ENZYME REGULATION BY SUBSTRATE; RAPID INACTIVATION OF GLUTAMATE DEHYDROGENASE BY CARBAMYL PHOSPHATE

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Three main types of regulation of enzyme activity by substrate and related compounds are known: a) primarily kinetic, via changes in concentration of substrates, cofactors and inhibitors acting at the active site(s); b) feedback; and c) allosteric. A less well studied type is that of elastoplasticity, in which enzymes interact with substrates with resultant enzyme modification and inactivation[1]. A modality of this latter type may be the acylation of proteins by substrates, a phenomenon recently discovered in our laboratory and exemplified in this note by glutamic dehydrogenase.

As illustrated in Fig 1, at the physiological concentrations of glutamic dehydrogenase and carbamyl phosphate present in liver (the mitochondria in the liver of man make at least 0.5 moles of carbamyl phosphate per day), this reagent rapidly inactivates the dehydrogenase.

As typified in Fig 2, the inactivation results in carbamylation of the protein. Thin layer chromatography of protein samples revealed that for this experiment carbamylation involved lysine exclusively. There are[2] some 7.6 gm of lysine per 100 gm of glutamic dehydrogenase or about 675 residues per 10^6 gm. Using a sensitive carbamyl method[3], and assuming that carbamylated lysine in the protein has the same chromogenic value as homocitrulline, it appears that carbamylation of ~4% of the lysine per mole of active enzyme subunit (~300,000 molecular weight) results in almost complete inactivation. It is unknown as yet whether removal of carbamyl phosphate at an earlier time after it has come in contact with the dehydrogenase would also yield increased instability. However, other aspects of the plasticity hypothesis are not involved, e.g., addition of TPNH in 0.1-0.3 millimolar range does not change the speed of inactivation. It is of interest, however that as previously shown, 0.5 to 1 molar urea in combination with 1 mM TPNH potentiates the cofactor induced inactivation[4]. It remains to be seen if under these

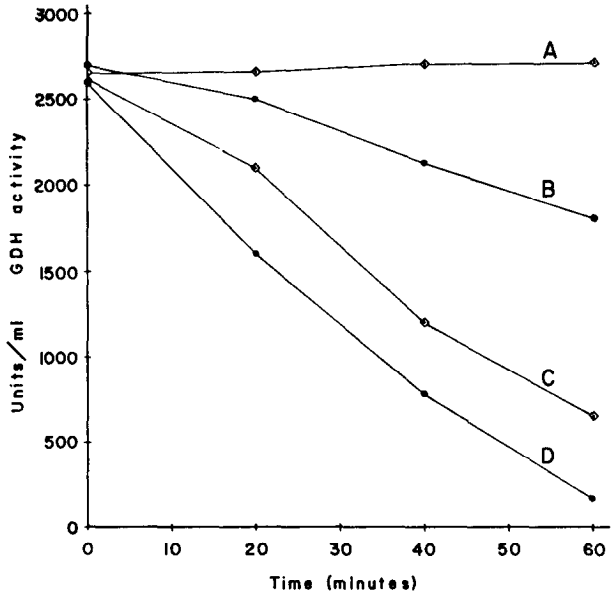


Figure 1. Effect of carbamyl phosphate on glutamate dehydrogenase activity. 2 mg of enzyme were incubated at 38° with carbamyl phosphate as indicated and 15 mM $(NH_4)_2SO_4$ at pH 7.4 in a total volume of 1 ml. A, control (no carbamyl phosphate); B, 12.7 mM carbamyl phosphate; C, 32 mM carbamyl phosphate; D, 114 mM carbamyl phosphate.

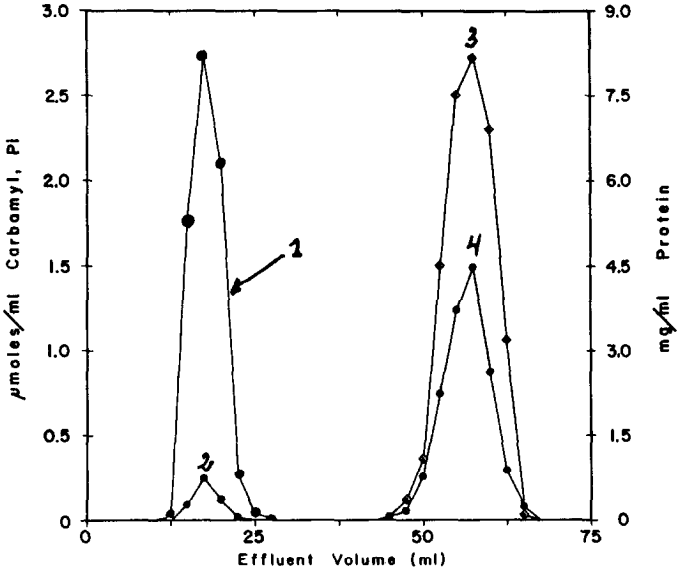
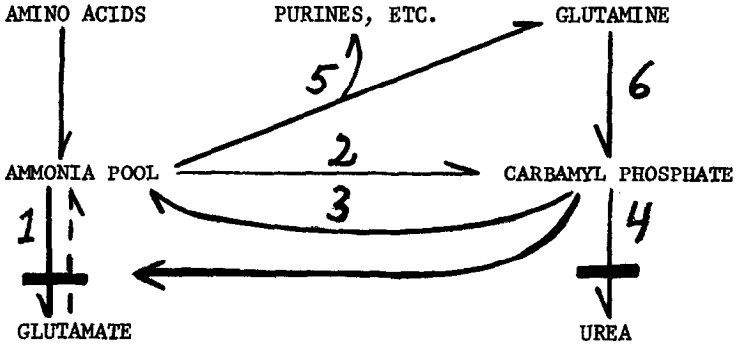


Figure 2. Elution of carbamylated glutamate dehydrogenase from Sephadex G-25 (fine). 60 mg of enzyme were incubated with 60 mg carbamyl phosphate in a total volume of 1.5 ml for 1 hr at 38°. At this time the enzyme had less than 2% of its original activity. The incubation mixture was then applied to a column of Sephadex G-25 (fine)~1.9 x 38 cm and eluted with 20 mM NaCl. Peak number 1, protein; peak number 2, carbamyl protein; peak number 3, inorganic phosphate; peak number 4, carbamyl phosphate.

latter conditions there is concomitant protein carbamylation. If so, the implications in uremia are obvious.

There has been speculation suggesting a close connection between glutamate dehydrogenase and urea synthesis, i.e., via NH_4^+ as a common intermediate, but the possibility that they were connected in the fashion shown in this paper has never been considered. In this regard the present findings are of further interest in suggesting a regulatory role for the very active and widely distributed acyl phosphatase[5] as illustrated in the scheme.



Scheme 1

Then according to the scheme when the main pathway for ammonia detoxication in ureotilic animals, i.e., urea production from carbamyl-P (reaction 4 in the scheme) is not operating at full capacity, for example due to insufficient ornithine and/or ornithine transcarbamylase, as indeed has been suggested in hepatic coma and/or cirrhosis, carbamyl-P may accumulate to such high levels that it inactivates glutamate dehydrogenase. There is presently considerable doubt regarding glutamate dehydrogenase as an enzyme connected with ammonia production; indeed some investigators believe that the primary function of this enzyme is aminoacid synthesis. Then a feedback type of mechanism might ensue leading to further blocking of reaction 1. It is at this point where the acyl Pase might exert a physiological function in preventing accumulation of deleterious levels of carbamyl-P particularly in sensitive areas (tissues with the exception of liver and kidney lack most if not all the enzymes for urea synthesis); moreover, recently a new pathway for carbamyl P for pyrimidine biosynthesis reaction(s) has been uncovered.

Possibly the acyl phosphates, other than carbamyl phosphate, which are attacked by acyl phosphatase[5], i.e., formyl phosphate, acetyl phosphate

(which in turn are synthesized by carbamyl P-synthetase[6]) and 1,3 P-glycerate (synthesized in glycolytic tissues) play similar roles in acylating and/or phosphorylating protein; we have preliminary evidence for this and for the presence of carbamylated proteins in the liver of ureotilic animals. Our findings may provide a new basis for clarification of protein turnover, i.e., acyl proteins may be more easily degraded by cathepsins or lysosomal enzymes. Furthermore, if extended, they may account for the occurrence of acyl amino acids and phosphoamino acids in proteins. These substituted aminoacids are not accounted for by the currently accepted genetic code and are generally believed to originate through modification of preformed poly peptides.

The phenomenon here described reflects the fact that enzymes (particularly those present in large concentrations) may react with substrate to yield covalent bonds unrelated to the reaction catalyzed by the enzyme "per se", and that this results in enzyme inactivation.

It has not escaped our attention that other recent findings, such as the acetylation of serum albumin by aspirin[7], or the phosphorylation of the dephospho form of phosphogluco-mutase to a phospho enzyme by 1,3 phosphoglycerate[8], are closely related phenomena, as is the adenylation of glutamine synthetase[9], although in the latter case the effect is under enzyme control.

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