AMINO ACID SEQUENCE AT THE ACTIVE CENTER OF SUCCINATE DEHYDROGENASE

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SUMMARY

The amino acid sequence of the coenzyme binding site of beef heart succinate dehydrogenase is serine-histidine-threonine-valine-alanine. Flavin adenine dinucleotide is covalently bound to a ring nitrogen of histidine through the 8α position of the isoalloxazine ring system.

It has been demonstrated that the covalently bound FAD at the active center of mammalian succinate dehydrogenase is linked to the peptide chain (1) through the 8α-carbon of the benzene ring of riboflavin (2-4). We have recently shown that the amino acid attached to the 8α-carbon is histidine and that the bond is to one of the two ring nitrogens of imidazole (5). Histidyl riboflavin has been synthesized (6) and shown to be identical with the compound isolated from succinate dehydrogenase.

The present paper describes the isolation and amino acid sequence of a flavin pentapeptide, obtained from beef heart succinate dehydrogenase.

MATERIALS AND METHODS

Succinate dehydrogenase of approximately 50% purity was obtained by a modification of the Bernath and Singer procedure (8). An acetone powder of ETP, instead of mitochondria, served as the source material and the glycine extract was precipitated with 0.5 saturated $(NH_4)_2SO_4$, dialyzed, then precipitated with trichloroacetic acid, digested with trypsin-chymotrypsin and hydrolyzed to the flavin mononucleotide level (3). After chromatography on

Florisil and on Sephadex G-25 (3), the main flavin peptide band, representing 80% of the flavin, was subjected to descending paper chromatography in n-butanol-acetic acid-H_2O (2:1:1, v/v) on Whatman No. 3 paper. The main flavin band was subjected to preparative TLC on silicic acid in the same solvent and was eluted with 5% (v/v) pyridine. The material thus obtained appeared homogeneous on analytical TLC and high voltage electrophoresis and on drastic acid hydrolysis yielded 1 mole each of alanine, serine, valine, threonine, and histidine (5) per mole of flavin.

TABLE 1

Edman Degradation of Flavin Pentapeptide[a)]

Constituent	Amino acyl residues remaining after degradation step				
STEP:	0[b)]	1	2[c)]	3	4
Histidyl riboflavin[d)]	0.99				
Serine	1.01	0.25	0.24	0.25	0.29
Threonine	0.99	0.98	1.00	0.30	0.28
Valine	1.05	1.01	1.03	0.98	0.37
Alanine	0.96	1.03	0.98	1.02	1.00

a) Ratios were determined without regard to destruction or slow release upon acid hydrolysis (110°, 6N HCl, 22 hrs). Under these conditions about 50% of the histidine is liberated; at 95° none, and at 125° 100% (5).

b) Acid hydrolysis on isolated peptide prior to Edman degradation.

c) It is assumed that histidyl riboflavin is removed in this step.

d) Determined spectrophotometrically, assuming $\varepsilon_{445} = 12 \times 10^3$. To conserve material histidine was not determined in subsequent steps.

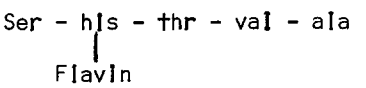
The amino acid sequence was determined by the Edman degradation. Thiocarbamylation was performed essentially by the procedure of Eriksson and Sjöquist (9). The phenylthiocarbamyl derivative was treated with trifluoro-

acetic acid for 1 hour at 25° (10) and the released amino acyl residue was deduced by the subtractive method by analysis of the amino acid composition of the remaining peptide (11).

RESULTS AND DISCUSSION

Upon acid hydrolysis, besides the amino acids listed in Table 1, small amounts (10 to 20% of the amount of peptide analyzed) of aspartate, glutamate, and glycine were obtained. These amino acids could have arisen in part as artifacts of the Edman procedure (12), and in part by destruction of histidyl riboflavin. Trace amounts of aspartate, glycine, glutamate, and serine were, in fact, obtained on acid hydrolysis of synthetic (6) histidyl riboflavin. It is quite unlikely that these artifacts are due to a contaminating peptide, since the proportion of these residues did not decrease on stepwise degradation by the Edman procedure and, further, only serine, threonine, valine and alanine were detected by the amino acid analyzer after extensive digestion with pronase.

In step 2 of the Edman procedure the proportion of the constituent amino acids listed in Table 1 did not change. Thus the amino acyl residue released in step 2 must be histidyl riboflavin. Continued degradation of the peptide (steps 3 and 4) indicates that the riboflavin moiety does not interfere in the procedure. Amino acid analysis prior to acid hydrolysis of an aliquot of the material remaining in step 4 yielded only one free amino acid, alanine, indicating that this is the carboxyl terminal residue. Thus, four of the residues are definitively assigned in the pentapeptide and histidyl riboflavin must therefore be the second residue:



The pentapeptide dealt with in the present paper differs by two amino acids (a second serine and glutamate) from the flavin peptide isolated by Kearney over a decade ago (1). The latter was regarded as a hexapeptide but

actually, it must have been a heptapeptide: histidine, the seventh amino acid, was not detected because the conditions of acid hydrolysis used (95°, 6N HCl) do not break the histidine-flavin bond (5) and thus free histidine was not liberated, although Kearney (1) correctly noted that acid hydrolysis yields, besides free amino acids, a riboflavin derivative (now known to be histidyl riboflavin), not free riboflavin. It remains for sequence studies on tryptic peptides to determine at which end of the pentapeptide the two additional amino acids are located. Studies along these lines are in progress. Hopefully, sequencing of longer peptides will also elucidate the location of the -SH groups which have been known for many years to be the substrate binding site (13).

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