THE PRESENCE OF $\alpha$-AMINO-$\beta,\gamma$-DIHYDROXYBUTYRIC ACID IN HYDROLYSATES OF ACTINOMYCIN Z1

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Actinomycin Z1, synthesized by Streptomyces fradiae contains only one residue of the amino acid, threonine. Hydrolysates of actinomycin Z1 were investigated using paper, gas and ion-exchange chromatographic procedures. Identification of an unknown amino acid in actinomycin Z1 (and other actinomycins of the Z series) as $\alpha$-amino-$\beta,\gamma$-dihydroxybutyric acid (hydroxythreonine) was confirmed by mass spectrometry.

The actinomycin Z complex, produced by a strain of Streptomyces fradiae, was first described by Bossi et al1) . Total hydrolysates of the actinomycin Z components were shown to contain threonine, valine, sarcosine, N-methylvaline and N-methylalanine, but no proline. Subsequently, BROCKMANN and MANEGOLD confirmed the presence in actinomycin Z1 of these amino acids in addition to three previously unidentified amino acids2) . Two of these were tentatively identified as L-4-oxo-5-methylproline2,3) and its 3-hydroxy derivative4), L-3-hydroxy-4-oxo-5-methylproline, while the third was said to be an hydroxy amino acid which differed from serine and threonine. While several publications5–7) have referred to N-methylthreonine as an amino acid component of actinomycin Z1 and have quoted a private communication from BROCKMANN, no experimental evidence for this finding has appeared in the literature.

We compared hydrolysates of actinomycins Z1, Z5 and the complete Z complex with authentic N-methylthreonine in several chromatographic systems, but none of these experimental procedures revealed the presence of any N-methylthreonine in hydrolysates. It was concluded that N-methylthreonine, which is a component of the peptide antibiotic stendomycine8), is not present in the actinomycins of the Z complex. We now report that the unknown hydroxy amino acid in actinomycin Z1 is $\alpha$-amino-$\beta,\gamma$-dihydroxybutyric acid (hydroxythreonine).

Materials and Methods

Compounds. Actinomycins Z1, Z5 and the Z complex were kindly provided by Prof. H.
BROCKMANN, Göttingen University, West Germany. Actinomycin Ro 2-6329 and L-threo-α-
a-mino-β,γ-dihydroxybutyric acid (hydroxythreonine) were obtained through the courtesy of
was a gift from Drs. M. BODANSKY, Case Western Reserve University, Cleveland, Ohio, and
A. FELIX, Hoffmann-La Roche Inc. All other compounds employed were purchased from
commercial sources.

Paper electrophoretic and chromatographic procedures. Actinomycins were hydrolyzed in
6 N HCl as described previously. Amino acids in hydrolysates were separated by high
voltage electrophoresis in one dimension and ascending chromatography in the second dimen-
sion, using Whatman 3 MM paper. After drying, amino acids were visualized with 0.2 %
ninhydrin/acetone.

Amino acid analyses. Analytic separations of amino acids were effected with a Beckman
Spinco automatic amino acid analyzer, Model 120 C, using 0.2 M sodium citrate buffer, pH 3.05
and 4.25. The flow rate of the buffer and ninhydrin solution were both 34 ml/hour.

Gas chromatography. For gas-liquid chromatography (GLC) a Shimadzu Model 4BM
chromatograph equipped with flame ionization detectors was employed. The carrier gas was
argon (40 ml/min.) and the columns were glass, 6 ft. X 3 mm I.D. Column A was packed with
3 % OV17 on Gas Chrom Q (100~120 mesh) and column B with 3 % OV225 on Gas Chrom
Q (100~120 mesh). An aliquot (0.1 μL) of actinomycin Z₁ hydrolysate was dried in high
vacuum and the residue treated in a sealed tube with 25 % bis-(trimethylsilyl)-trifluoroacetamide
in acetonitrile (25 °C) at 80°C for 45 minutes. Aliquots (2 μL) of the resulting solution were
injected directly into the gas chromatograph. Authentic L-threo-α-amino-β,γ-dihydroxybutyric
acid and other amino acids present in actinomycin were derivatized in the same way.

Gas chromatography-mass spectrometry. An LKB9000 combined gas chromatograph-mass
spectrometer equipped with a 6-ft. column of 1 % OV17 on Gas Chrom Q was used. Electron
impact mass spectra of various chromatographic peaks were obtained.

Results and Discussion

Paper electrophoresis, paper chromatography and the use of the amino acid analyzer
have confirmed the presence in actinomycin Z₁, Z₅ and the Z complex of sarcosine, valine,
N-methylalanine, threonine and N-methylvaline (see Table 1). The presence of cis-5-methyl-
proline in actinomycin Z₅ was reported earlier. In addition, the hydrolysate of actinomycin
Z₁ contains three unknown amino acids, one of which gives a yellow ninhydrin spot on paper
chromatograms, while the other two give purple spots. One of the latter amino acids
displayed an electrophoretic mobility intermediate between those of threonine and N-methyl-
threonine, and a lower Rf on paper chromatography than either of these hydroxy amino
acids. This result suggested that an additional hydroxy group might be present in the mole-
cule. On the amino acid analyzer the unknown had a retention time intermediate between
those of threonine and N-methylthreonine. In all these systems the unknown behaved in an
identical manner with hydroxythreonine and cochromatographed with an authentic sample of
the L-threo isomer of this amino acid. Similar results were observed with an hydrolysate of
actinomycin Z₅ and the Z complex.

The conclusion that actinomycin Z₁ contains hydroxythreonine was further supported by
gas chromatography after trimethylsilylation of the hydrolysate amino acids. On column A
(see Materials and Methods) at 140°C threonine and hydroxythreonine had retention times of
2.5 and 6.4 minutes, respectively; on column B at 135°C the corresponding retention times
were 2.0 and 4.4 minutes. On both columns the derivatized actinomycin Z₁ hydrolysate gave corresponding peaks. The identity of the hydroxythreonine peak was confirmed by combined gas chromatography-mass spectrometry (see Materials and Methods). Mass spectra from the authentic and actinomycin Z₁-derived hydroxythreonine peaks were identical (see Table 2). The principal fragmentations observed have been discussed by Westley et al. 9).

The results obtained by us have recently been confirmed by a modified fluorescamine analysis 12).

Acknowledgments

We are grateful to Drs. J. Berger and J.W. Westley for a supply of L-threo-a-amino-β,γ-dihydroxybutyric acid and for a sample of actinomycin Ro 2-6329. We wish to thank Dr. H. Fales and Mr. W. Comstock, National Institutes of Health, for the use of an LKB combined gas chromatography-mass spectrometer. This investigation was supported by Public Health Service Research Grants Nos. CA-06926 (to E.K.) and CA-11627 (to A.B.M.) by the National Cancer Institute, and by a grant-in-aid from the Schering Corporation (to E.K.).

References

3) Brockmann, H. & E.A. Stahler: 4-Oxo-5-methyl-prolin, ein Baustein des Actinomycin Z₁

Table 1. High-voltage electrophoretic (HVE), paper chromatographic (PC) and amino acid analyzer (AAA) comparisons of amino acids from actinomycins with standards (std.)

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>HVE</th>
<th>PC</th>
<th>AAA (min)</th>
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<tr>
<td>Sarcosine</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
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<tr>
<td>Valine</td>
<td>0.89</td>
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<tr>
<td>N-Methylalanine</td>
<td>0.86</td>
<td>0.86</td>
<td>0.86</td>
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<tr>
<td>Threonine</td>
<td>0.82</td>
<td>0.82</td>
<td>0.82</td>
</tr>
<tr>
<td>Hydroxythreonine</td>
<td>0.75</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>Cis-5-Methylproline</td>
<td>—</td>
<td>0.72</td>
<td>0.72</td>
</tr>
<tr>
<td>N-Methylvaline</td>
<td>0.69</td>
<td>0.68</td>
<td>0.69</td>
</tr>
<tr>
<td>Unknown</td>
<td>0.69</td>
<td>0.68</td>
<td>—</td>
</tr>
<tr>
<td>N-Methylthreonine</td>
<td>0.69</td>
<td>0.68</td>
<td>0.54</td>
</tr>
<tr>
<td>Imino acid*</td>
<td>0.48</td>
<td>—</td>
<td>—</td>
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* Identified by Brockmann and Stahler 4) as 3-hydroxy-4-oxo-5-methylproline.

Table 2. Mass spectra of trimethylsilylated hydroxythreonine.

<table>
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<th>m/e</th>
<th>Relative abundance</th>
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Naturwiss. 52: 391, 1965


