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KINETIC ANALYSIS OF A NEW HUMAN ORNITHINE CARBAMOYLTRANSFERASE VARIANT

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Summary

A new human enzymatic variant was found in a patient with ornithine carbamoyltransferase (carbamoylphosphate:L-ornithine carbamoyltransferase, EC 2.1.3.3) deficiency. This mutant enzyme has decreased affinity, with an abnormal K_m value for ornithine (3—5-times greater than control at all pH values). The maximal velocity (V) varied with pH as a normal enzyme but the sigmoid curve obtained (V vs. pH) is shifted towards alkaline pH values. The pK of the functional catalytic group is 8.3 instead of 6.65 of a control enzyme. At its optimum pH the V of the mutant enzyme is greater than the V of the normal enzyme. Other mutant enzyme proteins with abnormal affinity for ornithine have already been described. They all are different from the reported here.

The mutation of ornithine carbamoyltransferase (carbamoylphosphate: L-ornithine carbamoyltransferase, EC 2.1.3.3) is one of the more common genetic defects occurring in the urea cycle. X-linkage of ornithine carbamoyltransferase has been suspected by the studies of the pedigrees of the affected families and then demonstrated [1]. Ornithine carbamoyltransferase deficiency induces in boys an hyperammonemia the onset of which may occur in the first day of life, in infancy or childhood [2,3]. The ornithine carbamoyltransferase activity is the second highest (after arginase) enzymatic activity and is never a limiting step of the urea cycle; in addition the mutant enzymes may retain a noticeable percentage of the normal activity. These data explain how some affected hemizygote males survive beyond the neonatal period. The study of

kinetics of ornithine carbamoyltransferase variants is easy, even with a low residual activity. Several such studies have been reported: these mutations may lead to a decreased affinity for carbamoylphosphate [4—6] or for ornithine [7—10], the latter being apparently the more common abnormality observed. A new variant with a decreased affinity for ornithine and unusual activity at alkaline pH is reported here.

Materials and Methods

The patient was a two-year old boy hospitalized in a premortem state. A severe hyperammonemia was discovered and the child died quickly. The patient's liver tissue was removed shortly after death and stored immediately at —80°C. The liver of a child who died from a cause other than urea cycle deficiency was used as control. Liver fragments were weighted and homogenized in 14 vols. of cold 0.1% cetyltrimethyl ammonium bromide. In the kinetic studies one of the two substrates (ornithine and carbamoylphosphate) was at the final concentration of 5 mM while the other ranged from 0.04 mM to 5 mM. The effects of pH variation were studied in 0.2 M maleate-NaOH buffer (pH 6.0—6.75) or in 0.2 M triethanolamine-HCl buffer (pH 7.0—9.0); homogenates were diluted in 1% bovine serum albumin according to their activities previously determined with saturating concentrations of the two substrates. The final volume of 1 ml containing 100 µl of the diluted homogenate was incubated for 10 min at 37°C and the reaction was stopped by the addition of 500 µl of 10% trichloroacetic acid. Each assay was carried out twice together with a blank containing the substrates and boiled homogenate. The citrulline formed was assayed in the supernatant of centrifugation by an automated colorimetric method [11,12]. Other urea cycle enzymes were assayed by the method of Brown and Cohen [13]. Protein contents were determined according to the method of Lowry [14].

Results

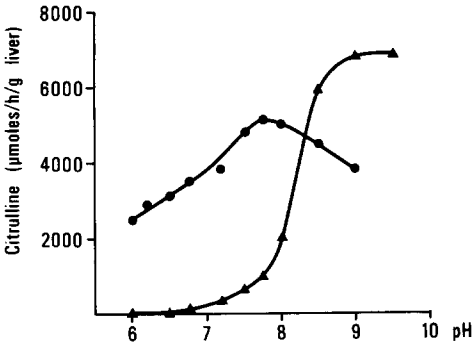
Table I shows that the activities of carbamoylphosphate synthetase, argininosuccinase and arginase were normal in the patient's liver while ornithine carbamoyltransferase activity determined at its usual optimum pH was relatively high for an authentic ornithine carbamoyltransferase deficiency (30% of the

TABLE I

ENZYMATIC ACTIVITIES IN THE UREA CYCLE

Results are expressed in µmol of product formed per h and per mg of protein (citrulline for ornithine carbamoylphosphate synthetase; urea for argininosuccinase and arginase). The number of determinations in parenthesis.

	Control	Patient
Carbamoylphosphate synthetase	0.90 ± 0.30 (10)	0.55 ± 0.90 (2)
Ornithine carbamoyl transferase	35.5 ± 9.6 (10)	11.9 ± 3.0 (5)
Argininosuccinase	1.0 ± 0.3 (10)	0.8
Arginase	435 ± 120 (10)	455



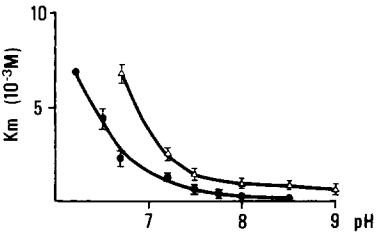


Fig. 1. Activities of the mutation (▲———▲) and the normal (●———●) ornithine carbamoyltransferase as a function of pH. The final concentrations of ornithine and carbamoylphosphate are 5 mM.

Fig. 2. Variations of K_m (ornithine) of mutant (△———△) and normal (●———●) ornithine carbamoyltransferase as a function of pH. The standard errors of the estimation of K_m values are shown on the graph.

control value) but the plot of this enzymatic activity vs. pH was quite abnormal (Fig. 1). The activity was near zero upto pH 6.75, then increased dramatically upto pH 9.0 and was not modified by increasing the alkalinity until pH 9.5. Moreover at pH 9.0 the mutant ornithine carbamoyltransferase activity was twice the activity of the normal enzyme. At pH 8.0 the K_m of ornithine carbamoyltransferase for carbamoylphosphate was normal in the patient's liver (0.13 mM as compared to 0.16 ± 0.08 mM obtained in the control liver). On the other hand the K_m for ornithine was found to be abnormal at pH 8.0 and was then measured at several pH values comparative to a normal enzyme. The results are shown in Table II and Fig. 2. The observed variations of K_m for ornithine as a function of pH for the normal enzyme were quite similar to those found by Snodgrass [15]. The K_m values for the zwitterion form of ornithine (which concentrations were calculated at each pH) were not modified by the pH variation. The K_m values observed for the variant ornithine carba-

TABLE II

EFFECTS OF pH ON K_m AND V OF ORNITHINE CARBAMOYLTRANSFERASE

Results are obtained with the method of least-mean squares with weighting in $1/V^2$. n.d., not determined.

pH	K_m ornithine		K_m zwitterion		V	
	Control	Patient	Control	Patient	Control	Patient
6.20	6.80	n.d.	0.022	n.d.	16	n.d.
6.50	4.20	n.d.	0.026	n.d.	24	n.d.
6.75	2.20	6.80	0.025	0.066	33	3
7.20	1.20	2.50	0.037	0.077	36	4
7.50	0.70	1.40	0.043	0.066	39	7
7.75	0.35	n.d.	0.037	n.d.	41	n.d.
8.00	0.22	1.00	0.038	0.190	40	16
8.50	0.14	0.95	0.043	0.390	38	47
9.00	n.d.	0.70	n.d.	0.470	n.d.	60

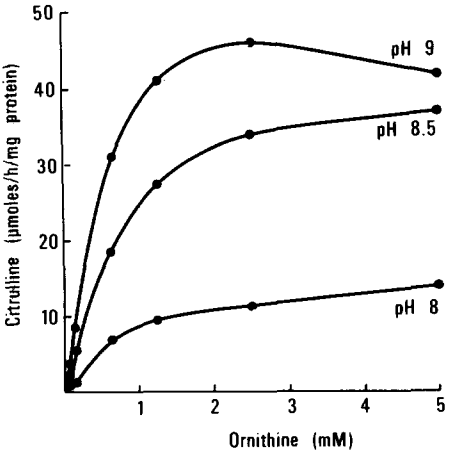


Fig. 3. Effect of the pH on the activities of the mutant ornithine carbamoyltransferase as a function of ornithine concentration.

moyltransferase were always higher than for the control enzyme whatever the pH used and the K_m values for the zwitterion of ornithine were unmodified at low pH values but increased at an alkaline pH. The inhibition by an excess of ornithine which normally appears at 5 mM for pH 7.5 and 2.5 mM for pH 8.0, was not found at these pH values for the variant enzyme but only at pH 9.0 for 5 mM ornithine (Fig. 3).

The V vs. pH plot was a sigmoid curve even for the normal or the mutant enzyme (Fig. 4). As for most enzymes, these curves reflect the protonation and

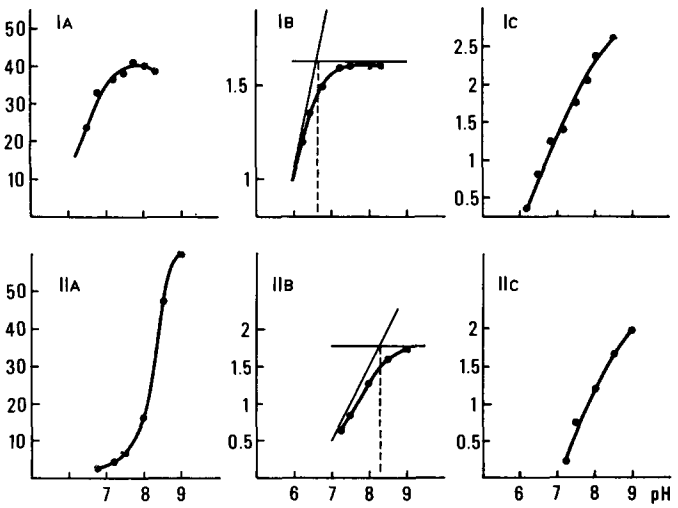


Fig. 4. Effect of pH (in abscissae) on kinetic constants. In ordinate: A, V; B, log V and C, log V/K_m. Upper curves (I) for normal ornithine carbamoyltransferase; lower curves (II) for mutant ornithine carbamoyltransferase.

deprotonation of a functional group directly implicated in the catalysis. The logarithmic plot of V vs. pH (Fig. 4) allows easier determination of the pH of this functional group: the intersection point of two lines, one of slope 1 and the second of slope 0. This point was found at 6.65 for the normal enzyme and 8.30 for the mutant one. For some enzymes the plot log V/log K_m vs. pH may help to determine the pH of the functional group of the free enzyme. This method cannot be used for ornithine carbamoyltransferase, either for the normal or the mutant enzyme because at the pH values used, there is no inflexion point to allow the drawing of a line of slope 0 (Fig. 4). The graph log K_m vs. pH (not shown) is no more explicit.

Discussion

The present study seems to imply that there is a new mutation of ornithine carbamoyltransferase characterized by: (a) a mild decrease of activity at the normal optimum pH (30% of the control value); (b) a curve of velocities vs. pH shifted toward alkaline pH values; (c) the same shift of the V curve and (d) at pH 9.0 a V higher with variant ornithine carbamoyltransferase than with the normal enzyme. This can be explained by two hypotheses: either the mutant enzyme is more stable than the normal one at alkaline pH (thereby allowing catalytic activity to be fully expressed at this pH) or there is a much higher level of the enzymic protein in the patient's liver than normal, due to a regulating mechanism which may be either increased synthesis or decreased breakdown of the protein enzyme. This latter hypothesis could be checked by an immunological assay of the mutant protein with specific rabbit antisera. Unfortunately this could not be done because we had no more hepatic material.

The pK of the functional catalytic group is shifted from 6.65 for the normal enzyme to 8.3 for the variant. This pK value of 6.65 is thought to be the pK of an imidazole group of histidine [15]. However, we cannot conclude that histidine in replaced in the abnormal ornithine carbamoyltransferase by another amino acid. A pK of 8.3 is close to the pK of a SH group of cysteine (8.8—9.1) but for histidine to be replaced by cysteine, two codon bases must be changed which is not consistent with a point mutation. The most likely hypothesis is that of a mutation leading only to a change in the conformation of the active site. This conformational change could explain the abnormalities found in the affinity for ornithine. The K_m values of the variant enzyme are always higher than control enzyme at all pH values but the values of K_m decrease with the pH at about the same rate. The inhibition by an excess of substrate is also the same as for a normal enzyme, but at higher pH. We suggest that in this variant, the active site could be less accessible to its substrate, while still behaving in the same way towards it.

In the literature nine human mutations with abnormal affinity of ornithine carbamoyltransferase towards its substrates are described: four with respect to ornithine [7—10], three towards carbamoylphosphate [4—6] and two towards both substrates [16,17]. Among the four variants with decreased affinity for ornithine, three have the highest activity at high pH rather than at the optimum pH of a normal ornithine carbamoyltransferase. In only one case [7] was the

catalytic pK determined at 8.7. In the two others, this pK may be approximately determined from the curve of activity. It is 7.7 for one [8] and 7.9 for the other [10]. The functional pK is always shifted towards alkaline pH values. In these four mutations, the K_m values for ornithine are always higher than those found in our own variant: 100-times the control in one [7], 15-times the control in two others and 5-times the normal in the fourth [8].

The mutant enzyme reported here seems to be completely different: the affinity for ornithine is less altered, the pK of the catalytic group is different, while a very high V at pH 9.0 is observed. All four mutants already described seem to differ from each other. However, one cannot be sure of this, due to insufficient kinetic studies in two cases [8,9].

Three mutations yielding a decrease in ornithine carbamoyltransferase affinity for carbamoyl phosphate have been described. For one of them the activity is very poor at pH 7.0 and is near normal at pH 8.5. For another [6] the optimum pH is 8.5 instead of 7.5 for controls. Also in one mutation [16] where the affinity for both the substrates appears to be increased, the maximum of activity is also shifted towards alkaline pH values. All the mutations in human ornithine carbamoyltransferase appear to shift the maximum of activity at alkaline pH, and this observation cannot account for a peculiar abnormality of K_m. Further studies on other ornithine carbamoyltransferase mutants are required to be sure of the generality of this phenomenon.

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