COMMUNICATIONS TO THE EDITOR

A RACEMASE FOR THREONINE IN *ESCHERICHIA COLI*

Sir:

Growth studies with biochemically deficient mutants of yeast[1] and bacteria[2,3] requiring threonine have suggested that some strains possess a threonine racemase.

I wish to report the demonstration in a cell-free extract from *Escherichia coli* of an enzyme capable of transposing groups on both asymmetric carbon atoms of threonine to convert D-threonine to L-threonine, for which ATP or yeast or muscle adenylic acid serves as a cofactor. In rather limited attempts it has not been possible to demonstrate a vitamin B_6 requirement.

Cells of *Escherichia coli*, strain K-12, grown on the medium of Davis and Mingioli[4] with 0.5% glucose were dried *in vacuo* over phosphorus pentoxide, ground with alumina and extracted with 0.01 *M* phosphate buffer at *p*H 7.1. The activity of the extracts toward threonine was determined after 16 hours dialysis against 0.01 *M* phosphate buffer, *p*H 7.1, at 2–4°. On storage the extracts lost the ability to deaminate L-threonine but retained that of converting L-threonine to the D-form and *vice versa*.

The formation of L-threonine starting from D-threonine was demonstrated by microbiological assay using a fastidious L-threonine-requiring mutant of *Escherichia coli*, ML52, and quantitatively estimated by ammonia liberation with an L-threonine deaminase from *Clostridium welchii*. The welchii enzyme proved to be specific for L-threonine and will not attack the L-allo, D- or D-allo forms. The conversion of L-threonine to D-threonine, followed by the disappearance of L-threonine, appeared to occur at the same rate as the reverse transformation.

TABLE I

CONVERSION OF D-THREONINE TO L-THREONINE BY EXTRACTS OF *Escherichia coli*, STRAIN K-12

The complete reaction system contained K-12 extract (3.5 mg. extract protein), 50 μM. of D-threonine, 10 μg of pyridoxal phosphate, welchii deaminase (2.4 mg. protein), 5.0 μM. of ATP and 1.0 ml. of 0.20 *M* phosphate buffer, *p*H 7.8; incubed at 37° for 3 hours in tubes flushed with nitrogen and stoppered; reaction stopped with 0.2 ml. of 50% trichloroacetic acid and precipitate removed by centrifugation; ammonia determined on supernatant by micro-Kjeldahl procedure.

Reaction system	μM. of NH_3
Complete	10.2
Minus K-12 extract	0.2
Minus D-threonine	1.2
Minus ATP	3.8
Minus pyridoxal phosphate	10.5
Adenosine-5-phosphate for ATP	10.7
Minus welchii deaminase	6.4[a]

[a] At end of 3-hr. incubation an aliquot was removed, from which K-12 enzyme was precipitated with 6 *N* HCl, precipitate removed by centrifugation, the supernatant returned to *p*H 7.8 with NaOH and welchii enzyme added for 90 min. at 37°.

The presence of welchii deaminase during the enzymatic racemization of D-threonine accelerated this conversion by removing L-threonine as it was formed. In such a system the disappearance of threonine and the appearance of α-ketobutyric acid could be followed by colorimetric estimation after paper chromatography[5,6] (Table I).

Adenosine-3-phosphate stimulates racemization as effectively as ATP and muscle adenylic acid. The Christman synthetic coenzyme for aspartic deaminase[7] has no effect. The quantity of enzyme in K-12 is depressed sharply by the presence in the growth medium of D-threonine. Fractionation of the crude extract for more precise characterization of the enzyme is in progress.

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TANTALUM SUBCHLORIDE[1]

Sir:

Among the subhalides mentioned in the literature, tantalum subchloride is one of the few whose existence has neither been confirmed nor denied. According to Ruff and Thomas,[2] thermal decomposition of the dichloride *in vacuo* leads to a pyrophoric compound with the empirical formula Ta_2Cl. We have attempted to prepare this compound by the procedure outlined by these authors.

Aluminum powder and tantalum pentachloride were mixed in stoichiometric proportions according to the equation

$$3TaCl_5 + 2Al = 2AlCl_3 + 3TaCl_3$$

and heated in evacuated Pyrex tubes for 100 hours at 300 to 350°. The aluminum trichloride was removed by sublimation, and the product, in all probability a mixture of $TaCl_4$ and $TaCl_3$, was transferred to small tantalum crucibles for subsequent thermal reduction. All manipulations were made in dry argon.

The X-ray powder diagram of the initial reduction product was extremely complex, but did not indicate the presence of aluminum, aluminum oxide or tantalum.

Thermal decomposition of this product at 600 to 700° produced a compound whose properties were identical to those reported by Ruff and Thomas for

(1) Under research grant from the National Research Corporation, Cambridge, Mass.
(2) O. Ruff and F. Thomas, *Z. anorg. Chem.*, **148**, 1 (1925).