PEPTIDE BOUND FLAVINS AND SUCCINIC DEHYDROGENASE ACTIVITY IN THE BRAIN

P. Cerletti, R. Strom, M.G. Giordano, F. Balestrero and M.A. Giovenco

Departments of Biological Chemistry of the Universities of Rome and Camerino, Italy

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In a previous paper (Cerletti et al.,I963) a method has been described for the determination of peptide bound flavin in tissues. This makes it possible to assay the content of succinic dehydrogenase (SD), even in unpurified preparations, independently of measurements of respiration.

The application of this procedure has allowed us to measure in various tissues the turnover number (T.N.) of the enzyme for succinate oxidation (moles succinate oxidized per min. per mole bound flavin). Values ranging around I7.000 have been obtained in heart preparations and somewhat lower values in other tissues. In the brain however, results were extremely ambiguous. Though consistent respiration rates for succinate were always measured, bound flavins could seldom be found.

This raised the question of the presence of bound flavin as the prosthetic group of the enzyme in brain. The problem was investigated and the first results are presented here.

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MATERIALS AND METHODS

The experiments were done on pig brain homogenates and on brain mitochondria prepared according to Brodie and McBain (I952).

The oxidation of succinate was measured by the manometric phenazine assay (Bernath and Singer, I962) with the addition in each vessel of 30 μmoles cysteine sulphinate and of I mg phospholipase A (from Naia naia, Light and Co.). This enzyme was omitted in assays on brain homogenates prepared in diluted buffered saline ($9.6 \times IO^{-3}$ M KCl in $I.2 \times IO^{-3}$ KH_2PO_4 pH 6.8).

The determination of peptide bound flavins was performed by differential fluorimetry at pH 3.I and pH 6.2 following tryptic chymotryptic digestion, as described previously (Cerletti et al., I963). Soluble flavins were removed by extracting with trichloroacetic acid (TCA) (IO% twice, then once I%) this treatment yielding, with brain, easier sedimentations.

In a group of experiments, digestion with phospholipase A (from Naia naia, Light and Co.), phospholipase C (from Cl.Welchii, Sigma) and phospholipase D (from cabbage, Light and CO.) (2 hours at 25°C) was applied previous to proteolytic digestion. The action of trypsin and chymotrypsin was in these cases much more rapid and complete.

Fluorescence measurements were made using a Farrand spectrofluorimeter. Samples were previously hydrolyzed I5 min. at IOO°C in HCl at pH I, so as to degrade to the mononucleotide level the flavine peptides released by proteolysis.

RESULTS AND DISCUSSION

Clear evidence for the existence of peptide bound flavin in the brain was obtained only when fluorescence excitation and emission were measured with sharply monochromatic light. When a conventional filter fluorimeter was used, the fluorescence in neutral medium was the same and sometimes even higher than at pH 3.

As shown in table I, the reason for this behaviour was that two fluorescence peaks partly overlapping with flavin fluorescence were given by brain extracts. The intensity of both was considerably higher than flavin fluorescence, and for one of them it was enhanced by raising the pH.

TABLE I

RELATIVE INTENSITY, AT DIFFERENT pH, OF FLUORESCENCE PEAKS SHOWN BY BRAIN MITOCHONDRIA

pH	max.exc.:450 mμ max.em. :530 mμ	max.exc.:350 mμ max.em. :425 mμ	max.exc.:300 mμ max.em. :400 mμ
3.2	I.000	7.000	I2.600
6.2	0.863	6.I80	I9.500
I0.5	0.836	5.880	20.330

When measured under appropriate conditions, the typical fluorescence behaviour of peptide bound flavins at the mononucleotide level, could be demonstrated.

From the amounts of flavin peptide thus determined a T.N. for succinate oxidation was calculated for the brain enzyme. Its value is of the same order of magnitude of that of the heart enzyme.

TABLE 2

TURN-OVER NUMBERS OF SD IN VARIOUS TISSUES

Values are expressed as moles of succinate oxidized/mole of flavin peptide/min.

Tissue	Total homogenate	Acetone powder	Mitochondria
Brain	I8,450	I5,450	I9,200
Heart	I6,250 ± 2,300	5,870 ± 850	
Liver	5,900 ± 520	2,290 ± 240	
Kidney	9,500 ± I,500	3,I50 ± 400	

This is the first report of a SD from different origin than the heart, but having the same catalytic rate for succinate oxidation. Incidentally it may be noted that in brain homogenates prepared in hypotonic KCl-phosphate, addition of phospholipase A was unessential for the penetration of phenazine methosulphate to the enzyme. The respiration rates were the same with or without phospholipase.

Another peculiarity of brain SD, was that it remained unaffected by treatment with acetone according to Ziegler and Doeg (I962), the minor decrease being very likely due to enzyme inactivation during the preparation of the powder. The T.N. for succinate oxidation decreased only on contact with alkali (I0 min. pH 9.6, 0°C), the drop being of the same order (about 60%) as that effected in other tissues by acetone.

This unique behaviour recalls the results about cyanide inactivation of SD. The enzyme of the brain is unaffected by CN^-, while particulate preparation of other tissues are inactivated by 50% or more (Giuditta and Singer, I957). The effect of cyanide has been interpreted as being due to some sort of interference with iron related to SD (Singer,I963).

At the present level of knowledge about SD the above mentioned facts have appearantly little in common. It is

however stimulating to keep them in mind in an effort to direct further research to give a definite answer to the problem.

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