THE INCORPORATION OF 1,7 C^{14} PIMELIC ACID INTO BIOTIN VITAMERS*

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Indirect evidence accumulated over the past twenty years implicate both pimelic acid and desthiobiotin as possible intermediates in the biosynthesis of biotin (duVigneaud, Dittmer, Hague and Long 1942; Eakin and Eakin 1942; Tatum 1945; Dittmer, Melville and duVigneaud 1944). The present report, which is part of a study on the mechanism of biotin biosynthesis (Eisenberg 1962), shows the incorporation of radioactive pimelic acid into the biotin vitamers synthesized by Phycomyces blakesleeanus. While this work was in progress, a similar study was reported with Aspergillus niger (Elford and Wright 1962).

EXPERIMENTAL AND RESULTS

Phycomyces blakesleeanus was grown in Schoper's medium to which was added 23.8 μM of 1,7 C^{14} pimelic acid (sp. ac. 2.5×10^6 cts/min/μM). The medium was innoculated with a heat activated spore suspension and shaken at 70 rpm with aeration at 30°C. After 10 days, the culture was filtered to remove the mycelial mat. The filtrate contained over 90% of the total biotin activity as measured by the yeast assay (Hertz 1943). The vitamers were identified by paper chromatography as biotin, biotin sulfoxide,

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desthiobiotin and an unknown product. All biotin vitamers were adsorbed onto 15 grams of Norite A and eluted with an alcohol, water, ammonia mixture (1:1:0.1). The eluates were combined and taken to dryness in vacuo. The residue was taken up in water and placed onto a Dowex-1-acetate column, 1.9 x 20 cm. The column was washed with 500 ml of water which removed the unknown vitamers (see below), and then developed with increasing concentrations of acetic acid (0.5 - 2.0 N). The biotin, biotin sulfoxide, and desthiobiotin were eluted together as a single peak with 1.5 N acetic acid while pimelic acid was eluted with 2.0 N acetic acid. The contents of those tubes showing biotin activity, as measured by the yeast bioautographic method, were pooled together, the solvent removed, and the residue taken up in water. The solution was placed on new Dowex-1-acetate column and the procedure repeated. The final solution obtained from the second column was then placed onto a Dowex 50-2X-H^+ column. The biotin vitamers with the exception of the unknown vitamers are not adsorbed on this column and are therefore readily eluted with water.

The contents of those tubes showing biotin activity were pooled and taken to dryness. The material was reduced with Raney Nickel (duVigneaud et al 1943) to convert all biotin and biotin sulfoxide to desthiobiotin. The resulting material was purified by ascending paper chromatography on large sheets of Whatman 3MM paper with n-butanol, acetic acid and water (60:15:25) as developing solvent. The areas showing biotin activity were eluted with butanol saturated with water, the solvent removed, and the residue taken up in water. This solution was

then placed into a Dowex-1-acetate column which was developed as previously described. The contents of those tubes showing biotin activity were pooled and the solution taken to dryness. The residue was quantitatively removed to a tared graduated centrifuge tube and made up to a known volume. 15.2 mg of d-desthiobiotin were added to the above solution and the material recrystallized to constant specific activity as shown in Table I.

TABLE I

RECRYSTALLIZATION OF d-DESTHIOBIOTIN TO CONSTANT SPECIFIC ACTIVITY

Activity cts/min	Weight mgs	Specific activity cts/min/mg
354,650	15.0	23,640
231,180	12.3	18,800
178,050	9.7	18,360
121,730	6.7	18,170

The water eluate from the first Dowex-1-acetate column was reduced in volume and a sample chromatographed on paper with n-butanol, acetic acid and water as the developing solvent. The yeast bioautographic assay revealed 3 components with Rf values of 0.32, 0.67, and 0.90 respectively. The second component (Rf 0.67) also appeared on the chromatogram of the original filtrate along with biotin, biotin-d-sulfoxide and desthiobiotin which have Rf values of 0.81, 0.56, and 0.88 respectively. A sample of the water eluate was also subjected to paper electrophoresis at pH 3.0 at 300 volts for 3 hours and two components appeared traveling toward the anode with mobilities of 1.1 and 7.8 centimeters. The identical components were also obtained with the original filtrate. A series of electrophoretic strips were pre-

pared and the more rapidly moving component eluted from the paper. This material was found to be radioactive. When this solution was first chromatographed and then subjected to electrophoresis after turning the paper 90°, two components appeared. One of the components had an Rf value of 0.61 and a mobility of 7.6 cm and the second had an Rf value of 0.90 and a mobility of 7.3 cm.

For further purification, the entire water eluate was placed onto a Dowex 50 H^+ column which was developed with increasing concentrations of HCl. Most of the material showing biotin activity was eluted with 1.0 N HCl and the major portion of the radioactivity was also associated with this fraction.

DISCUSSION

The difficulties entailed in the separation of biotin from desthiobiotin and the desire to maintain the highest specific activity for subsequent degradative studies, dictated the reduction of all biotin and biotin sulfoxide to desthiobiotin. The constant specific activity of the recrystallized desthiobiotin indicated the incorporation of pimelic acid into either biotin, desthiobiotin or both. The calculated specific activity of the desthiobiotin prior to addition of carrier was 5.4×10^6 counts per minute per micromole. Since this value is about twice that of the initial pimelic acid, it would indicate a multiple entry of pimelic acid into the biotin molecule. This will be further elucidated by the degradation studies which are now in progress.

Three biotin vitamers have been separated from biotin, biotin sulfoxide and desthiobiotin by their inability to be ad-

sorbed onto a Dowex-1-acetate column. The component with an Rf value of 0.32 and mobility of 1.1 is very suggestive of biocytin which appears occasionally on chromatograms when more concentrated solutions are used. The combination of paper chromatography and paper electrophoresis enabled the resolution of the fast moving electrophoretic component into two compounds which do not correspond to any of the known biotin vitamers on the basis of Rf values and mobilities. Radioactivity was found in these components whether isolated by electrophoresis or by column chromatography which would suggest that they may be either direct or indirect intermediates in the biosynthesis of biotin. Efforts are being directed at present to the separation of these components and to their chemical characterization.

REFERENCES

Dittmer, K., Melville, D. B., and duVigneaud, V., Science, 99, 203 (1944).

duVigneaud, V., Dittmer, K., Hague, E., and Lang, B., Science, 96, 186 (1942).

duVigneaud, V., Melville, D. B., Folkers, K., Wolf, D. E., Mosingo, R., Keresztesy, J. C., and Harris, S. A., J. Biol. Chem., 146, 479 (1942).

Eakin, R. E., and Eakin, E. A., Science, 96, 187 (1942).

Eisenberg, M. A., Fed. Proc., 21, 467 (1962).

Elford, H. L., and Wright, L. D., Fed. Proc., 21, 467 (1962).

Hertz, R., Proc. Soc. Exp. Biol. and Med., 52, 15 (1943).