## Glucosamine-6-phosphate deaminase*

The formation of hexose phosphate from N-acetylglucosamine (2-acetamido-2-deoxy-D-glucose) and ATP by cell-free extracts of *Escherichia coli* was reported by SOODAK[1]. He postulated that at least four steps were involved in the reaction sequence. Recently, the deamination of glucosamine-6-phosphate (Gm-6-P; 2-amino-2-deoxy-D-glucose-6-phosphate) by extracts of *E. coli* has been studied by several investigators (ROSEMAN[2], WOLFE AND NAKADA[3], ASAHINA AND YAMAHA[4]). The product of this reaction, however, has not been clearly defined.

In the present investigation the conversion of crystalline[2] Gm-6-P to hexose phosphate by cell-free extracts of *E. coli* B was studied. Our results indicate that fructose-6-phosphate (F-6-P) is the initial product.

*E. coli* B was grown in a mineral medium containing glucosamine as the sole source of carbon. The specific activity of cells grown on glucosamine is ten times that of cells grown on glucose.

The deamination of Gm-6-P to hexose phosphate proceeds rapidly in the presence of crude extract (Table I) but it is impossible to determine whether glucose-6-phosphate (G-6-P) or F-6-P is the first product of the reaction. An active phosphoglucose isomerase is present in the crude extract which rapidly interconverts the F-6-P and G-6-P formed; consequently, the deamination step appears to be the rate controlling reaction. In addition, some hydrolysis of the sugar phosphates occurs; this may explain both the fact that hexose phosphate formation proceeds to a maximum in ten minutes and then decreases, and the fact that the ratio of F-6-P to G-6-P is somewhat higher than expected.

Purification of the extract (Table II) removed all detectable phosphoglucose isomerase activity. Incubation of the purified enzyme with Gm-6-P (2.48 μmol/ml) yielded the following balance

TABLE I

DEAMINATION OF GLUCOSAMINE-6-PHOSPHATE WITH CRUDE *E. coli* EXTRACT

| | *Hexose-6-phosphate* | | | |
|---|---|---|---|---|
| *Time min* | *Total μmol/ml* | *G-6-P μmol/ml* | *F-6-P μmol/ml* | *F-6-P as % of total* |
| 5 | 1.56 | 0.91 | 0.65 | 42 |
| 10 | 2.29 | 1.40 | 0.89 | 39 |
| 15 | 2.10 | 1.25 | 0.85 | 40 |

The incubation mixture contained 0.2 ml of neutralized 0.05 *M* Gm-6-P; 1.0 ml of 0.05 *M* Tris buffer, pH 7.9; and 0.05 ml of enzyme. G-6-P was determined with "Zwischenferment" and TPN. F-6-P was determined by a modified ROE method.

TABLE II

PURIFICATION OF GLUCOSAMINE-6-PHOSPHATE DEAMINASE

| *Step No.* | *Purification factor* | *% Yield of activity* | *Isomerase activity* |
|---|---|---|---|
| 1. Crude extract | 1.0 | 100 | 4+ |
| 2. Protamine | 4.5 | 70 | 4+ |
| 3. Heat, pH | 8.3 | 70 | 4+ |
| 4. Calcium phosphate gel I | 15.0 | 44 | 1+ |
| 5. Calcium phosphate Gel II | 13.0 | 30 | 0 |

Protein was determined nephelometrically with sulfosalicylic acid. The incubation mixture was the same as indicated in Table I.

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data: Gm-6-P (—0.77 μmol/ml), F-6-P (+0.80 μmol/ml) and $NH_3$ (+0.79 μmol/ml). There was no detectable G-6-P in the incubation mixture when analyzed with yeast "Zwischenferment" and TPN.

LELOIR AND CARDINI[5] have shown that extracts of *Neurospora crassa* when incubated with F-6-P or G-6-P and glutamine yielded Gm-6-P and glutamic acid. By removing isomerase from extracts of this mold, BLUMENTHAL *et al.*[6] were able to demonstrate that F-6-P was required by the enzyme which synthesizes Gm-6-P.

Apparently, the *E. coli* enzyme does not simply reverse the reaction catalyzed by the *N. crassa* extract since (a) glutamic acid does not stimulate the reaction, and (b) a slight reversibility occurs when the purified enzyme is incubated with F-6-P and $NH_3$, whereas no detectable reversibility occurs with F-6-P and glutamine.

At the present stage of purification no cofactor requirements have been detected for the Gm-6-P deaminase.

Under optimal conditions the specific activity of the most purified preparation (45 ×) was 1.2 mmol F-6-P formed/mg of protein/15 minutes.

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## Sulfate-dependent exchange of pyrophosphate with nucleotide phosphate*

The enzymic formation of sulfate esters from inorganic sulfate and hydroxyl compounds requires the participation of adenosine triphosphate (ATP) in an initial activation of sulfate, followed by a transfer of the sulfate group to the hydroxyl acceptor[1-3]. In order to obtain information regarding the nature of the activated sulfate intermediate, studies of the compounds liberated from ATP concomitant with sulfate esterification have been undertaken.

The enzyme system used in these experiments was obtained from an homogenate of rat liver prepared as previously described[2], except that homogenization was in 0.05 *M* Tris acetate buffer, pH 7.5, instead of KCl solution. The supernatant solution obtained after centrifugation was further treated to reduce by 60–80 % the high level of non-sulfate-dependent orthophosphate ($P_i$) liberating activity which seriously interferes with studies of the reaction mechanism.

The results presented in Table I illustrate the sulfate-dependent exchange of pyrophosphate with nucleotide phosphate. The latter was separated from $P_i$ and pyrophosphate by the charcoal adsorption method of CRANE AND LIPMANN[4]. It can be seen that there was some exchange of $P_i$ and nucleotide phosphate which was not significantly increased by the addition of sulfate. An equivalent amount of pyrophosphate was incorporated only to the same extent as $P_i$ in the absence of added sulfate (reflecting the rapid breakdown of pyrophosphate to $P_i$ by a contaminating pyrophosphatase present in the enzyme preparation). However, the addition of sulfate in this case stimulated the incorporation of the label over 100 %, indicating that, in the presence of sulfate, pyrophosphate is incorporated directly into the nucleotide.

These results suggest that pyrophosphate is released in a reversible reaction during the process of sulfate activation. Further evidence for the involvement of pyrophosphate comes from studies of its ability to inhibit the esterification reaction. Under the conditions of the experiments presented in Table II, pyrophosphate inhibited significantly in as low a final concentration as $10^{-3}$ *M* and 100 % inhibition occurred at concentrations of $6 \cdot 10^{-3}$ *M* and higher. $P_i$ on the other hand had no effect on the rate at a concentration of $10^{-2}$ *M*.

Under conditions of incubation as indicated in Tables I and II no pyrophosphate formation

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