CATALYTIC CLEAVAGE OF THIOETHERS[1]

Sir:

It has been observed that lanthionine and cystathionine (but not cysteine, homocysteine or methionine) are cleaved in a catalytic manner in neutral or slightly alkaline media in the presence of chelating agents, metal ions and pyridoxal or pyridoxal phosphate. These results are to be contrasted with those of Snell and co-workers[2] in that the cleavages occur at physiological temperatures and at rates comparable with that of the purified enzyme.[3]

L(or meso)-Lanthionine was cleaved with the formation of cysteine, pyruvate and ammonia; hydrogen sulfide was not formed. The products from L(or L-allo)-cystathionine were homocysteine, pyruvate and ammonia; this cleavage was analogous to that of bacterial enzymes[4] but opposite to that of mammalian enzymes which produce cysteine from L-cystathionine. The cleavages were accelerated by chelating agents (Table I) and were metal specific.

TABLE I

CLEAVAGE OF THIOETHERS

0.005 *M* substrate incubated at 37°, *p*H 8.5–9.0, with 0.05 *M* chelating agent and 1×10^{-4} *M* metal ion and pyridoxal. Zinc and cupric ions were used with lanthionine and cystathionine, respectively. There was no cleavage in the absence of pyridoxal or metal ion.

Chelating agent	Per cent. cleavage (1 hr.) L-lanthionine[a]	L-cystathionine[b]
Tris	18	0
Oxalate	45	72
Citrate	57	42
Pyrophosphate	57	56
Glycine	45	40
EDTA	12	2

[a] Ref. 5. [b] Ref. 6, modified (buffer *p*H 7.2, zinc and ammonium salts omitted and 1% EDTA added.)

The effectiveness of chelating agents was related to the stability constant of the chelate complex. Optimal activity for the cleavage of cystathionine (cupric ion) and lanthionine (zinc ion) was observed at or near *p*H 9 and with chelates with stability constants near 10^{-12} and 10^{-9}, respectively. Only cupric ions were found to catalyze the cleavage of cystathionine. Zinc and cupric ions catalyzed the cleavage of lanthionine; zinc ion was more effective with pyridoxal and cupric ion was more effective with pyridoxal phosphate. Other ions tested (including aluminum) were without effect; thus, the metal chelates demonstrated considerable structural specificity. The rate and order of reaction was dependent upon concentration of substrate; first order reaction rates were observed at lower concentrations of substrate (0.005 *M*) and zero order reaction rates were observed at higher concentrations (0.025 *M*) with the system of Table I. A turnover number of 10,000 (maximal) was observed for the cupric ion in the cleavage of cystathionine in oxalate solutions with 10^{-5} *M* cupric ion. Thus, the simple chelate systems were found to have many properties comparable to those of enzymes.

The enzyme responsible for the cleavage of L-cystathionine to cysteine does not conform to the chelate models. First, no chelate model has been found to have the specificity of the mammalian enzyme and, second, the enzyme is not influenced in its activity by a wide variety of chelating agents. Cupric ion was found to activate the enzymatic cleavage but toward the formation of homocysteine; EDTA eliminated this activation without influence on the cleavage to cysteine. Thus, it would be necessary to assume that protein is substituting for metal ion if one is to regard the models as pertinent. The chelate systems may be related to the bacterial enzymes since the specificity of cleavage of cystathionine is identical and there is some evidence that a metal ion may be concerned.[7]

(1) Aided by grants from the Nutrition Foundation and from the U. S. Public Health Service. The following abbreviations are used: Tris, tris-(hydroxymethyl)-aminomethane; and EDTA, ethylenediaminetetraacetic acid.

(2) D. E. Metzler, M. Ikawa and E. E. Snell, THIS JOURNAL, **76**, 648 (1954).

(3) F. Binkley and D. Okeson, *J. Biol. Chem.*, **182**, 273 (1950).

(4) F. Binkley and A. Hudgins, *Federation Proc.*, **12**, 178 (1953).

(5) M. X. Sullivan and W. C. Hess, *J. Biol. Chem.*, **116**, 221 (1936).

(6) E. Brand, G. F. Cahill and B. Kassell, *ibid.*, **133**, 431 (1940).

(7) S. Wijesundera and D. D. Woods, *J. Gen. Microbiol.* (*Proc.*), **9**, 3 (1953).

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INVOLVEMENT OF ATP, 5-PHOSPHORIBOSYL-PYROPHOSPHATE AND L-AZASERINE IN THE ENZYMATIC FORMATION OF GLYCINAMIDE RIBOTIDE INTERMEDIATES IN INOSINIC ACID BIOSYNTHESIS

Sir:

The tentative identification of two aliphatic ribotide derivatives of glycinamide which are intermediates in the *de novo* synthesis of inosinic acid in enzyme systems from pigeon liver has recently been reported.[1] In agreement with these findings, we have isolated and characterized by chemical analysis two metabolic products of glycine, herein designated as glycinamide ribotide (GAR) and (α-N-formyl)-glycinamide ribotide (FGAR), both of which accumulate in relatively large amounts when *de novo* synthesis of purines in soluble enzyme fractions of pigeon liver is carried out in the presence of L-azaserine (O-diazoacetyl-L-serine). Both ribotides can be labeled by 1-C^{14}-glycine, while C^{14}-formate is found only in FGAR. Radioactive bicarbonate labels neither of these two compounds, although bicarbonate ions greatly enhance their formation.

GAR and FGAR behave as typical mononucleotides on ion exchange resins, in that they are not held by Dowex-50 cation exchanger (ammonium cycle), but are retained and can be eluted readily by dilute buffers on Dowex-1 anion exchanger (acetate cycle). A preparation of the barium salt of FGAR, which was obtained enzymatically from 1-C^{14}-glycine, purified by use of these ion exchange techniques and recrystallized several times from water-ethanol, has yielded the analyses reported in Table I.

(1) D. A. Goldthwait, R. A. Peabody and G. R. Greenberg, THIS JOURNAL, **76**, 5258 (1954).