Chemical Syntheses and Biocytinase Specificity for Sulfoxides and Sulfone of *d*-Biocytin[1] (34246)

DONALD B. MCCORMICK

Graduate School of Nutrition and Section of Biochemistry and Molecular Biology, Cornell University, Ithaca, New York 14850

Biocytin was first isolated from autolysates of yeast (1) and identified as ε-*N*-biotinyl-L-lysine (2). Its synthesis confirmed the structure (3). Subsequently, a derivative of biotin was isolated from human urine and tentatively identified as a biocytin sulfoxide on the bases of microbiological assay and mobility on paper chromatograms (4). An enzyme which catalyzes hydrolysis of biocytin to yield *d*-biotin and L-lysine was recognized in several natural extracts and partially purified from hog kidney (5). A similar activity was noted in blood and named biocytinase (6). The enzyme has been extensively purified from *Streptococcus faecalis* and the specificity for the amino acid moiety amide linked to biotin delineated (7).

The certain identity of biocytin sulfoxide and the further decision as to whether the *d*- or *l*-isomer was isolated from urine had not been confirmed by chemical syntheses. Also synthesis of biocytin sulfone had not been reported. An additional reason for syntheses of these derivatives was the lack of information on specificity of biocytinase for biocytin analogues in which the biotin moiety is altered.

The present report describes the syntheses and characterization of the sulfoxides and sulfone of biocytin, supplies additional evidence for the identity of the *d*-sulfoxide as the derivative occurring in urine, and notes the ability of biocytinase to catalyze cleavage of these oxidized forms of biocytin.

Materials and Methods. The *d*-biocytin was synthesized from *d*-biotin methyl ester and L-lysine by the improved method of Weijlard *et al.* (8) but purified by column chromatography (9). The sulfoxides of *d*-biocytin were made essentially according to Melville's procedure (10) for the synthesis of biotin sulfoxides. For this, 100 mg of biocytin was dissolved in 10 ml of glacial acetic acid and treated with 0.035 ml of 30% hydrogen peroxide (1 equivalent plus 10% excess). The solution was stored at room temperature overnight, evaporated to dryness under partial vacuum at 45°, the syrupy residue was taken up in 1 ml of water, and the product was precipitated by the gradual addition of 10 ml of acetone. Approximately 100 mg of material was obtained by filtering off the precipitate which was rinsed with acetone and dried *in vacuo* for over 90% yield. The sulfone of *d*-biocytin was made essentially according to the procedure of Hofmann *et al.* (11) for synthesis of biotin sulfone. For this, 100 mg of biocytin was treated exactly as for obtaining the sulfoxides except that 0.35 ml of 30% hydrogen peroxide (over 10 equivalents) was added. Approximately 90% yield of product was obtained after acetone precipitation.

Microanalyses were done by Schwarzkopf Microanalytical Lab. of Woodside, N Y. Melting points of the compounds were determined with a Fisher-Johns apparatus. For infrared characterization, 1-mg samples were compressed in 300 mg of potassium bromide and spectra were determined with a Perkin-Elmer Infracord. Thin-layer chromatography was performed with Brinkmann sheets of MN Silica Gel N-HR. Ethanol:water (1:1, v/v) and *n*-butyl alcohol:acetic acid:water (2:1:1, v/v/v) were used as ascending solvents. The 10-μg quantities of compounds,

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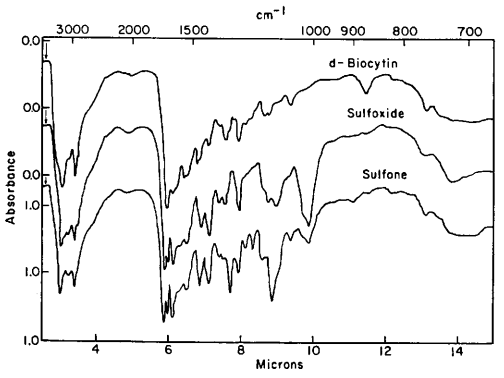

FIG. 1. Infrared spectra of *d*-biocytin and the sulfoxide and sulfone derivatives.

applied in 5-μl spots, were visualized as bluish areas after the developed sheets were sprayed with 0.2% Ninhydrin in butanol and dried with hot air.

Substrate behaviors of the compounds with biocytinase in blood was ascertained by a modification of the assay of Wright *et al.* (6). For this, 0.5 ml of a 1:10 dilution of fresh rat blood in water saturated with BAL (2,3-dimercapto-1-propanol) was stirred into 0.05 ml of 0.5 *M* sodium phosphate buffer, pH 6.5, containing 1 mg of compound. The mixtures were incubated at 37°, inactivated by boiling, centrifuged, and 5-μl aliquots of supernatant solutions withdrawn at intervals and applied to silica gel sheets for thin-layer chromatography as before. Enzymatic activity was noted by the appearance of lysine.

Results and Discussion. Elemental analyses for the synthetic, oxidized derivatives of *d*-biocytin were in good agreement with expected values. Sulfoxide ($C_{16}H_{28}N_4O_5S$) calculated as C, 49.46; H, 7.26; N, 14.42; S, 8.25; O, 20.59 was found to be C, 49.17; H, 7.09; N, 14.16; S, 8.30; O, 20.69. Sulfone ($C_{16}H_{28}N_4O_6S$) calculated as C, 47.51; H, 6.98; N, 13.85; S, 7.93; O, 23.73 was found to be C, 46.89; H, 6.89; N, 13.50; S, 8.00; O, 25.16. The mp (decomp.) for sulfoxide is 230–235° and for sulfone is 235–240°. The infrared spectra of sulfoxide and sulfone are compared with *d*-biocytin in Fig. 1. The sulfoxide exhibits strong absorbance at about 9.8 μ which is nearly absent in biocytin and moderate in the sulfone. The absorbance near 8.9 μ is greatest in the sulfone, moderate in the sulfoxide, and very weak in biocytin. Both of these regions are characteristic for alkyl sulfoxide and sulfone, and the change in their relative intensities is also as expected and found in analogous compounds, *e.g.*, sulfoxides and sulfone of *d*-biotin. The R_F values for the compounds presented in Table I indicate the order of mobilities in the alcohol–water solvents: biocytin>sulfone>*d*-sulfoxide>*l*-sulfoxide. This order was also found for the corresponding derivatives of biotin (12) and is true for chromtograms of paper or silica gel sheets. The chromatograms also establish the presence of both isomers of biocytin sulfoxide but with the *d*-sulfoxide accounting for 80–90% of the material synthesized under conditions which lead to a similar predominance of the *d*-sulfoxide from biotin (10). The R_F values of biocytin and the *d*-sulfoxide on Whatman No. 1 paper developed in butanol:acetic acid:water (4:1:5) are 0.28 and 0.15, respectively. These are identical to those reported for authentic biocytin and the urinary product suggested as sulfoxide (4). Hence, the identity of the natural sulfoxide is not only made more certain by chromatographic comparison with the synthetic compound, but it is additionally shown to be the *d*-isomer.

The ability of biocytinase in rat blood to catalyze hydrolysis of both sulfoxide and sulfone of biocytin is indicated by the semiquantitative data given in Table II. The amounts of lysine liberated from each of the substrates appears comparable. Biocytinase is not narrowly specific for a particular ring structure of the substrate. Hence, the

TABLE I. Mobilities of Compounds upon Thin-Layer Chromatography.

Compound	R_F value	
	Ethanol :water	Butanol:acetic acid:water
d-Biocytin	0.59	0.40
d-Biocytin *d*-sulfoxide	0.49	0.22
d-Biocytin *l*-sulfoxide	0.39	0.13
d-Biocytin sulfone	0.52	0.24
L-Lysine	0.17	0.19

TABLE II. Specificity of Biocytinase for Compounds.

Substrate	Hydrolysis after (hr): 0	1	2	20
d-Biocytin	—	±	+	++
d-Biocytin sulfoxide[a]	—	±	+	++
d-Biocytin sulfone	—	±	+	++

[a] Both *d*- and *l*-isomers.

presence of *d*-biocytin *d*-sulfoxide in urine probably is not due to a failure in enzymatic cleavage but may only reflect the ease in oxidizability of biocytin released.

Summary. The sulfoxides and sulfone of biocytin have been synthesized by treatment of biocytin in acetic acid with hydrogen peroxide at equimolar or tenfold higher concentrations, respectively. The derivatives were characterized by microanalyses, melting points, infrared spectra, and chromatographic mobilities. The *d*-sulfoxide predominates from chemical synthesis and is probably the compound which was found earlier in human urine. Biocytinase in rat blood can catalyze cleavage of both sulfoxide and sulfone forms of biocytin.

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