BIOCHEMISTRY

Enzymatic Cycle of Inorganic Nitrogen in Animal Tissues

It has previously been demonstrated that oximino compounds are widely distributed in organisms[1], and that two enzymes, named oximase[2] and transoximase[3], which participate in oxime metabolism, exist in some animals. In pursuing the behaviour of hydroxylamine in living cells, we have now established that there is an enzymatic cycle between ammonia and nitrate.

An example of the procedure of preparing the enzyme solution was as follows: 30 gm. of hen liver were homogenized with 90 ml. of 0·1 *M* phosphate buffer of *p*H 7·5, and after 30 min. at 35° C., the homogenate was centrifuged at 3,000 r.p.m. for 10 min. at 0° C. The supernatant was treated with acetone at −10° C., and the precipitate which was produced at 33–55 per cent concentration of acetone was gathered by centrifuging at 4,000 r.p.m. for 7 min. 1 gm. of this precipitate was dissolved in 20 ml. of water, dialysed against running water for 3 hr. and centrifuged at 3,000 r.p.m. for 7 min. The supernatant was used as enzyme solution; it contains about 0·2 mgm. protein nitrogen in 1 ml. As the enzyme is labile, the solution must be prepared daily from fresh liver.

To measure the reducing action of enzyme preparation, 1 ml. of 0·001 *M* substrate was mixed with 1 ml. of enzyme solution, 1 ml. of 0·013 per cent reduced diphosphopyridine nucleotide solution, 0·5 ml. of 0·005 per cent flavin adenine dinucleotide solution and 0·5 ml. of 0·1 *M* phosphate buffer of *p*H 7·5. A mixture containing no substrate served as control. The reaction temperature was 30° C. and the time 20 min. The enzymatic activity was determined spectrophotometrically and expressed in decrease (−) of absorption (−log *T*) at 340 mμ. Some of the results obtained are given in Table 1.

Nitrate, nitrite, hyponitrite and hydroxylamine are thus reduced by an enzyme system from hen liver. It was further found that the enzymes manifest the highest activity at *p*H 7·7 and 30° C., and that they are completely destroyed by heating at 50° C. for 5 min. Reduced triphosphopyridine nucleotide and flavin mononucleotide do not act as coenzymes for the reduction by liver enzymes.

Recently, hyponitrite reductase was demonstrated in *Neurospora* by Medina and Nicholas[4], and hydroxylamine reductase was extracted from a halotolerant *Micrococcus* by Taniguchi, Sato and Egami[5] as well as from *Neurospora* by Zucker and Nason[6]. It remains to be seen whether the enzyme system of hen liver contains the same reductases as those found in microbes.

For estimating the dehydrogenating action of liver enzyme system, the freshly prepared enzyme solution was heated beforehand at 40° C. for 5 min., because the reductases are more easily inactivated by heating than the dehydrogenases. The reaction mixture consisted of 1 ml. of 0·001 *M* substrate, 1 ml. of enzyme solution, 1 ml. of 0·013 per cent diphosphopyridine nucleotide solution and 1 ml. of 0·05 *M* *tris*-buffer of *p*H 7·5. The control contained no substrate. The increase (+) of −log *T* at 340 mμ was determined after a 20 min. incubation at 30° C. Some of the results are given in Table 2.

Table 1. Reducing Action of Liver Enzyme System

Substrate :	$NaNO_3$	$NaNO_2$	$Na_2N_2O_2$	NH_2OH	$(NH_4)_2SO_4$	Without substrate
$-\log T$	−0·105	−0·143	−0·124	−0·122	−0·024	−0·022

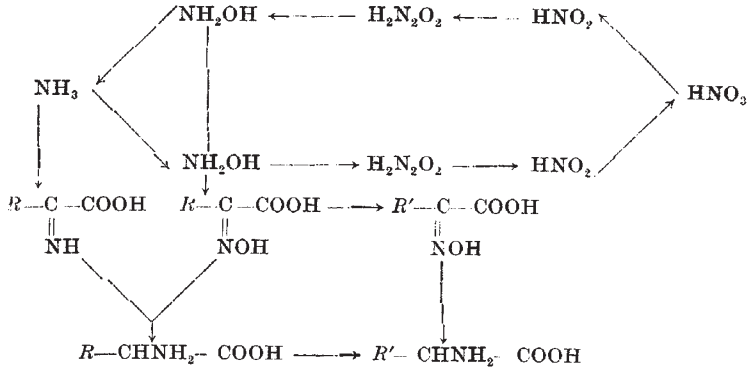
Table 2. Dehydrogenating Action of Liver Enzyme System

Substrate :	$(NH_4)_2SO_4$	NH_2OH	$Na_2N_2O_2$	$NaNO_2$	$NaNO_3$	Without substrate
$-\log T$	+0·120	+0·095	+0·138	+0·084	+0·021	+0·015

Ammonia, hydroxylamine, hyponitrite and nitrite are thus dehydrogenated by the liver enzyme preparation. The dehydrogenation, however, does not proceed in a mixture containing phosphate buffer. It was further observed that the enzymes display the highest activity at *p*H 7·3–7·5 and 30° C., and that they are destroyed by heating at 60° C. for 5 min. Triphosphopyridine nucleotide, flavin adenine dinucleotide and flavin mononucleotide show no coenzymatic action for the dehydrogenation.

Klausmeier and Bard[7] reported recently that an ammonium dehydrogenase is present in *Bacillus subtilis*. However, both tri- and di-phosphopyridine nucleotide act as coenzymes for the bacterial dehydrogenase and ammonium sulphate does not serve as its substrate.

The enzyme solution was also prepared from silkworms, and its reducing and dehydrogenating actions were studied in the same way. Similar results were obtained.



Scheme 1. Inorganic nitrogen cycle and amino-acid formation

The combination of inorganic nitrogen cycle and amino-acid formation, including the transoximase–oximase reaction, can thus be formulated as in Scheme 1.

K. Yamafuji
Y. Osajima
H. Omura

Agricultural Chemistry Institute,
Kyushu University,
Fukuoka, Japan. Sept. 5.

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