ACETYL AND CARBAMYL PHOSPHATE UTILIZATION WITH ASPARTATE TRANSCARBAMYLASE AND CARBAMATE KINASE

S. Grisolia, R. Amelunxen and L. Raijman
Department of Biochemistry and the McIlvain Laboratories
University of Kansas Medical Center Kansas City, Kansas

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Our recent investigations have demonstrated that several enzymes using carbamyl phosphate can also utilize acetyl phosphate (Grisolia and Harmon, 1962; Grisolia and Harmon, 1962; Grisolia et al, 1962; Novoa and Grisolia, 1962). Further examples of utilization of carbamyl-P and acetyl-P are presented here, with animal and bacterial aspartate transcarbamylase.

While the observations previously reported, indicate clearly that carbamyl-P utilizing enzymes can also use acetyl-P and although carbamate kinase from D_{10} Streptococci is an acetokinase (Grisolia and Harmon, 1962; Grisolia et al, 1962), our findings should not be construed to indicate that all acetyl-P enzymes are necessarily carbamyl-P enzymes. Some of our earlier findings (Grisolia and Harmon, 1962) appear to have been thus interpreted by other workers (Kreil-Kiss and Hoffmann-Ostenhof, 1963), although it was pointed out (Grisolia et al, 1962) that there was discrepancy in the utilization of acetyl-P and carbamyl-P with a number of E. coli mutants. In all cases the tested mutants had better activity with acetyl-P than with carbamyl-P, as opposed to the findings with the purified carbamate kinase from Streptococci (Grisolia et al, 1962).

Aspartate transcarbamylase was undetectable, with the methods used in the present work, in Baker's yeast extracts prepared by sonic disintegration of yeast or by breakage in the Braun homogenizer; nor was any activity observed in extracts from acetone powders of chicken or pigeon liver. However, low activity, of the order of 20 to 30 mμmoles per mg of protein per hour was detected with wheat germ extracts, and from 4 to 5 mμmoles per mg protein per hour with dog and ox intestinal mucosa and with rat liver extracts. On the other hand, as expected, preparations from E. coli were very active.

Aspartate transcarbamylase was purified from dog intestinal mucosa and from rat liver as follows: fresh gut was washed, the intestinal mucosa was scraped with a spatula and then homogenized with 4 volumes of water; the preparation was centrifuged at 25,000 x g for 10 min, and the precipitate

was discarded. The supernatant fluid was centrifuged at 100,000 x g for 1 hour, and the precipitate was discarded. The supernatant fluid was then centrifuged at 100,000 x g for 3 hours and the precipitate was suspended in a minimal volume of water. Essentially the same procedure was applied to beef intestinal mucosa. For rat liver the homogenization was carried out with 9 volumes of 0.154 M KCl and the first precipitate obtained by centrifugation at 100,000 x g was collected since it had the bulk of enzymatic activity and the higher specific activity. Although the preparations could not be further purified by a wide variety of methods, they were useful for the present work since they showed negligible blanks, and were very stable in the cold.

Cell free preparations from E. coli B were obtained with the aid of the Braun cell homogenizer (Grisolia et al, 1962); for E. coli 185-482, the growth conditions and purification procedure of Shepherdson and Pardee (1962) were followed.

TABLE I

RELATIVE RATES OF UTILIZATION OF ACETYL-P AND OF CARBAMYL-P WITH ANIMAL AND BACTERIAL ASPARTIC TRANSCARBAMYLASE.

The conditions of assay were essentially as described by Shepherdson and Pardee (1962), except that, when used, acetyl-P replaced carbamyl-P. Carbamyl aspartate was measured colorimetrically by an unpublished procedure of Pardee and Yates. Acetyl aspartate synthesis was estimated by the disappearance of both acetyl-P and aspartate as previously described for acetyl-P transfer with ornithine transcarbamylase.

Preparation	Carbamyl Aspartate Synthesis	Acetyl Aspartate Synthesis	Ratio Carbamyl Aspartate / Acetyl Aspartate
	mμmoles	mμmoles	
Dog Intestinal Mucosa	7	0.3	23
Rat Liver	14	0.7	20
E. coli B	3800	10	380
E. coli 185*	33000	120	275
E. coli 185**	178000	386	460
E. coli 185***	312000	680	460

*Grown in the presence of uracil as a repressor.
**Grown in the presence of uracil and of dihydroorotate as a repressor; corresponding to Fraction I of Shepherdson and Pardee (1962).
***Grown in the presence of uracil and of dihydroorotate as a repressor; corresponding to Fraction II of Shepherdson and Pardee (1962). The activity of this fraction was 1/5 that of the crystalline enzyme.

As shown in Table I, the mammalian preparations can use acetyl-P, although at a slower rate than carbamyl-P. It is not possible to decide at present whether or not the activity with acetyl-P is due to the aspartate transcarbamylase activity of animal preparations. However, the data presented in the Table indicate that with bacterial preparations both activities are truly related, particularly since the enzyme from E. coli 185 approximates the activity of crystalline, homogeneous, bacterial transcarbamylase (Shepherdson and Pardee, 1962). It should be noted that better accuracy is obtained with preparations of high specific activity, particularly for the estimation of acetyl aspartate synthesis. While in all cases there is better activity with carbamylphosphate than with acetylphosphate the animal preparations show better relative reactivity for acetylphosphate than the bacterial preparations, as previously noted with ornithine transcarbamylase. It is of interest that no activity was detected with β-methylaspartic acid since it was thought that this reagent might serve as an acceptor for carbamyl phosphate (Woolley and Koehelik, 1961).

Table II further illustrates that the ratio of acetyl-P to carbamyl-P utilization is not constant among several bacterial preparations and that

TABLE II

SYNTHESIS OF ATP FROM ACETYL-P AND CARBAMYL-P WITH STRAIN D_{10} GROUP D STREPTOCOCCI AND E. COLI PREPARATIONS.

The strains were obtained from Dr. W. Maas and grown under the conditions recommended by J. Schwartz (1959). The bacterial cells were harvested, washed, sonically disrupted (Grisolia et al, 1962) and the supernatant fluids after centrifugation assayed as previously described.

Strain	ATP synthesis from Acetyl-P	ATP synthesis from Carbamyl-P	Ratio $\frac{\text{Acetyl-P}}{\text{Carbamyl-P}}$
	μmoles per mg protein per min.		
Streptococcus D_{10}	67	156	0.43*
E. coli R 185-823	7	0.8	8.76
E. coli R prototroph	12	0.8	15.60
E. coli K_{12}-wt	10	0.5	20.06

*This ratio did not change with a 40 fold purification resulting in homogeneous preparations (Grisolia et al, 1962).

acetyl-P is used much faster than carbamyl-P with E. coli extracts than with carbamate kinase. In addition, unpublished observations of L. Raijman and S. Grisolia with animal carbamate kinase-acetokinase have shown that formate can be utilized more effectively than acetate when the enzyme is supplemented with acetylglutamate and Mn^{++}. The classical acetokinase uses acetate faster than formate (Rose, 1955). There are several types of acetokinases distinct from the classical acetokinase as indicated by different ratios of substrate utilization. (E. Stadtman, personal communication). Indeed, our previous observations and the data presented here indicate that while all carbamyl-P enzymes thus far tested have activity with acetyl-P, there may be acetyl-P enzymes free of carbamyl-P activity.

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