# REQUIREMENT FOR $HCO_3^-$ BY ATP: UREA AMIDO-LYASE IN YEAST

P. A. Whitney and T. G. Cooper

Department of Biology, Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Received June 29, 1970

Summary: The cofactor requirements of the ATP:urea amido-lyase reaction in Saccharomyces cerevisiae were investigated. The results indicate that $HCO_3^-$ is a required reactant of the reaction as well as one of the products.

Saccharomyces cerevisiae grown on urea as the sole source of nitrogen has been found to contain an enzyme catalyzing an ATP dependent, avidin-sensitive cleavage of urea similar to that reported in Chlorella and Candida utilis (1). The reaction suggested for this cleavage was:

$$\text{Urea} + \text{ATP} \xrightarrow{Mg^{++}\ K^{+}} CO_2 + 2\ NH_3 + \text{ADP} + P_i \qquad \text{(I)}$$

In view of the current literature on the mechanistic details of biotin containing enzymes, it is difficult to envision how biotin could participate in reaction I. We, therefore, partially purified the enzyme from Saccharomyces and investigated its cofactor requirements. Our results indicate that the enzyme requires $HCO_3^-$ in addition to the cofactors previously documented (1). It is suggested that the reaction proceeds in the absence of added $HCO_3^-$ because (A) $HCO_3^-$ contaminates most solutions with a pH greater than 6.5 and (B) the reaction is autocatalytic, i.e., "$CO_2$" is both a reactant and a product.

## MATERIALS AND METHODS

M-25, a prototrophic diploid strain obtained from a cross between two haploid strains, XT1172-S185 ($\alpha$, $ad_6$, $le_1$) and XT1172-S62 (a, $ly_1$, $hi_6$, $ur_1$) kindly supplied by T. R. Manney was grown with aeration at 30° on Difco yeast carbon base with $10^{-2}$ M urea as the sole nitrogen source

The cells were harvested during late exponential phase and broken in a Waring blendor by the method of Burgess (2). Following removal of cellular debris by centrifugation, a neutralized 2% solution of protamine sulfate was added to the extract to a final ratio of 0.1 mg per mg of protein. A 35-45% ammonium sulfate fraction was obtained and following a 1.5 hour dialysis the protein was fractionated at pH 7.5 on $Ca_3(PO_4)_2$ gel. The 0.1 M phosphate eluate from the gel was finally chromatographed on DEAE cellulose using a 0-0.3 M KCl stepped gradient. The enzyme was eluted in the 0.2 M step of the gradient. This procedure yielded a preparation approximately 250 fold purified over the soluble extract.

## RESULTS AND DISCUSSION

To ascertain the possible existence of an acceptor for the carbon moiety of urea, cells were grown in the presence of $^{14}C$-urea. The total radioactivity in the system was monitored and found to be constant and, as shown in Figure 1, more than 99% of that taken up from the medium was evolved as $CO_2$. These data rule out a preferential incorporation of the carbon moiety into organic acids, but do not eliminate the possibility of its participation in highly transitory complexes.

In the reactions where biotin participates as a prosthetic group, 1'-N carboxybiotin is the active intermediate. Kaziro and Ochoa (3) demonstrated that the carboxylation of biotin requires ATP, $Mg^{++}$, and $HCO_3^-$. Therefore, it is reasonable to suggest that $HCO_3^-$ may be functioning in the present system. In a preliminary experiment we showed that the cleavage of urea has an absolute requirement for ATP and $Mg^{++}$ and is stimulated from 4-7 fold by $HCO_3^-$. If this $HCO_3^-$ stimulation reflects a requirement it would mean that "$CO_2$" is both a reactant and a product of the reaction in a stoichiometry of 1:2.

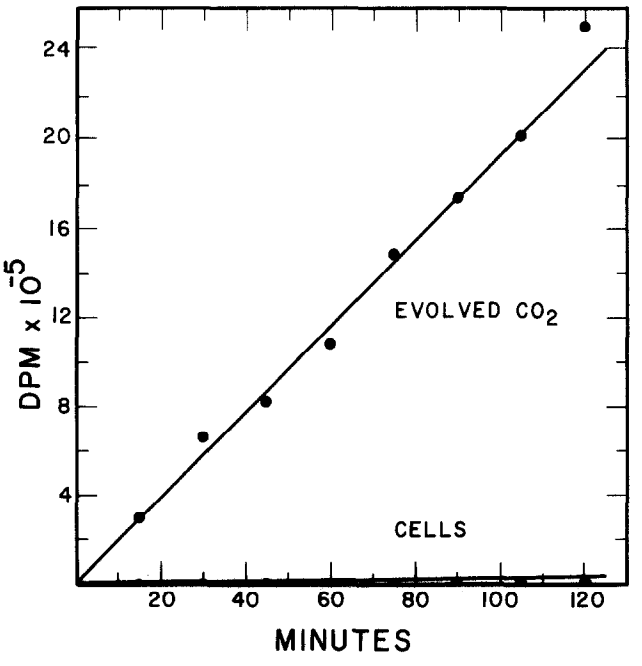


Figure 1. Time course of $^{14}C$-urea utilization in Saccharomyces cerevisiae. Exponentially growing cells at a density of approximately 2 x $10^7$/ml in minimal medium (5) with 0.6% glucose as the carbon source and $10^{-3}$ M urea as the nitrogen source were incubated in the presence of $^{14}C$-urea (activity 0.625 microcuries/micromole) in an apparatus modified from that of Harley and Beevers (4). The evolved $CO_2$ was collected continuously in hyamine hydroxide. At the times indicated the amount of radioactivity in the medium, the cells and the hyamine hydroxide was determined.

---

If it is assumed that (A) the enzyme has an absolute requirement for $HCO_3^-$, (B) $HCO_3^-$ contaminates solutions with a pH above 6.5, (C) none of the $CO_2$ is lost from the system and (D) the enzyme follows Michaelis-Menten kinetics, then it is possible to calculate the theoretically expected rate of cleavage as a function of time by substituting the appropriate values into the Michaelis-Menten equation. The theoretically expected results for several concentrations of added $HCO_3^-$ appear in Figure 2A. As shown, under conditions of little or no added $HCO_3^-$, the rate of the reaction is continually increasing, whereas it is linear if a large amount of $HCO_3^-$ is initially present. When the ATP:urea amido-lyase reaction was assayed spectropho-

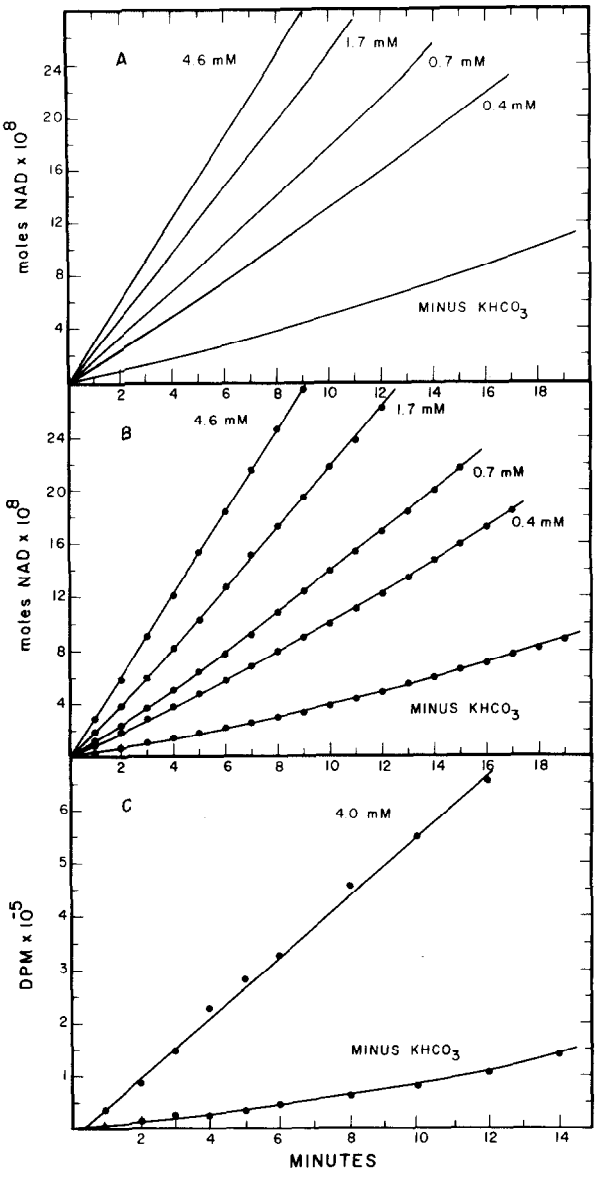


Figure 2. The theoretical and experimental time course of the ATP:urea amido-lyase reaction with varying initial $HCO_3^-$ concentrations.

A. The theoretical curves were obtained by integrating the Michaelis-Menten equation under conditions of continuously varying substrate concentration with a stoiciometry of 2:1 for product $CO_2$: reactant $HCO_3^-$. Each curve represents the addition of the indicated amount of $CO_2$ to a contaminating concentration of $HCO_3^-$. It was assumed that the contaminating concentration of $HCO_3^-$ was 0.132 mM, that the $K_m$ of the enzyme for $HCO_3^-$ was 1.034 mM and that $V_{max}$ was 7.88 micromoles/minute/mg of protein. Under conditions of no added $HCO_3^-$, $S_o$, the initial $HCO_3^-$ concentration, was 0.132 mM; when $HCO_3^-$ was added, $S_o$ = 0.132 plus the amount indicated in the figure.

B. Spectrophotometric assay of the amido-lyase reaction coupled to NADH oxidation via the pyruvate kinase and lactic dehydrogenase reactions. The complete reaction mixture contained, in a volume of 0.86 ml: 116 mM tris buffer; 92.6 mM KCl; 5.8 mM $MgSO_4$; 2.3 mM ATP; 2.3 mM urea; 5.8 mM dithiothreitol; 2.3 mM P-enolpyruvate; 0.6 mM NADH; 19 micrograms of pyruvate kinase (Calbiochem.); 26 micrograms of lactic dehydrogenase; bicarbonate at the indicated concentrations and 55 micrograms of DEAE cellulose purified enzyme. The final pH was 7.9. Non-urea dependent NADH oxidation (7% of maximum value) was determined and subtracted from all values reported.

C. The radiochemical assay contained in a volume of 1.0 ml: 100 mM tris buffer; 80 mM KCl; 5 mM $MgSO_4$; 2 mM ATP; 4 mM urea (activity 1 microcurie/micromole); $HCO_3^-$ at the concentration indicated and 425 micrograms of enzyme protein. The final pH was 7.9. The reaction was carried out in a closed vessel, terminated by addition of 0.2 ml of 70-72% perchloric acid, the evolved $CO_2$ collected in 0.2 ml hyamine hydroxide and counted in a scintillation counter.

---

tometrically as NADH oxidation by coupling this reaction to those of pyruvate kinase and lactic dehydrogenase, the data in Figure 2B were obtained. In Figure 2C the reaction is measured by monitoring the production of $^{14}CO_2$ from $^{14}C$-urea as a function of time. It is clear that the data are in very good agreement with the theory. It can also be predicted that under conditions of no added $HCO_3^-$, increasing the protein concentration should also increase the second derivative of the progress curve. As shown in Figures 3A and B the experimental results are in complete agreement with the theoretically expected results. These observations are consistent with the suggestion that $HCO_3^-$ or $CO_2$ is both a required reactant and a product of the ATP:urea amido-lyase reaction. Therefore we suggest that reaction I should be written:

$$\text{Urea} + \text{ATP} + HCO_3^- \xrightarrow{Mg^{++}\ K^+} 2\ CO_2 + 2\ NH_3 + \text{ADP} + P_i$$

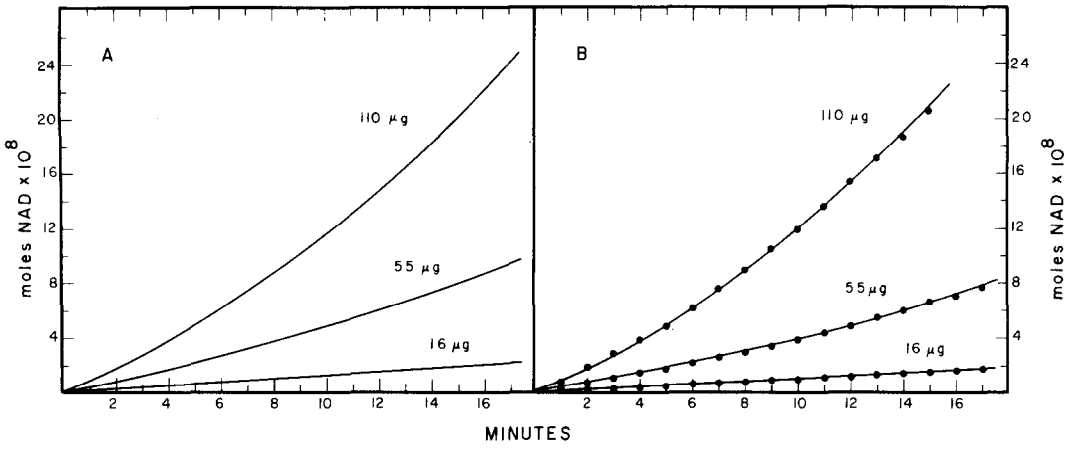


Figure 3. The theoretical and experimental time course of the ATP:urea amido-lyase reaction with varying protein concentrations in the absence of added $HCO_3^-$.

A. The theoretical curves were obtained as in Figure 2A. $S_o$ was held constant at 0.132 mM and the protein concentration ($V_{max}$) was varied as indicated.

B. The spectrophotometric reaction mixture was identical to that of Figure 2B except that $HCO_3^-$ was omitted in all cases and the protein concentrations were those indicated.

ACKNOWLEDGEMENT

The authors express their sincere gratitude to Professor Boris Magasanik for his helpful advice and enthusiastic support of this work. The suggestions of Dr. R. S. Krooth concerning the apparatus employed in the radiochemical assays were also appreciated. This work was supported by grants: NSF GB-5322X2, NIH 5-R01-GM-07446, and NIH 1-R01-AM-13894 to Boris Magasanik and an Air Force Office of Scientific Research - National Research Council Award to T. G. Cooper. P. A. Whitney was supported by a Microbiology Training Grant (5T1-GM-00602) to the M.I.T. Department of Biology.

BIBLIOGRAPHY

1. Roon, R. J. and Levenberg, B., J. Biol. Chem., 243, 5213 (1968).
2. Burgess, R. R., J. Biol. Chem., 244, 6160 (1969).
3. Kaziro, Y. and Ochoa, S., J. Biol. Chem., 236, 3131 (1961).
4. Harley, J. L. and Beevers, H., Plant Physiol., 38, 117 (1963).
5. Wickerham, L. J., J. Bacteriol., 52, 293 (1946).