Biochima et Biophysica Acta, 438 (1976) 574—583

BBA 67847

UREA AMIDOLYASE OF *CANDIDA UTILIS*

CHARACTERIZATION OF THE UREA CLEAVAGE REACTIONS

PETER A. CASTRIC [a] and BRUCE LEVENBERG [b, *]

[a] *Department of Biological Sciences, Duquesne University, Pittsburgh, Pa. 15219 and*
[b] *Department of Biological Chemistry, The University of Michigan, Ann Arbor, Mich. 48104 (U.S.A.)*

(Received December 16th, 1975)

Summary

Evidence is presented that the enzymes catalyzing the three reactions involved in urea cleavage in *Candida utilis*, biotin carboxylation, urea carboxylation, and allophanate hydrolysis occur as a complex of enzymes. The allophanate-hydrolyzing activity could not be separated from the urea-cleaving activity using common methods of protein purification. Further, urea cleavage and allophanate hydrolysis activities are induced coordinately in cells grown on various nitrogen sources.

The reactions involved in urea cleavage can be distinguished from one another on the basis of their sensitivities to (a) heat, (b) pH, and (c) chemical inhibitors. Evidence is presented for the product of the first reaction in urea cleavage, biotin carboxylation. Production of carboxylated enzyme is ATP dependent and avidin sensitive. Carboxylated enzyme is not observed in the presence of 1 mM urea.

Introduction

Urea cleavage in *Candida utilis* [1] as well as certain other fungi [1—3] and some green algae [1,4] is mediated by urea amidolyase (EC 6.3.4-). The examination of urea cleavage by urea amidolyase [5,6] has allowed the proposal of the following sequence of reactions for this process:

1. $ATP + HCO_3^- + \text{enzyme-biotin} \overset{Mg^{2+},K^+}{\rightleftharpoons} ADP + P_i + \text{enzyme-biotin-}CO_2^-$

2. $\text{Enzyme-biotin-}CO_2^- + H_2N{-}\overset{\overset{O}{\|}}{C}{-}NH_2 \rightleftharpoons \text{enzyme-biotin} + H_2N{-}\overset{\overset{O}{\|}}{C}{-}NH{-}\overset{\overset{O}{\|}}{C}{-}O^-$

* Deceased.

$$3.\ H_2NCNHCO^- \xrightarrow{H_2O} 2NH_4^+ + 2HCO_3^-$$

$$\text{Net reaction: ATP} + H_2NCNH_2 \xrightarrow[HCO_3^-, H_2O]{Mg^{2+}, K^+} \text{ADP} + P_i + 2NH_4^+ + HCO_3^-$$

The urea amidolyase component catalyzing reactions 1 and 2 (urea carboxylase) may be separated from the component catalyzing reaction 3 (allophanate hydrolase) in the green alga *Chlorella ellipsoidea* by chromatography on brushite [7]. In *Saccharomyces cerevisiae*, however, these components are firmly associated in a complex of enzymes as is indicated through the use of several techniques of protein purification [8].

The present report further examines the physical association of the three reactions catalyzed by urea amidolyase from *C. utilis*. Further, these reactions are distinguished by their selective sensitivities toward inhibitors. Finally, the formation of the product of reaction 1, carboxylated enzyme, is examined.

Materials and Methods

Chemicals. Avidin (11.6 units/mg) was purchased from Worthington Biochemical Corp. Omnifluor was purchased from New England Nuclear Corporation. Sephadex G-25 and G-200 and Sepharose 4B were purchased from Pharmacia Fine Chemicals. DEAE-cellulose was purchased from W. and R. Balston Ltd. Enzyme grade urea was purchased from Schwarz/Mann. The ^{14}C compounds used in this study were obtained from the following sources: [*amido*-^{14}C]-acetamide, International Chemical and Nuclear Corp.; [*Ureido*-^{14}C]allophanate was synthesized from [^{14}C]urea by the method of Dains and Wertheim [9]; $KH^{14}CO_3$, New England Nuclear Corporation; [^{14}C]formamide and [^{14}C]urea, Schwarz Bioresearch. All other chemicals were of reagent grade.

Growth conditions and preparation of enzyme. The medium and growth of *C. utilis* (ATCC 8205) have been described earlier [10]. Extracts of late log phase cells were prepared by sonication as previously described [10] or by autolysis. Autolysis consisted of twice freezing and maintaining packed cells at −60°C for 1 h and thawing to room temperature. The cells were air dried at 23°C for 4 days and the hard glaze formed was ground in a mortar and pestle to a fine powder. The enzyme was extracted by addition of 10 ml buffer (0.02 M potassium phosphate, pH 7.5) for each gram of powder used. After stirring for 1 h at 4°C the suspension was centrifuged for 15 min at 20 000 × *g*. No changes in apparent molecular weight, purification properties, or catalytic activity were noted between the two methods of cell breakage. From this point on the purification procedure used was that of Roon and Levenberg [10] and, unless otherwise stated, the enzyme preparation used was from the last step of this purification procedure. This procedure results in a 150-fold purification which is not homogeneous as judged by polyacrylamide electrophoresis.

In the induction experiments starter cultures were grown using the nitrogen sources indicated for the experimental culture. All growth periods were from

18 to 20 h which gave the following cell wet weights (g/l): $(NH_4)_2SO_4$, 4.9; urea, 3.5; acetamide, 2.7; formamide, 3.8; guanidine, 3.6; biuret, 0.9.

Enzyme assays. Enzymatic urea cleavage was assayed by the Thunberg method which measures the rate of release of $^{14}CO_2$ from [^{14}C]urea [10]. The Thunberg method for assay of allophanate hydrolysis which measures the rate of release of $^{14}CO_2$ from [*ureido*-^{14}C]allophanate has been described [11]. The exchange reaction measures reaction 2 coupled to allophanate hydrolysis and was assayed by measuring the release of $^{14}CO_2$ from [^{14}C]urea facilitated by biotin carboxylation by allophanate in the absence of ATP, Mg^{2+}, and K^+. This procedure has been described previously [11].

The assay for urea carboxylation in the absence of allophanate hydrolysis, as used in the experiments presented in Table IV, were carried out as follows. The reaction mixture and conditions were identical as that for urea cleavage, including the use of [^{14}C]urea as a substrate. The reaction was stopped by adding absolute alcohol up to a concentration of 50%. The sample was passed through a 0.5 × 2 cm Dowex 1 (OH^-) column. The column was rinsed with deionized water and then eluted with 1 M HCl which results in decarboxylation of allophanate. These samples were dried and the radioactivity, as [^{14}C]urea, was determined by scintillation counting.

The assay for acetamide or formamide cleavage was as follows. The reaction mixture contained 2.5 μmol disodium ATP, 4.0 μmol $MgSO_4$, 40 μmol KCl, 2 μmol $KHCO_3$, 50 μmol Tris · HCl, substrate ([^{14}C]acetamide, 10 μmol, 1.0 μCi, or [^{14}C]formamide, 10 μmol, 2.5 μCi), and enzyme preparation (0.4 unit from the final purification step) in a volume of 0.5 ml at a pH of 8.0. The reaction was started by the addition of enzyme to the reaction mixture in stoppered tubes which were prewarmed at 30° C for 5 min and stopped by the addition of 0.5 ml absolute alcohol. Samples of the reaction mixture and authentic reference compounds were spotted on Whatman No. 3 paper and developed in ethanol (absolute)/NH_4OH (0.44 M) (9 : 1, v/v). The strips containing the reference compounds were separated and the ammonium salts of the reference acids were detected using bromo cresol green spray [12] and the reference amides were detected by Ag^+ reduction [13]. Sample strips were then separated and scanned for radioactivity using a Vanguard Chromatogram scanner. Areas of the sample strip matching the authentic references were cut and counted by scintillation counting.

Units of enzyme activity are described as μmol of product formed per min of incubation under standard assay conditions. Specific activity is described as units per mg protein. Protein concentration was determined by the method of Lowry et al. [14] using bovine serum albumin as a standard.

Radioisotope counting. For scintillation counting material to be measured was added to vials containing 1.0 ml water. Scintillation fluid (Triton X-100, 333 ml; toluene, 667 ml; Omnifluor, 4 g) was added in the amount of 10 ml. Radioactivity was measured in a liquid scintillation counter using a channels ratio quench correction curve. Radioactivity in this case is reported as disintegrations per min. Radioactivity when measured using a gas flow counter system is reported as counts per min.

Column chromatography. DEAE-cellulose chromatography was performed as in the final step of purification of the enzyme [10]. Gel filtration with Sepharose

4B was carried out using the protein from $(NH_4)_2SO_4$ step (step 3) of this purification scheme. A column containing Sepharose 4B (2 × 22 cm) was equilibrated with 0.1 M Tris/potassium phosphate buffer, pH 7.5, containing 10% glycerol and was eluted with the same buffer at 4°C. 2.5-ml samples were collected at a rate of 22 ml/h.

Results

Co-purification of urea amidolyase reactions. C. utilis cells grown as described in Materials and Methods were harvested and then disrupted by sonication. This extract was subjected to the first four steps of the purification of Roon and Levenberg [10]. As is seen on Table I the urea cleavage and the allophanate hydrolysis activities in this extract are purified to the same extent. In addition, the ratio of these activities remain constant throughout the procedure.

Co-chromatography of urea amidolyase reactions. An enzyme preparation purified to step 4 of the above purification scheme was chromatographed on a DEAE-cellulose column, the fifth and final step of the above-mentioned purification scheme. Fig. 1 shows the elution profile obtained by this procedure. No allophanate hydrolysis activity is seen without a proportional amount of urea cleavage activity. The ratio of the activities, where these activities are significantly large, is proportional across the activity peaks. Gel filtration of enzyme preparations from step 3 of the above purification table was carried out using Sepharose 4B. The profiles seen in Fig. 2 indicate again that the urea cleavage and the allophanate hydrolysis activities elute coordinately and that the ratio over the activity peaks is constant.

Co-induction of urea amidolyase reactions. C. utilis can grow, under the culture conditions described, using acetamide, formamide, guanidine, urea, or $(NH_4)_2SO_4$ but not thiourea, *N*-methyl urea, or hydantoic acid as the sole source of nitrogen. Extracts of cells grown with these nitrogen sources show that for over a several hundred-fold range of induction the ratio of urea cleavage to allophanate hydrolysis remains constant (Table II). This indicates no selective induction or repression of either activity. Further, this proportionality

TABLE I

CO-PURIFICATION OF UREA CLEAVAGE AND THE ALLOPHANATE HYDROLYSIS ACTIVITIES FROM EXTRACTS OF *C. UTILIS*

Purification steps	Specific activity		Urea cleavage
	Urea cleavage (unit/mg)	Allophanate hydrolysis (unit/mg)	Allophanate hydrolysis
1a. Crude extract	0.0068	0.0150	0.45
1b. 100 000 x *g* supernatant	0.0119	0.0230	0.52
2. Protamine supernatant	0.0117	0.0262	0.45
3. $(NH_4)_2SO_4$ precipitate	0.0335	0.0793	0.42
4. Sephadex-G-200 eluate	0.02870	0.6700	0.43

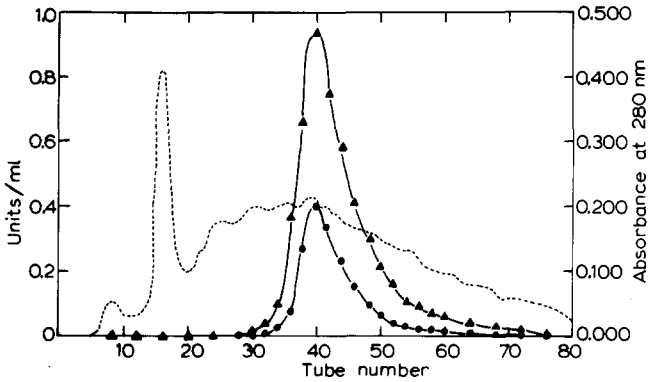

Fig. 1. The co-elution of the allophanate hydrolysis and the urea cleavage reactions from DEAE-cellulose. A linear KCl gradient beginning at tube 16 (0.08 M) and ending at tube 80 (0.30 M) was employed. ▲——▲, allophanate hydrolysis; ●——●, urea cleavage reaction; ------, absorbance at 280 nm.

is retained through log and into stationary phase with urea as nitrogen source (Castric, P.A., unpublished).

Extracts of cells grown using guanidine as the sole nitrogen source catalyze the release of no CO_2 from guanidine unless Jack Bean urease is added indicating the conversion of guanidine to urea. This might indicate that the rather high urea amidolyase specific activity seen here results from urea production from guanidine. Biuret may be catabolized to urea resulting in urea amidolyase production or may induce this enzyme because of its structural similarity to urea. It has been shown that in *S. cerevisiae* [15], which will not use biuret as a nitrogen source, there is an induction of urea amidolyase by this compound indicating the latter possibility. Acetamide and formamide may act as inducers in a likewise manner but have been indicated previously to be substrates for the urea carboxylase reactions [1,6]. Since the implicated urea amidolyase inducer molecule in *S. cerevisiae* is allophanate [16], the above-mentioned amides would likely have to be carboxylated to act as inducers.

Incubating 100-fold purified urea amidolyase preparations with [^{14}C]-acetamide or [^{14}C]formamide followed by paper chromatography and isotope

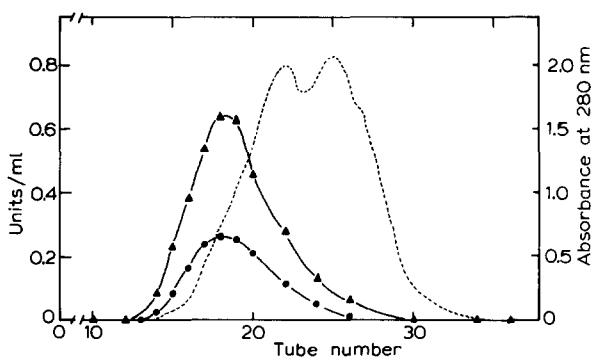

Fig. 2. The co-elution of the allophanate hydrolysis and the urea cleavage reactions from Sepharose 4B. ▲——▲, allophanate hydrolysis; ●——●, urea cleavage; ------, absorbance at 280 nm.

TABLE II

THE EFFECT OF THE GROWTH MEDIUM NITROGEN SOURCE ON UREA CLEAVAGE AND ALLOPHANATE HYDROLYSIS ACTIVITIES

The enzyme preparations are from step 1b (100 000 × *g* supernatant liquid) of the urea amidolyase purification procedure [10]. The nitrogen sources were all in initial concentrations of 10 mM.

Nitrogen source	Specific activity		Urea cleavage
	Urea cleavage (unit/mg protein)	Allophanate hydrolysis (unit/mg protein)	Allophanate hydrolysis
$(NH_4)_2SO_4$	0.0001	0.0033	0.33
Urea	0.0109	0.0268	0.41
Acetamide	0.0424	0.0910	0.47
Formamide	0.0141	0.0318	0.44
Guanidine	0.0092	0.0214	0.43
Biuret	0.0031	0.0076	0.41

counting as described in Materials and Methods results in no production of radioactive acetate or formate. This indicates that while these amides may serve as carboxylation substrates, they are not further acted upon by urea amidolyase.

Effect of temperature on urea amidolyase reactions. Since the data presented indicate that urea amidolyase is a complex of enzymes it is of interest to be able to distinguish among these activities. Fig. 3 shows the effect of incubation of the partially purified complex at 52°C on enzyme activity. As can be seen the allophanate hydrolysis activity is relatively heat stable while the earlier activities are rapidly lost.

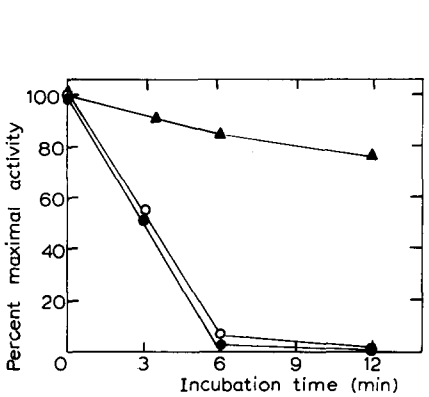

Fig. 3. Effect of heat on the enzymatic activities associated with urea amidolyase. The incubation mixture containing 0.5 mg protein of the 150-fold purified enzyme preparation, 20 μmol potassium phosphate (pH 7.5), and 0.25 ml glycerol in a total volume of 1 ml was incubated at 52°C. Portions of the incubation mixture containing 15 μg protein for the urea cleavage and allophanate hydrolysis reactions and 30 μg protein for the exchange reaction were removed at the times indicated and used to initiate the assay reaction. ▲——▲, allophanate hydrolysis; ○——○, exchange reaction; ●——●, urea cleavage reaction.

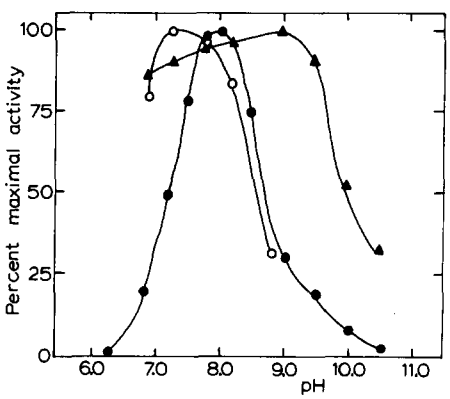

Fig. 4. Effect of pH on the enzymatic activities associated with urea amidolyase. For pH values up to and including 9.0, the reaction mixtures minus enzyme were prepared with Tris (pH adjusted with 6 M HCl) giving a final concentration of 0.2 M Tris at the pH values indicated. At pH values above 9.0, a glycine (0.1 M) carbonate (0.1 M) buffer was used. The 150-fold purified enzyme preparation (30 μg) was added to initiate the reaction. ▲——▲, allophanate hydrolysis; ○——○, exchange reaction; ●——●, urea cleavage reaction.

Effect of pH on urea amidolyase reactions. Urea cleavage, as described previously [1] for the *C. utilis* enzyme has a pH optimum of slightly under 8.0. Fig. 4 shows that the exchange reaction has an acidic shift of optimum activity of about 0.5 pH unit with reference to the urea cleavage reaction. The allophanate hydrolysis activity can be distinguished by its pH optimal of 9.0 and by its relative insensitivity to pH between 6.9 and 9.5.

Effect of chemical inhibitors on urea amidolyase reactions. The reactions involved in urea cleavage may be also distinguished from one another by selective chemical inhibition (Tables III and IV). ADP, known to be a competitive inhibitor with respect to ATP, and NaF inhibit urea cleavage while they have less or no effect on the exchange and allophanate hydrolysis reactions. Formamide and acetamide, however, inhibit both the urea cleavage and exchange reactions but not allophanate hydrolysis. This indicates that these compounds most likely inhibit urea breakdown at the site of urea carboxylation. Table IV shows that incubation of *C. utilis* extracts in 2.5 M urea results in the complete loss of allophanate hydrolase and therefore urea cleavage. However, avidin-sensitive, ATP-dependent urea carboxylase retains significant activity.

Urea amidolyase-biotin-CO_2 intermediate. If urea amidolyase acts in a manner analgous to other biotin enzymes one would expect to be able to isolate a carboxylated form of the enzyme (the product of reaction 1). Fig. 5 shows that when partially purified enzyme is incubated with ATP, Mg^{2+}, K^+, and $H^{14}CO_3^-$ and then subjected to gel filtration a portion of the radioactivity is found associated with the high molecular weight material excluded from the gel. When ATP is omitted from the reaction mixture little radioactivity is eluted at the

TABLE III

EFFECT OF INHIBITORS ON UREA AMIDOLYASE ACTIVITIES

The complete reaction mixture (minus substrate) containing the 150-fold purified enzyme preparation (30 μg) was pre-incubated 15 min at 30° C with the inhibitor. The assays were then initiated with the addition of substrate.

Inhibitor	Concentration (mM)	Activities remaining (%)		
		Urea cleavage	Exchange reaction	Allophanate hydrolysis
None	—	100	100	100
ADP	1	84	102	102
	5	29	100	100
	10	3	100	93
NaF	0.25	89	94	95
	0.50	74	89	90
	1.00	43	77	77
Formamide	10	96	90	104
	50	55	45	106
	100	38	21	102
Acetamide	50	78	81	95
	100	65	65	101
	400	31	26	97
Thiourea	100	94	—	90
	200	76	—	83
	400	7	—	0

TABLE IV

EFFECT ON 2.5 M ON UREA AMIDOLYASE ACTIVITIES

C. utilis crude extracts were incubated for 30 min at 30°C in the presence of 2.5 M urea. These samples were desalted by passage through a Sephadex G-25 column (1.3 × 20 cm) at 4°C. Portions of the untreated extract (1.1 mg protein) and the treated extract (0.9 mg protein) were used to initiate the assay reaction.

Enzyme	Radioactivity (dpm)		
	Urea cleavage	Urea carboxylase [a]	Allophanate hydrolysis
Untreated	36 485	—	56 084
Urea treated	13	24 818	73
Urea treated minus ATP	—	2 076	—
Urea treated plus avidin [b]	—	2 193	—

[a] The radioassay described in Materials and Methods was employed.
[b] Avidin (100 μg) was preincubated with the enzyme for 2 min at 30°C prior to the assay.

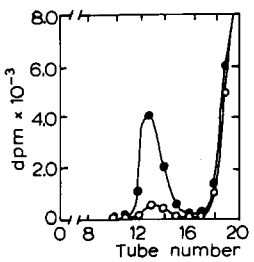

Fig. 5. Formation of enzyme-biotin-CO_2^-. The complete incubation mixture was prepared as follows: 1.4 mg of pyruvate carboxylase-free urea amidolyase preparation (from the leading edge of the elution profile for urea cleavage activity on DEAE-cellulose, the final step of the purification scheme) was incubated with 5 μmol ATP; 5 μmol $H^{14}CO_3^-$ (specific activity, 54.9 Ci/mol); 55 μmol Tris; 30 μmol potassium phosphate in a volume of 1 ml at a pH of 7.8 for 2 min at 30°C. This solution was chilled in an ice bath for 5 min and then applied to a Sephadex G-25 column (1.3 × 20 cm) equilibrated with 0.1 M Tris phosphate buffer (pH 7.9) containing 25% glycerol. This same buffer was used to elute the column at 4°C. 1-ml samples were collected and radioactivity was determined by scintillation counting. The void volume was determined by the use of Blue dextran 2000. ●———●, complete reaction mixture; ○———○, complete reaction mixture minus ATP.

TABLE V

REQUIREMENTS FOR THE FORMATION OF ENZYME-BIOTIN-CO_2^- BY UREA AMIDOLYASE

Incubation mixture	Column equilibration buffer [a]	$^{14}CO_2$ incorporated × 10^{-3} [b]
Complete	Complete	11.0
Complete	Complete plus 1 mM urea	1.6
Complete minus ATP	Complete	1.8
Complete plus avidin [c]	Complete	2.9
Complete plus avidin-biotin [d]	Complete	10.1

[a] 0.02 M potassium phosphate buffer, pH 7.5, containing 25% glycerol.
[b] These values represent radioactivity incorporated into the void volume material using the procedure described in Fig. 5.
[c] The enzyme preparation was incubated with 100 μg avidin for 2 min at 30°C prior to its addition to the other components of the incubation mixture.
[d] The enzyme preparation was treated with avidin as in c except that the avidin was pre-incubated for 5 min at 30°C with 750 μg (+)-biotin prior to incubation with the enzyme preparation.

void volume. Likewise little radioactivity is incorporated when the column used to separate the reaction mixture is equilibrated with 1 mM urea or when the enzyme preparation is incubated with avidin prior to the addition of cofactors and substrates (Table V). However, if the avidin is preincubated with saturating amounts of biotin prior to incubation with the enzyme the inhibiting effect of avidin is not seen.

Discussion

The activities making up urea cleavage by the urea amidolyase of *C. utilis* form a complex of enzymes. This is indicated by the constant ratio between urea cleavage and allophanate hydrolysis during enzyme purification and chromatography. The co-ordinate induction of these reactions supports this conclusion. Work done by Whitney and Cooper [8] shows that the *S. cerevisiae* urea amidolyase also occurs in the form of an enzyme complex. The ratio of their activities during purification give allophanate hydrolysis specific activities 3—5-fold higher than the specific activities of urea carboxylase. The data presented here for *C. utilis* indicate a 2—2.5-fold excess of allophanate hydrolysis. This difference in ratios may be accounted for by lability of the *S. cerevisiae* urea carboxylase during purification or may mean that there is a basic difference in subunit makeup or mode of catalysis between the urea amidolyase of the two organisms.

S. cerevisiae will use neither formamide nor acetamide as a sole source of nitrogen, however, formamide is able to induce urea amidolyase synthesis in this organism [15,16]. In the present study it is shown that *C. utilis* will use these amides both as nitrogen sources and as inducers of urea amidolyase. In addition *C. utilis* produces amidases capable of hydrolyzing acetamide and formamide [17,18], which have been observed in the strain used in this study (Castric, P.A., unpublished). Since these amides are apparently not degraded by urea amidolyase the situation exists in this organism where compounds of one pathway may function as non-metabolizable inducers for another pathway.

While the urea amidolyase activities are physically associated in a complex they may be distinguished from one another by selective inhibition. The heat inactivation experiments indicate that urea carboxylase is much more sensitive to this denaturant than allophanate hydrolase. This is in agreement with heat sensitivity studies of the *S. cerevisiae* urea amidolyase [19]. Which, if either, of the sequential reactions catalyzed by urea carboxylase is the sensitive component cannot be said. However, the exchange reaction is definitely heat sensitive and this may result in the loss of the biotin carboxylation reaction seen with *S. cerevisiae* studies.

The broad range of pH values which gives near maximal activity for allophanate hydrolase indicates that this activity is not the reaction which gives the overall pH curve its shape. Since the exchange reaction is active on the acidic side of the urea cleavage pH optimal, biotin carboxylation, reaction 1, is the limiting reaction at these pH values. On the basic side of the urea cleavage pH optimal the exchange reaction is limiting but the data shown cannot assess the pH effect on biotin carboxylation.

The data indicate inhibitors for each reaction involved in urea cleavage. ADP most likely affects ATP binding [1] and thus may serve in a regulatory capacity.

Acetamide and formamide inhibit the exchange reaction. This inhibition is competitive with respect to urea (Castric, P.A. and Levenberg, B., unpublished) and since these amides serve as carboxylation substrates, they affect the urea binding site. The lack of inhibition seen with these amides with *S. cerevisiae* urea amidolyase [6] may have been due to the low concentrations of inhibitor used. Thiourea inhibition of allophanate hydrolysis is in agreement with work done with *S. cerevisiae* urea amidolyase [19]. The *C. utilis* enzyme complex, however, is much less sensitive to this inhibitor.

Urea treatment results in the complete loss of urea amidolyase activity. However, significant amounts of urea carboxylase, reactions 1 and 2, remain. This may be the result of the inherent stability of the proteins catalyzing biotin and urea carboxylation or may represent protection through the binding of urea to the enzymes urea binding site. The latter would seem likely as urea protects urea amidolyase also against heat denaturation (Waheed, A. and Castric, P.A., unpublished).

The demonstration of enzyme-boitin carboxylation, the exchange reaction, and allophanate hydrolysis and as well as studies with the inhibitors of these reactions indicate that three distinct sequential catalytic activities are involved in the cleavage of urea by *C. utilis*. These activities represent three enzyme classes: a ligase (biotin carboxylation), a transferase (urea carboxylation), and a hydrolase (allophanate hydrolysis). The last activity would indicate a unique subunit makeup among biotin enzymes. Discernment of the quaternary structure of this enzyme complex must await purification and dissociation of the components involved.

Acknowledgements

This work was supported by grants to B. Levenberg (Research grant GM-13325 and equipment grant AM-12734) and P. Castric (Research grant GM-20419) from the United States Public Health Service. Helpful advice from Dr. E.E. Dekker and technical assistance by K.F. Castric, A.E. Hunt, and R. Jones are gratefully acknowledged.

References

1 Roon, R.J. and Levenberg, B. (1972) J. Biol. Chem. 247, 4107—4113
2 Shorer, J., Zelmanowicz, I. and Barash, I. (1972) Phytochemistry, 11, 595—605
3 Whitney, P.A. and Cooper, T.G. (1970) Biochem. Biophys. Res. Commun. 40, 814—819
4 Leftly, J.W. and Syrett, P.J. (1973) J. Gen. Microbiol. 77, 109—115
5 Roon, R.J., Hampshire, J. and Levenberg, B. (1972) J. Biol. Chem. 247, 7539—7545
6 Whitney, P.A. and Cooper, T.G. (1973) J. Biol. Chem. 248, 325—330
7 Thompson, J.F. and Muenster, A.-M.E. (1971) Biochem. Biophys. Res. Commun. 43, 1049—1055
8 Whitney, P.A. and Cooper, T.G. (1972) Biochem. Biophys. Res. Commun. 49, 45—51
9 Dains, F.B. and Wertheim, E. (1920) J. Am. Chem. Soc. 42, 2303—2309
10 Roon, R.J. and Levenberg, B. (1970) Methods Enzymol. 17, 317—324
11 Roon, R.J. and Levenberg, B. (1970) J. Biol. Chem. 245, 4593—4595
12 Block, R.J., Durrum, E.L. and Zweig, G. (1958) A Manual of Paper Chromatography and Paper Electrophoresis, 2nd edn., p. 217, Academic Press, New York
13 Lederer, E. and Lederer, M. (1957) Chromatography, 2nd edn., p. 254, Elsevier Publishing Co., New York
14 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275
15 Sumrada, R. and Cooper, T.G. (1974) J. Bacteriol. 117, 1240—1247
16 Whitney, P.A., Cooper, T.G. and Magasanik, B. (1973) J. Biol. Chem. 248, 6203—6209
17 Domnas, A. (1961) Can. J. Microbiol. 7, 657—666
18 Brady, B.L. (1969) J. Gen. Microbiol. 59, 47—55
19 Whitney, P.A. and Cooper, T.G. (1972) J. Biol. Chem. 247, 1349—1353