DIFFERENCES IN THE AMINO ACID COMPOSITIONS OF THE SUBUNITS OF SUCCINATE DEHYDROGENASE

Norman T. Felberg and Thomas C. Hollocher

Graduate Department of Biochemistry, Brandeis University
Waltham, Mass. 02154

Received July 11, 1972

Summary: The subunits of highly purified succinate dehydrogenase preparationswere isolated by SDS electrophoresis and their amino acid compositions determined. The subunits differ so widely in composition that the larger subunit probably could not be derived from a dimerization of the smaller one.

Recently, succinate dehydrogenase (succinate:(acceptor)oxidoreductase, EC 1.3.99.1) has been prepared in a highly purified form (1,2). The enzyme has a subunit structure of two peptides. The FP or flavoprotein subunit, with a molecular weight of about 70,000, contains peptide-bound flavin, non-heme iron, and labile sulfide in a mole ratio of 1:4:4. The IP or iron protein subunit, with a molecular weight of about 27,000, contains non-heme iron and labile sulfide in a mole ratio of 4:4 (1,3,4). Homogeneous preparations of the enzyme are reported to contain 10.3 $\pm$ 0.4 nmoles flavin x (mg protein)$^{-1}$ (1).

We wish to report that a further major difference between the subunits lies in their amino acid compositions.

Methods and Materials: Succinate dehydrogenase was prepared according to Baginsky and Hatefi (2) and Davis and Hatefi (1) from beef heart. Flavin concentrations were determined after tryptic digestion of the heat denatured enzyme according to Hatefi and Stemple (5) (oxidized minus reduced $\Delta\epsilon_{450}$ = 10.3 l xmmole^{-1}). Protein was determined by dry weight, by the Folin-Lowry method using crystalline bovine serum albumin (Sigma) as the standard (6), and by ultraviolet absorption via the method of Edelhoch (7) in the case of the separated subunits. The enzyme preparations contained no more than 10% contaminants which were separated from the two subunits on disc electrophoresis in sodium dodecyl sulfate (SDS) by the method of Weber and Osborn (8).

Following electrophoretic separation, FP was located by virtue of the fluorescence of its covalently bound flavin and IP was located by the fluo-

Copyright © 1972 by Academic Press, Inc.
All rights of reproduction in any form reserved.

rescent stain, 8-anilino-1-naphthalene sulfonic acid (Eastman magnesium salt), according to Hartman and Udenfriend (9). The bands were cut out and then homogenized and eluted in 0.2% SDS according to Weber and Osborn (8). Pellets obtained from acetone precipitation were hydrolyzed in 6 N HCl in sealed, evacuated tubes for 4 hrs at $145^{o} \pm 2^{o}$ according to Roach and Gehrke (10). The subunits were also separated by column chromatography on agarose in 6 M guanidinium chloride (11) and on Sephadex G-200 in 0.15% SDS (12).

Cysteine and methionine contents were estimated as cysteic acid and methionine sulfone after performic acid oxidation according to Hirs (13). Analyzer constants for cysteic acid and methionine sulfone were calculated through the use of oxidized Ribonuclease-A (Bovine, Sigma, type I-A). Tryptophan was determined according to Edelhoch (7) in subunits separated by column chromatography on agarose in 6 M guanidinium chloride. Amino acid analyses were performed on a Beckman 120 C analyzer, employing the enhanced sensitivity circuit.

Results and Discussion: Assuming molecular weights of 70,000 (FP) and 27,000 (IP), as independently determined in this laboratory, the apparent mole ratio IP/FP in the separated fractions was determined to be 1.20-1.25. This compares with an expected ratio of 1.0 (1) for a 1:1 dimer and with a previously published value of about 1.3 (4). Procedures to detect a major contaminant in the IP fraction were without success. We conclude, rather, that the IP fraction is very nearly pure and that the excess of IP is an artifact resulting from the use of perchlorate in the purification procedure. Chaotropic anions can dissociate succinate dehydrogenase into subunits, of which IP appears to be the more soluble (1). A slight differential extraction of IP relative to FP from complex II appears to occur. While it has been suggested that the excess of IP is due to contaminants (4), more recent studies in that laboratory substantiate our findings (14).

The amino acid compositions of the subunits of succinate dehydrogenase are presented in TABLE I. The values for threonine, serine, cysteine and tyrosine (partially labile amino acids (15)) and for methionine are considered semiquantative and are not included in the comparisons below. A comparison of FP and IP, normalized to 100,000 g of protein (columns 4 and 5), show several significant differences. Except for the above five amino acids, we consider the standard deviation of the composition ratios (column 4 divided by column 5) to be about 5%, and a deviation from 1 of $\pm$ 15% or about three standard deviations to be probably significant. Lysine, histidine, valine, isoleucine, and phenylalanine show major differences; aspartic acid, glycine, and proline show smaller differences. It seems likely that the values for

cysteine may also prove to be different. Arginine, glutamic acid, alanine, tryptophan, and leucine fail to show significant differences.

TABLE I

Amino acid composition of the subunits of succinate dehydrogenase

Amino acid	FP[a]	IP[b]	FP + IP[c]	FP[d]	IP[d]
Lysine	27.0	21.7	48.7	38.5	80.4
Histidine	21.3	3.8	25.1	30.4	14.1
Arginine	41.1	13.4	54.5	58.6	49.7
Aspartic Acid	57.6	28.8	86.4	82.1	106.8
Threonine	36.9	11.3	48.2	52.6	41.9
Serine	31.4	10.0	41.4	44.8	37.1
Glutamic Acid	68.9	25.6	94.5	98.3	94.9
Proline	27.2	13.1	40.3	38.8	48.6
Glycine	69.9	20.1	90.0	99.7	74.5
Alanine	54.3	17.9	72.2	77.4	66.4
Cysteine[e]	21.7	16.9	38.6	30.9	62.6
Valine	42.0	5.7	47.7	59.9	21.2
Methionine[e]	14.1	7.9	22.0	20.1	29.3
Isoleucine	25.8	14.5	40.3	36.8	53.8
Leucine	52.1	19.1	71.2	74.3	70.8
Tyrosine	22.3	6.2	28.5	31.8	23.0
Phenylalanine	23.3	5.3	28.6	33.2	19.6
Tryptophan[f]	3.8	1.7	5.5	5.4	6.3

[a] - Residues per 70,000 g protein.

[b] - Residues per 27,000 g protein.

[c] - Addition of FP and IP (columns 1 plus 2).

[d] - Residues per 100,000 g protein.

[e] - Determined after performic acid oxidation.

[f] - Determined spectrophotometrically according to Edelhoch (7).

Acknowledgements: We acknowledge the technical assistance of L. M. Buckley. This work was supported by grants from the National Science Foundation (T.C.H.) and Training Grant NB-5241 from the National Institutes of Neurological Diseases and Stroke, National Institutes of Health (N.T.F.).

References

1. Davis, K. A. and Hatefi, Y., Biochem., 10, 2509 (1971).
2. Baginsky, M. L. and Hatefi, Y., J. Biol. Chem., 244, 5313 (1969).
3. Reghetti, P. and Cerletti, P., FEBS Letters 13, 181 (1971).
4. Coles, C. J., Tisdale, H. D., Kenney, W. C., and Singer, T. P., Biochem. Biophys. Res. Commun., 46, 1843 (1972).
5. Hatefi, Y. and Stempel, K. E., J. Biol. Chem., 244, 2350 (1969).
6. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem., 193, 265 (1951).

7. Edelhoch, H., Biochemistry, 6, 1948 (1967).
8. Weber, K. and Osborn, M., J. Biol. Chem., 244, 4406 (1969).
9. Hartman, B. K. and Udenfriend, S., Anal. Biochem., 30, 391 (1969).
10. Roach, D. and Gehrke, C. W., Abstracts, 160th Meeting Amer. Chem. Soc., Chicago, September (1970).
11. Fish, W. W., Mann, K. G., and Tanford, C., J. Biol. Chem., 244, 4989 (1969).
12. Yurewicz, E. C., Ghalambor, M. A., Duckworth, D. H., and Heath, E. C., J. Biol. Chem., 246, 5607 (1971).
13. Hirs, C. H. W., Methods in Enzymology, 11, 59 (1967).
14. Singer, T. P., personal communication of 23 June 1972.
15. Moore, S. and Stein, W. H., Methods in Enzymology, 6, 819 (1963).