ON THE NATURE OF THE ENZYMATIC REACTION BETWEEN γ-SUBSTITUTED AMINO ACIDS AND MALEIMIDES

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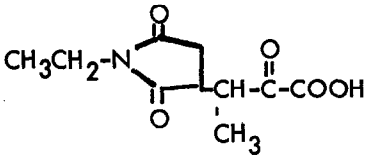
In pyridoxal-P potentiated enzymatic "γ-elimination" reactions (reaction 1), it was found that when N-ethylmaleimide (EM) was added to the reaction mixture

$$ACH_2CH_2CH(NH_2)COOH + H_2O \rightarrow AH + NH_3 + CH_3CH_2COCOOH \quad (1)$$

$$ACH_2CH_2CH(NH_2)COOH + H_2O + EM \rightarrow AH + NH_3 + KEDB \quad (2)$$

(reaction 2) a large part of the α-ketobutyrate was replaced by a new reaction product (Flavin and Slaughter, 1964a). Recently evidence has been presented that the latter is α-keto-3-(3'-(N'-ethyl-2',5'-dioxopyrrolidyl)) butyric acid, or KEDB (Flavin, 1965).



KEDB was presumed to arise through an attack on the EM double bond by an intermediate with carbanion character on the third carbon, and 2 schemes were proposed for such a reaction. The first (see structures V to VIII in Flavin, 1965) involved reaction of EM with an early enzyme-bound intermediate, prior to the separation of the terminal electronegative substituent "A". The second scheme (see footnote 2 in Flavin, 1965) involved reaction of EM with a late intermediate, the Schiff's base of aminocrotonate, and implied enzymatic catalysis of the conversion of aminocrotonate to α-ketobutyrate in reaction 1 (though this feature may not be essential). This note presents some preliminary results which appear to rule out the first scheme.

These experiments were carried out with a Neurospora cystathionine γ-cleavage enzyme (Flavin and Segal, 1964), and were aided by the discovery that the wild-type enzyme content was increased 20-fold following sulfur deprivation (Flavin and

Slaughter, unpublished). It had been postulated that the cellular utility of this enzyme might be in a response to sulfur deprivation (Delavier-Klutchko and Flavin, 1965). Maximum enzyme levels were found after growth with 0.2 μatom of sulfur per ml, whether supplied as sulfate, cysteine, or methionine. The level of the cystathionine β-cleavage enzyme (Flavin and Slaughter, 1964b), which functions in methionine biosynthesis, was not affected by sulfur deprivation.

The γ-cleavage enzyme also catalyzes β-elimination (reaction 3) from lanthionine

$$A\text{-}CH_2CH(NH_2)COOH + H_2O \rightarrow AH + NH_3 + CH_3COCOOH \quad (3)$$

and cystine, and EM has been reported not to react with any intermediates in this case (Flavin and Slaughter, 1964a). To distinguish between reaction schemes 1 and 2, it was desirable to find a β-methyl analogue of these substrates which could be decomposed by the enzyme. Threonine and β,β-dimethyl lanthionine were found not to be substrates, but β-mercapto-α-aminobutyric disulfide (a gift from Dr. H.R.V. Arnstein) was slowly decomposed, and DL-erythro-β-chloro-α-aminobutyrate (a gift from Dr. Marco Rabinovitz) was rapidly decomposed (reaction 4). Both of these

$$A\text{-}\overset{CH_3}{\overset{|}{C}}HCH(NH_2)COOH + H_2O \rightarrow AH + NH_3 + CH_3CH_2COCOOH \quad (4)$$

$$A\text{-}\overset{CH_3}{\overset{|}{C}}HCH(NH_2)COOH + H_2O + EM \rightarrow AH + NH_3 + KEDB \quad (5)$$

substrates, when incubated at pH 7.6 with enzyme and 0.005 M labeled EM, yielded KEDB (reaction 5), and in proportion to α-ketobutyrate the amounts were similar to those obtained when the substrate was L-homocystine or O-succinyl-DL-homoserine. Since α-aminocrotonate would be a common intermediate in both reactions 1 and 4 this result is compatible with reaction scheme 2, and it is incompatible with scheme 1 because there is no substituent on the fourth carbon in reactions 4-5.

It had been reported that α-ketobutyrate itself did not react with EM, in the presence of the enzyme, to yield KEDB (Flavin and Slaughter, 1964a). This experiment was with concentrations (0.002 M) similar to those of other substrates, since its purpose was to show that free α-ketobutyrate was not an intermediate in the formation of KEDB in reaction 2. Following the discovery of reaction 5, this experiment has been repeated with high concentrations (0.06 M) of freshly prepared ammonium α-ketobutyrate, and with this concentration appreciable amounts of KEDB were formed. Reaction 6 required ammonia. Preliminary results suggest that it can take

$$CH_3CH_2COCOOH + EM \rightarrow KEDB \quad (6)$$

place spontaneously, though the yield of KEDB appears to be increased by the addition of enzyme fraction.

The principle objection to reaction scheme 2 has been that EM did not appear to react with intermediary α-aminoacrylate in reaction 3 (Flavin, 1965). This conclusion is now being re-examined. There are some indications that a product analogous to KEDB may be formed from pyruvate under the conditions of reaction 6.

References

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