indicate that at some point between pH 6 and 7 the group potential of acetyl AMP is at least as great as that of acetyl imidazole. Since acetyl imidazole is some 5,000 calories more energy-rich than acetyl glutathione or acetyl coenzyme A (CoA)[4], which are, in turn, approximately equivalent to the pyrophosphate link of ATP[6,7], acetyl AMP must be roughly 5,000 calories above ATP. If allowance is made for the effect of pH on the equilibrium and on the concentration of reactants[8] this difference rises to 7–8,000 calories at a pH near 8. With such an energy barrier, any accumulation of acyl AMP in the enzymic reaction of ATP and carboxylic acid would be expected to be exceedingly small.

Acetyl AMP also reacts with glycine, leucine, cysteine, glycyl glycine, and, at a slower rate, ammonia and tris(hydroxymethyl)aminomethane. The products of these reactions were identified as N-acetyl compounds by hydroxamic acid formation at 100° C[9] and, in the case of acetyl glycine and leucine, by chromatography[9]. The disappearance of acetyl AMP in 0.02 *M* glycine in phosphate buffer, pH 6.8, at 37° C, for example, follows first order kinetics with a half-time of 43 min. At a pH below the pK_a of the amino group the rates of reaction of these compounds and of imidazole increase with pH, suggesting that the free bases are the reactive species.

Acyl AMP reacts relatively slowly with glutathione and CoA to form the corresponding thioesters, which can be identified by their difference spectra. The rate of acyl transfer from acyl AMP to the sulfhydryl group of CoA or glutathione is greatly increased by low concentrations of imidazole (Fig. 2). Imidazole also catalyzes an analogous acyl transfer from acetyl phosphate to CoA, and the phosphorolysis and arsenolysis of acetyl AMP, which occur slowly or not at all