The Table shows the total (free and bound) pyridine nucleotide concentrations found in the 2 groups of rats at the end of the 10-min contraction period. The value for muscle DPNH showed a statistically significant difference between the 2 groups. The alteration in the DPNH value was sufficient to cause a significant difference in the pyridine nucleotide oxidized-to-reduced ratio. As a result of intra-group variation in work performance and oxidized-to-reduced ratio, the sum of the oxidized pyridine nucleotides divided by the sum of the reduced nucleotides did not (and mathematically should not) equal the mean of the individually calculated oxidized-to-reduced ratios.

The statistical relationships between the muscle oxidized-to-reduced ratio (y) and work performance (x) were as follows: non-trained animals, $y = 7.7811 - 0.0041x$; trained animals, $y = 9.9797 - 0.0006x$. Work performance equaled 2645 ± 142 meter-grams for the non-trained animals and 2715 ± 145 for the trained animals. These relationships indicate that, within each group, the greater the work performance the more capable the cell was of tolerating a more reduced state. Furthermore, for any given work performance level, the muscle of the trained animal had a higher oxidized-to-reduced ratio than the muscle of the non-trained animal.

It seems likely that the higher oxidized-to-reduced ratio in the muscles of the trained rats indicates an increased capacity of the mitochondria to keep the cell in a more oxidized state. Currently, we are investigating alterations in the oxidation-reduction state of the various subcellular compartments within the muscle cell[5].

Pyridine nucleotide concentrations

	Non-trained	Trained
DPN	970.8 ± 75.4	1027.3 ± 90.0
DPNH	262.8 ± 51.5[a]	124.9 ± 19.4
TPN	8.1 ± 1.5	10.2 ± 3.1
TPNH	106.4 ± 30.9	66.9 ± 15.9
Oxidized/reduced	4.4 ± 0.7[a]	7.3 ± 1.2
Total pyridine nucleotides	1348.1 ± 109.4	1229.3 ± 94.2

Values are in μmoles/kg of tissue ± S.E.M. $N = 18$. The pyridine nucleotide oxidized to reduced ratios represent the mean of the ratios (DPN+TPN)/(DPNH+TPNH) for each experiment. [a] $p < 0.05$.

Zusammenfassung. Die Pyridinnukleotid-Gehalte von trainierten und untrainierten Muskeln werden nach zehnminütiger Arbeit miteinander verglichen. Das Verhältnis der oxidierten zu reduzierten Pyridinnukleotiden ist beim trainierten Muskel stärker oxidiert als beim nicht trainierten Muskel.

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Inactivation of Cystathionase and of Cysteine Sulfinic Acid Decarboxylase by Proteolytic Enzymes: Effect of Pyridoxal Phosphate

It has been reported[1,2] that the activities of crude and of partially prufied preparations of rat liver cystathionase (L homoserine hydro-lyase EC 4.2.1.15) and cysteine sulfinic acid (CSA) decarboxylase (L cysteine sulfinate carboxy-lyase EC 4.1.1.29) are, at least in part, protected against heat denaturation and urea inactivation by their coenzyme, pyridoxal phosphate (PLP), whereas pyridoxine and other derivatives (pyridoxamine, pyridoxal, pyridoxamine phosphate) afford no protection. It appeared of interest to undertake studies which would attempt to determine whether a similar protection can be observed in other situations, more closely related to the conditions of physiological degradation of tissue proteins. In this paper, studies of the action of proteolytic enzymes, trypsin (Worthington), α-chymotrypsin (Seravac Lab.) and pronase (Calbiochem) on partially purified cystathionase and CSA decarboxylase are described. Furthermore, to gain additional insight as to the role that PLP plays, the effect of PLP on these reactions of proteolysis was investigated and is also described. A preliminary report of some of these findings has already appeared[3].

Cystathionase and CSA decarboxylase were extracted from Wistar male rat liver and partially purified according to the procedures already described[2] with slight modifications: in the preparation of CSA decarboxylase, the supernatant was heated to 55 °C for 5 min and centrifuged before addition of ammonium sulphate[4]; in the preparation of cystathionase, dithiothreitol (DTT) was omitted in the solutions used and the preparation was in each case lyophilized.

It is noteworthy that cystathionase was obtained essentially in the form of apoenzyme, for, before or after lyophilization, it exhibited a very small activity unless PLP was added to the incubation mixture, whereas the activity of purified CSA decarboxylase was the same when measured with or without addition of PLP.

The measurements of enzymic activities were carried out according to the procedures previously described[5,6], and the modifications already reported[1,2].

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For the digestion of CSA decarboxylase a routine procedure has been set up: incubation of the enzyme at 25 °C in 0.02 *M* phosphate buffer pH 7.86 with a solution of trypsin (or α-chymotrypsin, or pronase) prepared in the same buffer, the concentration of the proteolytic enzyme being, in each case, 40 μg/ml enzyme. Whenever the effect of PLP on these reactions of proteolysis was investigated, PLP (final concentration $10^{-4}M$) was added to the enzymic preparation before addition of the proteolytic enzyme. After 1 h and 2 h of incubation, portions of the incubated mixtures were withdrawn and 10-fold diluted for subsequent enzyme assay. As the concentration of trypsin (or α-chymotrypsin or pronase) in the assay was 4 μg, we controlled that addition of such a quantity of proteolytic enzymes to a non-incubated enzyme was without significant effect on the enzymic activity.

Furthermore, as CSA decarboxylase is highly susceptible to heat denaturation[1,2], we also measured the activity of the enzyme, similarly incubated with and without PLP for 2 h at 25 °C, without proteolytic enzymes.

For the digestion of cystathionase a routine procedure was devised: incubation of the enzyme dissolved in 0.02 *M* phosphate buffer pH 7.86 at 37 °C with a solution of the proteolytic enzyme at a concentration of 30 μg/ml enzyme. Whenever the effect of PLP (or of pyridoxine and other derivatives of pyridoxine) was investigated, PLP $10^{-4}M$ (or other derivatives at the same concentration) was added to the enzymic preparation before addition of the proteolytic enzyme. Aliquots of incubation mixtures were withdrawn after 30 min and 60 min of incubation and 10-fold diluted for subsequent enzyme assay. Appropriate controls were run under conditions similar to the experimental ones.

In Figure 1 are reported, expressed as percentage of the activity of the untreated enzyme assayed in the same conditions, the residual activity of CSA decarboxylase measured after 1 h and 2 h of incubation without proteolytic enzyme (curves Ia and Ib), with trypsin (curves IIa and IIb), with chymotrypsin (curves IIIa and IIIb) and with pronase (curves IVa and IVb).

As shown in Figure 1, incubation of partially purified CSA decarboxylase at 25 °C (curve Ia), in absence of proteolytic enzymes led to a slight fall of activity, for the enzyme after 2 h of incubation retained roughly 90% of the initial activity. The stability of the enzyme is slightly better when the incubation was carried out in presence of PLP (curve Ib).

On the other hand, the results clearly suggest that the inactivation of CSA decarboxylase by proteolysis differed markedly according to the proteolytic enzyme used. Indeed treatment of the enzyme with trypsin for 2 h decreased the activity of approximately 25% (curve IIa) whereas, in the same conditions, the incubation with chymotrypsin (curve IIIa) did cause 50% of inactivation, and the incubation with pronase (curve IVa) resulted in 75% of inactivation.

It is obvious that PLP was without effect on proteolysis of CSA decarboxylase since the residual activity was, respectively, whatever the proteolytic enzyme used, identical whether the incubations were carried out with or without addition of PLP to the mixture.

In Figure 2 are shown results of representative experiments using cystathionase. Incubation of cystathionase without proteolytic enzymes for 1 h at 37 °C led to a decrease (about 20%) of activity (curve Ia), and addition of PLP (curve Ib) prevented this fall of activity. It is of interest that when DTT was included in the mixtures used for preparation of the enzyme, or added to the enzyme before incubation, such a decrease of activity was not observed.

Incubation of the enzyme with trypsin for 1 h decreased the activity of approximately 40% (curve IIa) whereas incubation with pronase (curve IIIa) and with chymotrypsin (curve IVa) resulted in, respectively, 60% and 70% of inactivation. However, when PLP was added to the enzyme before incubation with proteolytic enzymes, a partial protection was observed, and this protection was

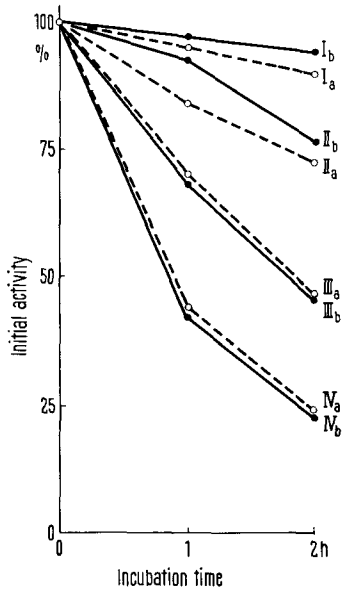

Fig. 1. Time course of proteolysis of cysteine sulfinic acid decarboxylase incubated at 25 °C. a, ○—○—○, without PLP; b, +—+—+, with PLP; I, control; II, with trypsin; III, with chrymotrypsin; IV, with pronase.

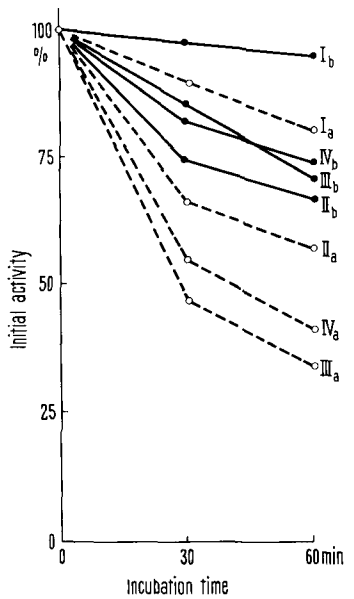

Fig. 2. Time course of proteolysis of cystathionase incubated at 37 °C. a, ○—○—○, without PLP; b, +—+—+, with PLP; I, control; II, with trypsin; III, with chymotrypsin; IV, with pronase.

approximately of the same magnitude, whatever the proteolytic enzyme used.

Furthermore, we observed that pyridoxine and other derivatives afford no protection of cystathionase against proteolysis, and that inclusion of 3 µg of trypsin (or chymotrypsin or pronase) in the assay of a non-incubated enzyme resulted in a slight fall of activity (approximately 10–15%).

The evidence at present available indicates that, for both enzymes, chymotrypsin is the most efficient among the physiological proteolytic enzymes.

Addition of pyridoxal phosphate is without effect on the inactivation of CSA decarboxylase, whereas the digestion of cystathionase is decreased when the incubations were carried out in presence of pyridoxal phosphate. Finally, we have also observed that with regard to this protection, pyridoxal phosphate is specific. It must be kept in mind that CSA decarboxylase was obtained as holoenzyme, whereas cystathionase was available as apoenzyme. It is therefore tempting to speculate that the molecular conformation of cystathionase is modified whether the enzyme exists in the form of apoenzyme or in the form of holoenzyme, and that apocystathionase is more susceptible to inactivation by proteolytic enzymes than holocystathionase.

Conformational changes in enzymic proteins leading to modified susceptibility to proteolysis have already been achieved for other enzymes[7-9].

Anyway these results clearly suggest that the level of PLP in the liver has a role in imparting stability of cystathionase in this tissue. Secondly, as cystathionase is more susceptible to thermal denaturation when DTT was not included in the incubated mixture during the assay, this finding suggests that the integrity of some sulfhydryl groups contribute to the stability of the enzyme.

On the other hand, PLP partially protects CSA decarboxylase against heat denaturation and thermal inactivation[1,2] whereas it is ineffective against proteolysis. Whatever the reason for the different behaviour of PLP in the 2 situations, it seems of interest to emphasize this fact, for the explanation of which more research is needed.

Résumé. Les résultats obtenus lors de l'étude de la protéolyse, par la trypsine, l'α-chymotrypsine et la pronase, de préparations partiellement purifiées de cystathionase et de décarboxylase de l'acide cysteine sulfinique sont décrits. Il apparait que, pour la cystathionase, la sensibilité à la protéolyse est différente selon que l'on utilise l'apoenzyme ou l'holoenzyme.

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Einfluss des Schwefeldioxids auf den Gehalt freier Saccharide und Aminosäuren in Erbsen-Keimpflanzen

Der Mechanismus von toxischen Einwirkungen des Schwefeldioxids auf die Pflanzen wurde noch nicht völlig geklärt, obwohl dieses Problem schon in vielen Studien bearbeitet wurde[1-12]. In manchen Arbeiten wurde der Einfluss des SO_2 auf den Stoffwechsel der Saccharide und die Photosynthese erwähnt[3-11]. Es kommt zu einer Verminderung der Konzentration der Saccharide, wobei besonders die Konzentration der Saccharose abfällt[5,7] und die Stärke hydrolytisch zersetzt wird[11]. Ausserdem wurden in den mit SO_2 vergifteten Pflanzen auch Störungen des Aminosäurestoffwechsels beobachtet und es zeigte sich eine Verminderung des Glutaminsäure-Gehalts[7].

In Rahmen unserer Versuche über die biochemischen Grundlagen der SO_2-Toxizität bei Pflanzen benutzten wir Erbsenkeimlinge, also Pflanzen mit intensiv verlaufendem Metabolismus, bei denen die Empfindlickeit gegen toxische Einwirkung des SO_2 gross ist. Die Intoxikation mit SO_2 wurde bei grünen sowie etiolierten Keimlingen durchgeführt, um abzuklären, ob SO_2 den Saccharidstoffwechsel via Photosynthese wirklich stört. Die Veränderungen des Saccharidgehalts und der freien Aminosäuren in den vergifteten Erbsenkeimpflanzen werden diskutiert.

Wir benutzten 14–15tägige Erbsenkeimpflanzen, die bei Licht oder Dunkelheit auf mit destilliertem Wasser befeuchtetem Filtrierpapier kultiviert wurden. Die Intoxikation der Keimpflanzen mit SO_2 wurde in grösseren Erlenmeyer-Kolben (2000 cm^3), die mit Schliffverschluss versehen waren, durchgeführt. Die Keimpflanzen wurden auf feuchtes Filtrierpapier auf den Boden des Kolbens gegeben und die nötige 1% Atmosphäre mittels Zersetzung von festem Na_2SO_3 mit 20% Schwefelsäure in kleiner Glasschale hergestellt. Die Einwirkung des SO_2 dauerte bei Licht und Dunkelheit 24, 48, 72 und 96 h. Die Kontrollpflanzen ohne SO_2 waren während derselben Zeit in gleichen Gefässen an der Luft. Nach Beendigung der gewählten Intoxikationszeit wurden die Versuchs-

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