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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

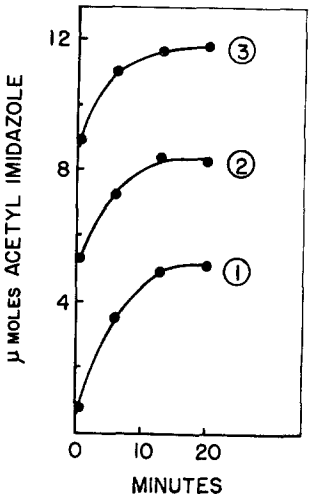

Fig. 1. Formation of acetyl imidazole from acetyl AMP and imidazole. The reaction mixture contained: 9.6 μmoles acetyl AMP, 20 μmoles imidazole, 1.1 μmoles AMP and added acetyl imidazole in tubes 2 and 3 as shown, in a volume of 1.0 ml. Incubated at 26° C. Acetyl imidazole formation measured at 240 mμ in 0.1 ml aliquots diluted to 5.1 ml against a blank containing no imidazole. Acetyl AMP disappearance at the end of the reaction was estimated at about 75% in each tube by paper electrophoresis and elution of the reaction products. Final pH 6.67 ± 0.02.

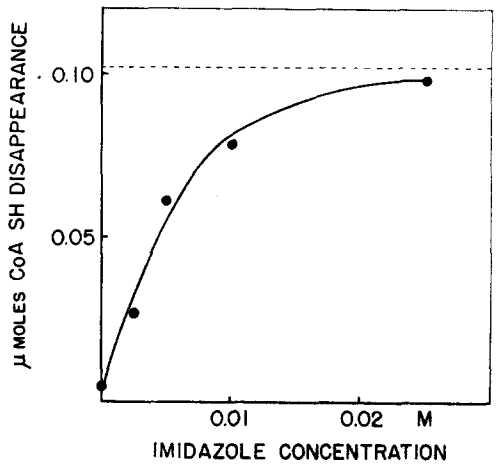

Fig. 2. The effect of imidazole on the acylation of CoA by acyl AMP. The reaction mixture contained: 1.0 μmole hexanoyl AMP[3], 0.1 μmole CoA, 2 μmoles $MgCl_2$, 1.0 μmole ethylenediaminetetraacetic acid, 20 μmoles tris(hydroxymethyl)aminomethane, pH 8.0, and imidazole as indicated in a volume of 0.2 ml. Incubated 15 min at 37° C. Acetylation of CoA measured as disappearance of CoA SH[7].

in the absence of imidazole. The catalysis of acyl transfer from acyl AMP to CoA may be compared to that carried out by the fatty acid activating enzymes and the corresponding reaction with acetyl phosphate and CoA parallels that catalyzed by transacetylase[10].

Several reports have appeared recently describing the imidazole catalyzed hydrolysis of *p*-nitrophenyl acetate, a reaction which appears to be similar to those described here, except that water acts as the acyl acceptor[11,12].

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