INCORPORATION OF THE AMINO ACID MOIETIES OF AMINO ACID-ADENYLIC ACID ANHYDRIDES INTO PROTEINS[1]

Sir:

Recent studies have led to the suggestion that AA-AMP[2] anhydrides are intermediates in incorporation of AA into proteins in cell-free systems.[3,4] In an effort to elucidate the nature of such incorporation, we have prepared a number of AA–AMP anhydrides by treating N-carbobenzoxy-amino acids with AMP in the presence of N,N′-dicyclohexylcarbodiimide; the blocking group was removed from the purified carbobenzoxy-AA-AMP anhydride by catalytic hydrogenation.[5] The products exhibited consistent ionophoretic mobility,[6] gave the corresponding AA-hydroxamates, yielded equivalent quantities of AA and AMP on hydrolysis, and formed ATP enzymatically.[6]

When C^{14}-AA-AMP anhydrides were incubated with enzyme preparations, the protein subsequently isolated contained significant radioactivity (Table I). No incorporation occurred when the anhydride was hydrolyzed prior to study. When the enzyme preparations were heat-denatured, incorporation was greater than with unheated enzyme. The C^{14}-AA of the proteins (heated and unheated) were released over a period of 16 hours by hydrolysis with 6 *N* HCl at 105°; treatment of these proteins with 1-fluoro-2,4-dinitrobenzene, and then acid hydrolysis[7] gave DNP-amino acid preparations containing 70 to 80% of the C^{14} originally incorporated.

TABLE I: TRANSFER OF C^{14} FROM C^{14}-AMINO ACID-AMP ANHYDRIDES TO PROTEINS

Reaction mixtures[a]	Incorporation into protein (c.p.m./mg.) Glycine	Tryptophan
E + AA-AMP	30	16
E + AA + AMP	0.3	0
E (heated; 100°; 10 min.) + AA-AMP	286	166
E (heated; 100°; 10 min.) + AA + AMP	0.3	0

[a] Contained enzyme preparation (E; 2 ml. of supernatant solution (containing microsomes) obtained by centrifuging a 25% rat liver homogenate at 12,000 × *g*[8]), gly-1-C^{14}-AMP (2.5 μmoles; 3.6×10^5 c.p.m.) or DL-try-3-C^{14}-AMP (1 μmole; 1.9×10^5 c.p.m.) in a volume of 2.5 ml.; incubated at 38° for 30 minutes. E catalyzed incorporation of gly-C^{14} and try-C^{14} in the presence of an ATP-generating system, as described by Zamecnik and Keller.[9]

Transfer of C^{14}-AA from C^{14}-AA-AMP to heated proteins may be regarded as non-enzymatic acylation of protein amino groups, perhaps mainly those of lysine residues. That heated proteins incorporated more C^{14} than did unheated proteins suggests that denaturation exposes more reactive groups. To what extent labelling of unheated proteins is enzymatic remains to be determined. Non-enzymatic acylation of proteins may also occur when C^{14}-AA are incubated with enzyme and ATP-generating systems; thus, the high reactivity[8] of AA-AMP anhydrides may explain some of the incorporation previously reported.[9,10,11] However, it is not excluded that under physiological conditions, selective mechanisms (*e.g.*, binding to enzymes) may direct transfer of the AA-moieties of AA-AMP anhydrides to specific acceptors and ultimately to specific positions in the peptide chains of proteins.[12]

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(2) Abbreviations: amino acid, AA; adenylic acid, AMP; amino acid-adenylic acid anhydride, AA-AMP; adenosine triphosphate, ATP; glycine, gly; tryptophan, try; ribonucleic acid, RNA.

(3) M. B. Hoagland, *et al.*, *J. Biol. Chem.*, **218**, 345 (1956).

(4) P. Berg, *Federatiau Proc.*, **16**, 152 (1957).

(5) Ribose acylated derivatives appear excluded by paper ionophoresis in borate and other buffers and by periodate titration. Acylation of the 6-amino group was excluded by treating carbobenzoxy-try-AMP with nitrous acid to give carbobenzoxy-try-inosinate.

(6) M. Karasek, *et al.*, accompanying paper.

(7) H. Frankel-Conrat, *et al.*, *Methods of Biochemical Analysis*, **2**, 359–425 (1955).

(8) Purified liver RNA preparations (A. Gierer and G. Schramm, *Nature*, **177**, 702 (1956) also were labelled readily when incubated with C^{14}-AA-AMP; after incubating such C^{14}-RNA with heated and unheated proteins, significant radioactivity was found in the proteins subsequently isolated. The nature of this incorporation is being investigated.

(9) P. C. Zamecnik and E. B. Keller, *J. Biol. Chem.*, **209**, 337 (1954).

(10) M. B. Hoagland, *et al.*, *Biochim. et Biophys. Acta*, **24**, 215 (1957).

(11) The concentrations of AA-AMP anhydrides presumably formed in incorporating systems consisting of AA and ATP-generating systems would be lower than in the present studies; it is therefore difficult to make a meaningful comparison of the labelling in the two systems.

(12) While this work was in progress, we learned that C. Zioudrou, S. Fujii and J. S. Fruton have independently synthesized C^{14}-tyrosine-AMP and C^{14}-glycyltyrosine-AMP by a similar procedure, and observed labelling of heated and unheated rat liver mitochondria by these compounds and their N-carbobenzoxy derivatives. They also conclude that non-enzymatic acylation is responsible for the labelling. (We thank Dr. Fruton for communicating these findings to us prior to publication.)

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ENZYMATIC SYNTHESIS AND REACTIONS OF TRYPTOPHAN-ADENYLIC ACID ANHYDRIDE[1]

Sir:

Several enzyme preparations catalyze AA[2] activation reactions which yield AA-hydroxamate when enzyme is incubated with AA, ATP, Mg^{++}, and hydroxylamine.[3,4,5,6] Although available evidence[7,8,9] is consistent with intermediate formation of AA-AMP anhydrides similar to those postulated in acetate[10] and fatty acid[11,12] activation and in phenylacetylglutamine and hippurate synthesis,[13]

(1) Supported in part by grants from the National Science Foundation and National Institutes of Health.

(2) Abbreviations: amino acid, AA; amino acid-adenylic acid anhydride, AA-AMP; adenosine triphosphate, ATP; pyrophosphate, P-P; tryptophan, try.

(3) M. B. Hoagland *et al.*, *J. Biol. Chem.*, **218**, 345 (1956).

(4) P. Berg, *ibid.*, **222**, 1025 (1956).

(5) E. W. Davie *et al.*, *Arch. Biochem. Biophys.*, **65**, 21 (1956).

(6) J. A. Demoss and G. D. Novelli, *Biochim. Biophys. Acta*, **22**, 49 (1956).

(7) J. A. Demoss *et al*, *Proc. U. S. Nat. Acad. Sci.*, **42**, 325 (1956).

(8) P. Berg, *J. Biol. Chem.*, **222**, 1015 (1957).

(9) M. B. Hoagland *et al.*, *Biochim. Biophys. Acta*, **26**, 215 (1957).

(10) P. Berg, *J. Biol. Chem.*, **222**, 1015 (1956).

(11) W. P. Jencks and F. Lipmann, *ibid.*, **225**, 207 (1957).

(12) H. S. Moyed and F. Lipmann, *J. Bact.*, **73**, 117 (1957).

(13) K. Moldave and A. Meister, *J. Biol. Chem.*, **229**, 463 (1957).