TABLE II

RELATIVE RADIOACTIVITIES OF YEAST AMINO ACIDS[a]

Amino acids	Time in minutes 7	15	30	60	120	180	240	360	480
Aspartic acid		+	+++	++	+	+	+	+	+
Glutamic acid		+	+++	+++	+++	++++	++++	++	+
Proline		+	+	+	+	+	+	+	+
Glycine–serine		+	+	+	+	+	+	++	++
Alanine		+	+	+	+	+	+	+	+
Threonine				+	+	+	+		
Phenylalanine		+	+	+	+	+	+	+	+
Tyrosine		+	+	+	+	+	+	+	+
Valine					+	+	+		
Arginine				+	+	+	+	+	+
Lysine			+	+	+	++	+++	+++	+++

[a] Total radioactivity as determined by radioautography: ++++, very heavily labeled; +++, heavily labeled; ++ moderately labeled; +, detectable; substrate: glucose-u-C^{14} in NH_4–salt medium.

fraction of glucose metabolized glycolytically	= 87%
fraction of glucose metabolized through phosphogluconate decarboxylation	= 13%
fraction of pyruvate degraded to acetate	= 90%
fraction of pyruvate utilized in C_4 synthesis	= 10%
fraction of acetate carboxyl carbon utilized in biosynthesis	= 72%
fraction of acetate methyl carbon utilized in biosynthesis	= 88%

The value of G_e so obtained is in good agreement with that observed by Blumenthal, Lewis and Weinhouse,[4] using a different method. In view of the simplicity, reproducibility and the kinetic information provided by the present approach it is believed that it will prove useful in identifying and estimating catabolic pathways of carbohydrates in various species of microörganisms.

The Incorporation of Glucose Carbon into Amino Acids.—In Table II are given the relative total radioactivities of yeast amino acids derived from glucose-u-C^{14}. Activity appeared in seven amino acids, particularly aspartic and glutamic acid and alanine, 15 minutes after the administration of the labeled substrate. This is indicative of the rate of a sequence of reactions including glycolysis, the citric acid cycle and transamination. The much heavier labeling level in glutamic acid supports the previous finding[10] that carbon reserves may be accumulated as glutamic acid in yeast cells during glucose assimilation. The labeling in these amino acids appeared in line with the respective known biosynthetic pathways in this organism.

(10) C. H. Wang, B. E. Christensen and V. H. Cheldelin, *J. Biol. Chem.*, **201**, 683 (1953).

CORVALLIS, OREGON

[CONTRIBUTION NO. 2043 FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY]

The Effect of Added Sucrose Upon the α-Chymotrypsin-catalyzed Hydrolysis of Chloroacetyl-L-tyrosinamide in Aqueous Solutions at 25° and *p*H 7.75[1]

BY HENRY J. SHINE AND CARL NIEMANN[2]

RECEIVED OCTOBER 10, 1955

The initial rate of the α-chymotrypsin-catalyzed hydrolysis of chloroacetyl-L-tyrosinamide in aqueous solutions at 25° and *p*H 7.75 and 0.02 *M* in the THAM component of a THAM-HCl buffer is increased by the addition of 0.5 *M* sucrose and it has been shown that the behavior of the above system in the presence of 0.5 *M* sucrose is qualitatively the same as that observed previously for the same system in the presence of 0.3 or 0.4 *M* sodium chloride.

In a recent communication from these laboratories[3] it was shown that the initial rate of the α-chymotrypsin-catalyzed hydrolysis of chloroacetyl-L-tyrosinamide in aqueous solutions at 25° and *p*H 7.75 and 0.02 *M* in the THAM[4] component of a THAM–HCl buffer is increased by the addition of either sodium or potassium chloride and that the increase in initial velocities is given by the relation $\log (v_0/v_0^0) = 0.30 \pm 0.01\sqrt{M}$ for values of *M*, *i.e.*, the molarity of the reaction system with respect to added sodium or potassium chloride, up to and possibly exceeding 1.5 *M*. Furthermore, from a study of the effect of 0.3 and 0.4 *M* sodium chloride in systems in which the concentration of the specific substrate was varied, it was found that the value of K_S was essentially independent of the concentration of the added sodium chloride at the two levels investigated, whereas the value of k_3 was increased by the amount given by the relation $\log (k_3/k_3^0) = 0.30 \pm 0.01\sqrt{M}$. Since it was not obvious that the effects noted above were to be interpreted solely in terms of the properties of electrolytes, it was decided to examine the consequences of the addition of several non-electrolytes such as sucrose, glucose and fructose to the same basic reaction system. The results of this latter investigation are the subject of this communication.

(1) Supported in part by a grant from Eli Lilly and Co.

(2) To whom inquiries regarding this article should be sent.

(3) H. J. Shine and C. Niemann, THIS JOURNAL, **77**, 4275 (1955).

(4) Tris-(hydroxymethyl)-aminomethane.

It is known[5,6] that for the α-chymotrypsin-catalyzed hydrolysis of chloroacetyl-L-tyrosinamide in aqueous solutions at 25° and *p*H 7.75 and 0.02 *M* in the THAM component of a THAM–HCl buffer that $K_S = 27 \pm 2 \times 10^{-3}$ *M* and $k_3 = 4.0 \pm 0.2 \times 10^{-3}$ *M*/min./mg. protein-nitrogen/ml. For the condition that [E] = 0.0677 mg. protein-nitrogen/ml. and $[S]_0 = 20 \times 10^{-3}$ *M* it follows that if the initial stages of the above reaction system can be described in terms of equation 1, as appears to be the case,[5,6] the expected initial velocity of the

$$-d[S]/dt = k_3[E][S]/(K_S + [S]) \qquad (1)$$

system specified above is $0.115 \pm 0.010 \times 10^{-3}$ *M*/min. It will be seen from the data given in Table I that the addition of 0.1 *M* sucrose, glucose or fructose to the reaction system described above led to values of v_0, *i.e.*, the corrected initial velocity[7] in the presence of added sugar, which were identical within the limits of experimental error with the value of v_0^0, *i.e.*, the expected initial velocity of the basic reaction system which contained no added sugar. However, when the concentration of added sugar was 0.5 *M* the observed values of v_0 (*cf.* Table I) were significantly greater than the corresponding value of v_0^0 and it was apparent that, at least in the case of added sucrose and fructose, the addition of a substantial amount of either of these compounds to the basic reaction system caused a significant increase in the initial velocity.

In order to gain further knowledge of the effects produced by added sugar the α-chymotrypsin-catalyzed hydrolysis of chloroacetyl-L-tyrosinamide in aqueous solutions at 25°, *p*H 7.75, 0.02 *M* in the THAM component of a THAM–HCl buffer and 0.5 *M* in sucrose was studied at an enzyme concentration of 0.0677 mg. protein-nitrogen/ml. and at specific substrate concentrations of from 10 to 30×10^{-3} *M*. It will be seen from the data summarized in Table II that the value of the ratio v_0/v_0^0 appears to be independent of the concentration of the specific substrate within the limits of experimental error and that the mean value of v_0/v_0^0 in this particular instance is 1.24, with a standard deviation of ±0.04. It is noteworthy that both sucrose and sodium chloride cause an increase in v_0^0, that in both cases the value of the ratio v_0/v_0^0 appears to be independent of the concentration of the specific substrate and that in the same basic reaction system v_0^0 is increased by 24% by 0.1 *M* sodium chloride or by 0.5 *M* sucrose.

For reaction systems that can be represented by eq. 2, where $K_S = (k_2 + k_3)/k_1$, and whose course can

$$E_f + S_f \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_3} E_f + P_{1f} + P_{2f} \qquad (2)$$

be described by eq. 1 it follows that if v_0/v_0^0, as defined in this communication, is independent of the concentration of the specific substrate, K_S must remain constant and k_3 must increase if the value of the ratio v_0/v_0^0 is greater than unity. In order to demonstrate that the results obtained in this study were consistent with the above conclusion the values of $1/v_0$ and $1/[S]_0$ which are given in Table II were fitted by the method of least squares to the line $y = a + bx$. With $y = 1/v_0$ and $x = 1/[S]_0$ this procedure gave a value of $a = 3204$ with a standard deviation of ±158 and a value of $b = 76.5$ with a standard deviation of ±2.5. From these latter values it follows that in this instance $K_S = b/a = 24 \pm 2 \times 10^{-3}$ *M* and $k_3 = 1/a[E] = 4.6 \pm 0.25 \times 10^{-3}$ *M*/min./mg. protein-nitrogen/ml. Therefore, for the systems under discussion the values of K_S in the absence and presence of 0.5 *M* sucrose are 27 ± 2 and $24 \pm 2 \times 10^{-3}$ *M*, respectively, and the corresponding values of k_3 are 4.0 ± 0.2 and $4.6 \pm 0.25 \times 10^{-3}$ *M*/min./mg. protein-nitrogen/ml., respectively. It is clear from a comparison of the above values that the value of K_S has remained constant within the limits of experimental error and that the value of k_3 has been increased by a significant amount by the presence of the added sucrose. A similar behavior was noted previously[3] in the case of added sodium chloride.

While the same qualitative effects are produced by the addition of an electrolyte such as sodium chloride and by a non-electrolyte such as sucrose to a common reaction system, it should be noted that the consequences of the addition of sucrose appear to stand in marked contrast to those noted upon the addition of another non-electrolyte, *i.e.*, methanol, to a comparable reaction system. Kaufman and Neurath[8] examined the α-chymotrypsin-catalyzed hydrolysis of acetyl-L-tyrosinamide in aqueous solutions at 25° and *p*H 7.8 which were 0.079 to 0.10 *M* in an unspecified phosphate buffer, 0.0, 2.08, 3.74 and 5.17 *M* in methanol and with sufficient lithium chloride added to the reaction system to bring the total ionic strength to 0.292. While these experimental conditions led to the simultaneous variation of at least three possible reaction parameters, *i.e.*, the concentration of the unspecified phosphate buffer, the concentration of lithium chloride and the concentration of methanol, the results obtained by the above investigators indicated that variation of the methanol concentration between the limits of 0.0 *M* and 5.17 *M* caused no change in the value of k_3, *i.e.*, $2.92 \pm 0.22 \times 10^{-3}$ *M*/min./mg. protein-nitrogen/ml., but did cause a uniform increase in the value of K_S from a value of 32.6×10^{-3} *M* in the absence of methanol to a value of 80.6×10^{-3} *M* in the presence of 5.17 *M* methanol. Thus, the addition of one non-electrolyte, *i.e.*, methanol, has been found to increase the value of K_S without causing a significant change in the value of k_3, whereas the addition of a second non-electrolyte, *i.e.*, sucrose, has been found to increase the value of k_3 without causing a significant change in the value of K_S.

Kaufman and Neurath[8] offered an explanation of the effects observed upon the addition of methanol to a system containing α-chymotrypsin and acetyl-L-tyrosinamide, but since their argument was based in large part upon the assumption that for the case at hand $K_S = k_3/k_1$, which since has been questioned[9] and in fact renounced by one of the above authors,[10] there appears to be no point in reviewing

(5) H. J. Shine and C. Niemann, THIS JOURNAL, **74**, 97 (1952).
(6) R. J. Foster and C. Niemann, *ibid.*, **77**, 1886 (1955).
(7) R. R. Jennings and C. Niemann, *ibid.*, **75**, 4687 (1953).
(8) S. Kaufman and H. Neurath, *J. Biol. Chem.*, **180**, 181 (1949).
(9) H. T. Huang and C. Niemann, THIS JOURNAL, **73**, 1541 (1951).
(10) N. M. Green and H. Neurath in H. Neurath and K. Bailey, "The Proteins," Academic Press, Inc., New York, N. Y., 1954, p. 1123.

their argument. Alternatively, we believe that it is probable that $K_S \doteq k_2/k_1$ in the absence of methanol and that in the presence of methanol K_S no longer can be interpreted solely in terms of an enzyme specific substrate interaction, but that the value of K_S observed in the latter instance is a constant composed on one hand of a value of K_S in the sense ordinarily used, and on the other hand of a value of a partition coefficient describing the partition of the specific substrate between the two components of the solvent system. In other words, in the presence of methanol the catalytically active site of the enzyme and the non-aqueous component of the solvent system are competing for the specific substrate. Since it is appreciated that the above hypothesis ignores any consequences of the possible interaction of the non-aqueous solvent component with the active site of the enzyme or of possible changes in the state or confirmation of the enzyme in a solvent system of varying composition it is clear that any definitive explanation of the effects produced by added methanol, or of sucrose, or of any other non-electrolyte, must be deferred until additional information is at hand.

Experimental

Enzyme Experiments.—The chloroacetyl-L-tyrosinamide preparation was that which was described previously.[5] The α-chymotrypsin preparation was an Armour product lot no. 90402. All experiments were conducted in aqueous solutions at 25° and *p*H 7.75. The reaction mixtures were 0.02 *M* in the THAM component and 0.01 *M* in the HCl component of a THAM–HCl buffer and of varying molarity with respect to the added sugar. The extent of reaction was determined titrimetrically by the method of Iselin and Niemann[11] as modified by Huang and Niemann.[9]

TABLE I

THE EFFECT OF 0.1 *M* AND 0.5 *M* SUCROSE, GLUCOSE AND FRUCTOSE ON THE α-CHYMOTRYPSIN-CATALYZED HYDROLYSIS OF CHLOROACETYL-L-TYROSINAMIDE[a,b]

Sugar, *M*	Sucrose v_0[c]	Sucrose v_0/v_0^0	Glucose v_0[c]	Glucose v_0/v_0^0	Fructose v_0[c]	Fructose v_0/v_0^0
0.1	0.115	1.00	0.115	1.00	0.122	1.06
.1	.106	0.92	.114	0.99	...	..
.1	...	..	.111	.97	...	..
		0.96		.99		1.06
.5	.138	1.20	.130	1.13	.126	1.10
.5	.143	1.24	.119	1.03	.157	1.37
.5	.135	1.17	.127	1.10	...	..
		1.20		1.09		1.23

[a] In aqueous solutions at 25° and *p*H 7.75 and 0.02 *M* in the THAM component of a THAM–HCl buffer and under conditions where [E] = 0.0677 mg. protein-nitrogen/ml. and $[S]_0 = 20 \times 10^{-3}$ *M*. [b] For the designated conditions and with $K_S = 27 \pm 2 \times 10^{-3}$ *M*[5,6] and $k_3 = 4.0 \pm 0.2 \times 10^{-3}$ *M*/min./mg. protein-nitrogen/ml.[5,6] The expected initial velocity in the absence of added sugar, *i.e.*, v_0^0, is $0.115 \pm 0.010 \times 10^{-3}$ *M*/min.[3]. [c] Corrected initial velocities evaluated by the method of Jennings and Niemann[7] and given in units of 10^{-3} *M*/min.

TABLE II

THE EFFECT OF 0.5 *M* SUCROSE ON THE α-CHYMOTRYPSIN-CATALYZED HYDROLYSIS OF CHLOROACETYL-L-TYROSINAMIDE[a]

$[S]_0$[b]	v_0[c]	v_0^0[d]	v_0/v_0^0	$1/v_0$	$1/[S]_0$
10	0.095	0.073	1.30	10526	100.00
10	.092	.073	1.26	10870	100.00
12.5	.106	.086	1.23	9434	80.00
12.5	.107	.086	1.24	9346	80.00
15	.122	.097	1.26	8197	66.66
17.5	.129	.106	1.22	7752	57.14
17.5	.128	.106	1.21	7813	57.14
20	.143	.115	1.24	6993	50.00
20	.135	.115	1.17	7407	50.00
20	.138	.115	1.20	7246	50.00
25	.163	.130	1.25	6135	40.00
25	.165	.130	1.27	6061	40.00
30	.176	.142	1.24	5682	33.33
30	.184	.142	1.30	5435	33.33

[a] In aqueous solution at 25° and *p*H 7.75 and 0.02 *M* in the THAM component of a THAM–HCl buffer with [E] = 0.0677 mg. protein-nitrogen/ml. [b] Given in units of 10^{-3} *M*. [c] Corrected initial velocities evaluated by the method of Jennings and Niemann[7] and given in units of 10^{-3} *M*/min. [d] Calculated on the basis of $K_S = 27 \times 10^{-3}$ *M* and $k_3 = 4 \times 10^{-3}$ *M*/min./mg. protein-nitrogen/ml. and given in units of 10^{-3} *M*/min.

Evaluation of Experimental Data.—The data obtained from each experiment were presented in the form of $([S]_0 - [S]_t)$ *vs.* *t* and $\ln([S]_0/[S]_t)$ *vs.* *t* plots and the corrected values of v_0 which were obtained from each of these plots by the method of Jennings and Niemann[7] were averaged to give the mean values of v_0 which are summarized in Tables I and II. It was found that any given value of v_0 obtained from a corrected $([S]_0 - [S]_t)$ *vs.* *t* plot was in good agreement with the corresponding value of v_0 obtained from a $\ln([S]_0/[S]_t)$ *vs.* *t* plot, in general, the difference between the two values being less than ±2%. It also should be noted that all of the experiments described in this study were conducted under conditions which were compatible with the methods used for the evaluation of the experimental data, *i.e.*, $[E] = 1.92 \times 10^{-5}$ *M*, $E'_S = [E]/K_S = 0.07 \times 10^{-2}$ and $S'_S = [S]/K_S = 0.4\text{–}1.2$.[6]

The authors wish to express their indebtedness to Dr. R. A. Bernhard and Mr. Charles Goebel for their assistance in the evaluation of the experimental data.

PASADENA 4, CALIFORNIA

(11) B. M. Iselin and C. Niemann, *J. Biol. Chem.*, **182**, 821 (1950).