Journal of Chromatography, 329 (1985) 300–306
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17 777

Note

1-DOPA decarboxylase and dopamine-β-hydroxylase isolated from sympathetic splenic nerves estimated by sodium dodecyl sulphate polyacrylamide gel electrophoresis with and without mercaptoethanol

HUSSEIN S. HUSSEINI and HARTMUT O. BALZER*

Dept. I, Centre of Pharmacology, J. W. Goethe-University, Theodor-Stern-Kai 7, D-6000 Frankfurt/Main (F.R.G.)

(First received March 4th, 1985; revised manuscript received March 28th, 1985)

1-DOPA decarboxylase (1-AADC; EC 4.1.1.28) with molecular weight (M_r) 150 000 and the subunits of dopamine-β-hydroxylase (DBH; EC 1.14.17.1) with molecular weight 67 000–75 000 were analysed by HPLC on TSK-G 3000 SW columns and isolated preparatively by Fractogel liquid chromatography[1,2]. The activity of the DOPA decarboxylase was increased twelve-fold compared with the total protein content, according to a modification of the method described by Christenson *et al.*[3] as found by Husseini and Balzer[2].

New aspects of the interpretation of the protein fractions separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) were found by the preparative isolation of the soluble proteins in the sympathetic nerves. The fractions separated by Fractogel columns[2] were investigated by SDS–PAGE and correlated with multiple different fractions in the presence and absence of 2-mercaptoethanol (ME).

EXPERIMENTAL

SDS–PAGE

The LKB 2117-multiphor system was used. SDS–PAGE was carried out on 5% polyacrylamide gels with and without ME, according to a modification of the method of Fehrnström and Moberg[4]. The content of the samples (8–10 mg of protein/ml) was dissolved in phosphate buffer (0.01 *M*; pH 7.1). In the experiments with ME, 10 μl were added and heated to 100°C for 5 min. In the absence of ME the results were the same whether heat was applied or not. Protein estimation was carried out according to the method of Lowry *et al.*[5].

For construction of the calibration curve the calibration kit from Pharmacia was used. The molecular weights were plotted semi-logarithmically *versus* the relative mobilities of the proteins to the bromophenol blue control band. The bands were stained with Coomassie blue (Serva) and analysed with a Quick-Scan densitometer at 595 nm. The calibration curves are shown in Fig. 1.

0021-9673/85/$03.30

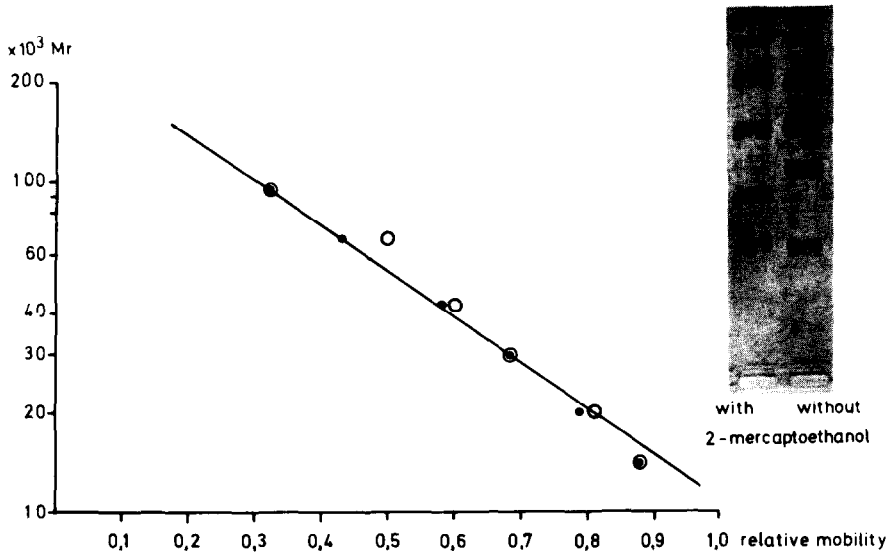

Fig. 1. Calibration curve obtained from the proteins of the Pharmacia kit. Semi-logarithmic plot of molecular weight *versus* mobility relative to bromophenol blue, as estimated by SDS–PAGE on 5% slab gel with (●) and without (○) 2-mercaptoethanol.

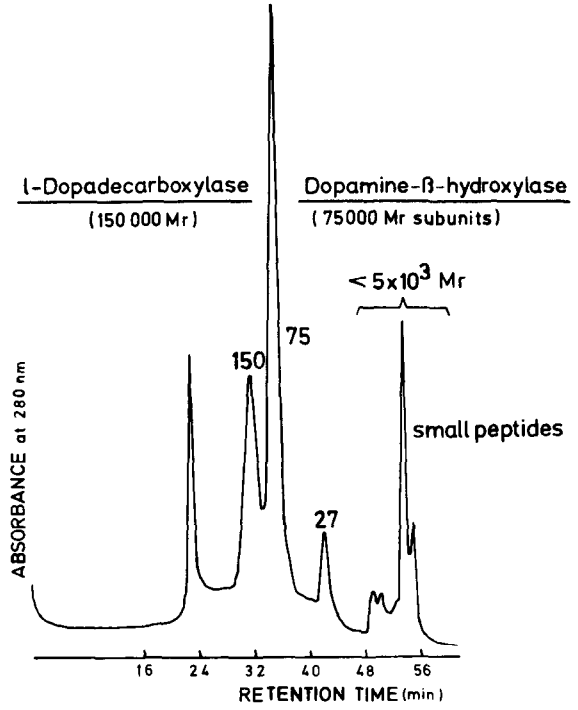

Fig. 2. Separation of soluble proteins isolated from nerve vesicles of the sympathetic splenic nerves by HPLC on TSK 3000 SW columns. l-DOPA decarboxylase (M_r 150 000) and dopamine β-hydroxylase subunits (M_r 75 000) were identified according to the method of Husseini and Balzer[2].

Sample preparation

The nerve vesicles of the sympathetic bovine splenic nerves were prepared according to a modification of the method described by Gasparis *et al.*[6]. The supernatant of the phosphate buffer after centrifugation at 12 000 *g* contains the nerve vesicles[7,8], and the soluble proteins were dissolved in phosphate buffer after centrifugation at 230 000 *g*[1,2,6,9].

The chromaffin granules of the bovine adrenals were prepared by a modification of the method of Smith and Winkler[10–12] and lysed by hyperosmotic shock (0.01 *M* potassium chloride). The resulting supernatant after 105 000 *g* centrifugation was used for determination of the soluble protein content of the chromaffin granules.

RESULTS AND DISCUSSION

The calibration proteins showed a linearity on semi-logarithmic plots when the SDS–PAGE experiments were carried out in presence of ME. In absence of ME the molecular weights of bovine serum albumin (BSA) and egg albumin decreased and

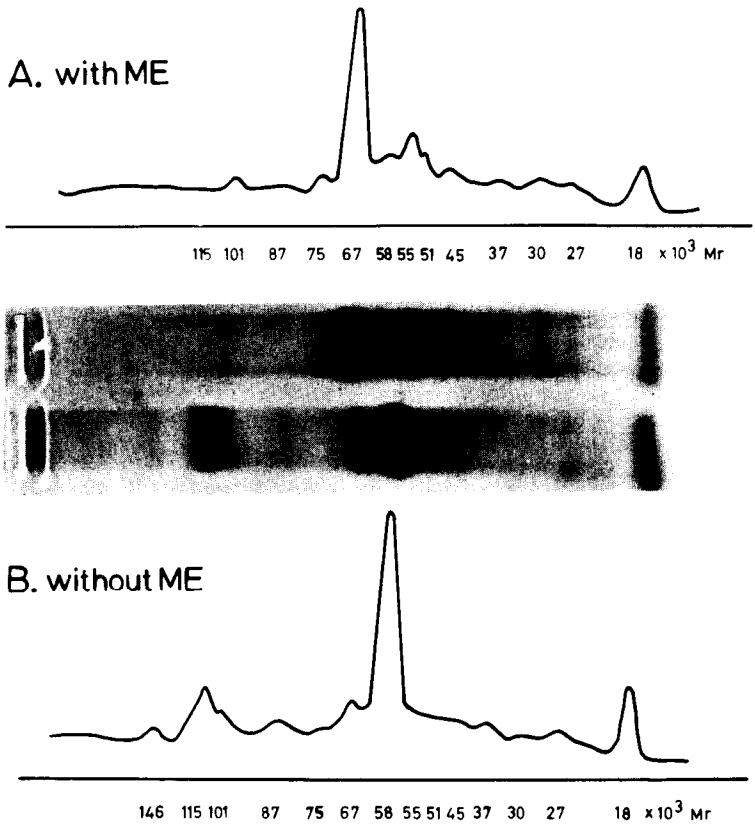

Fig. 3. Separation of soluble proteins isolated from nerve vesicles of the sympathetic splenic nerves by SDS–PAGE. Scan profiles (Quick-Scan densitometer at 595 nm) from 5% slab gels with (A) and without (B) 2-mercaptoethanol.

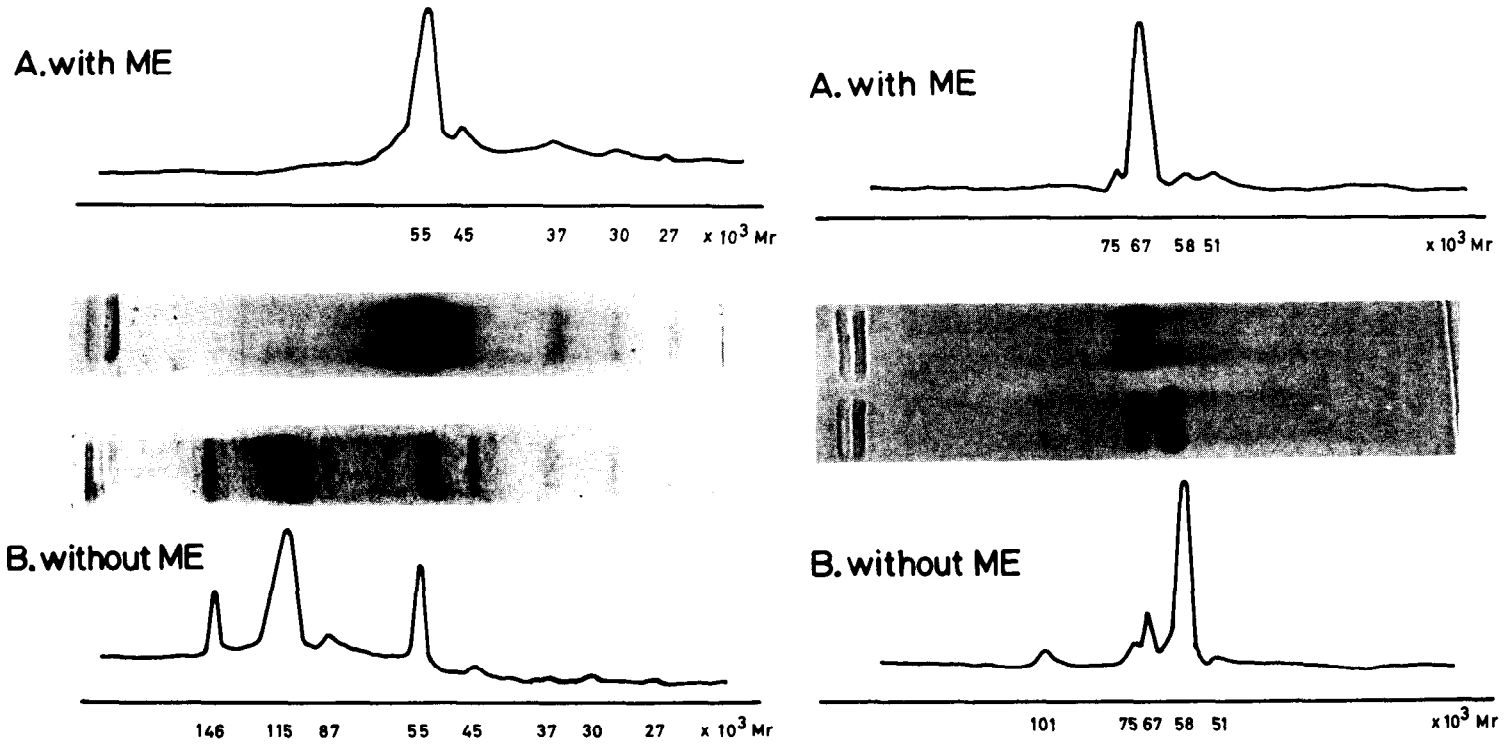

Fig. 4. Isolated 1-DOPA decarboxylase protein by preparative Fractogel chromatography[2] analysed by SDS–PAGE. Scan profiles (at 595 nm) from 5% slab gels with (A) and without (B) 2-mercaptoethanol.

Fig. 5. Isolated dopamine β-hydroxylase subunits by preparative Fractogel chromatography[2] analysed by SDS–PAGE. Scan profile (at 595 nm) from 5% slab gels with (A) and without (B) 2-mercaptoethanol.

the M_r values were off the linear curve as found in the experiments in presence of ME (Fig. 1). The molecular weights experimentally estimated with and without ME were calculated from the linear curve plotted in the presence of ME.

With HPLC separation on TSK 3000 SW, the molecular weights of the soluble proteins from sympathetic nerves were 150 000 and 67 000–75 000, and that of the small peptides was 5000. The M_r 150 000 protein was identified as the DOPA decarboxylase, and the DBH subunits show an apparent molecular weight of 67 000–75 000 (Fig. 2).

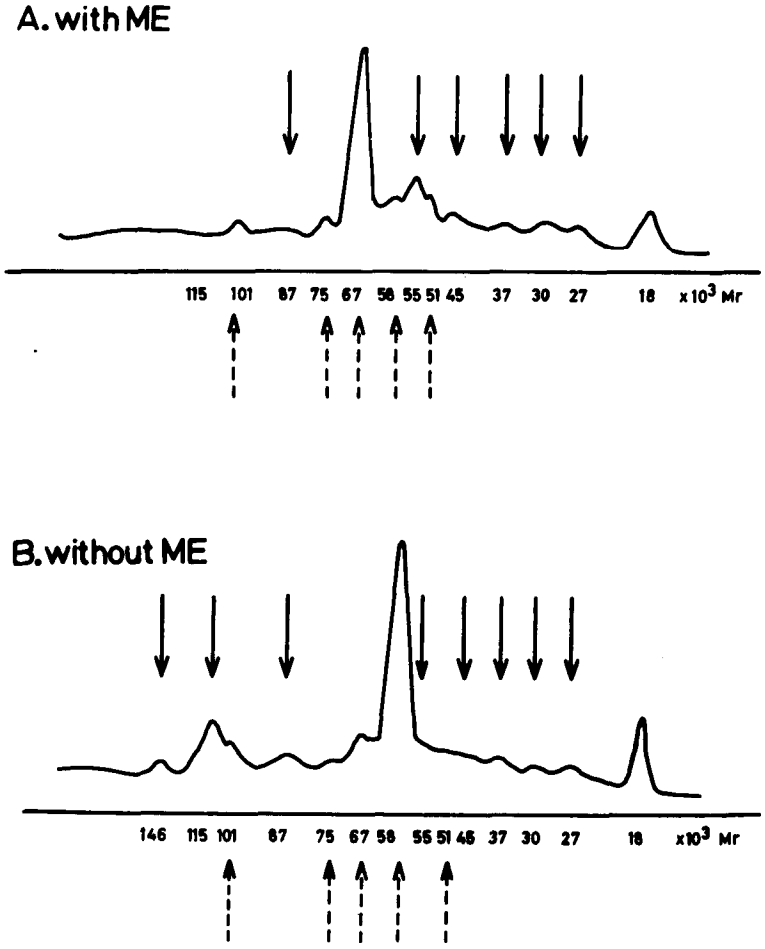

Fig. 6. 1-DOPA decarboxylase protein fractions (↓) and dopamine-β-hydroxylase fractions (↑) integrated in the total soluble protein fractions estimated by SDS–PAGE with (A) and without (B) 2-mercaptoethanol.

With SDS–PAGE estimation the main protein was of M_r 67 000 in the presence of ME, and 58 000 in the absence of ME (Fig. 3).

The DOPA decarboxylase and DBH units preparatively separated by Fractogel chromatography[2] were used to identify the protein fractions estimated by SDS–PAGE.

The isolated DOPA decarboxylase analysed by SDS–PAGE shows a main fraction with a molecular weight of 55 000 and smaller amounts of fractions of M_r 45 000, 37 000, and 27 000. In the experiments without ME the main fraction (M_r 55 000) decreased and new fractions of M_r 146 000, 115 000 and 87 000 were detected (Fig. 4). The estimation of the isolated DBH subunits shows a main fraction with a molecular weight of 67 000 and lesser fractions of M_r 75 000, 58 000 and 51 000. Without ME the main protein fraction was of M_r 58 000, in lesser fractions were of M_r 75 000, 67 000 and 51 000, and a new fraction of M_r 101 000 was detected (Fig.

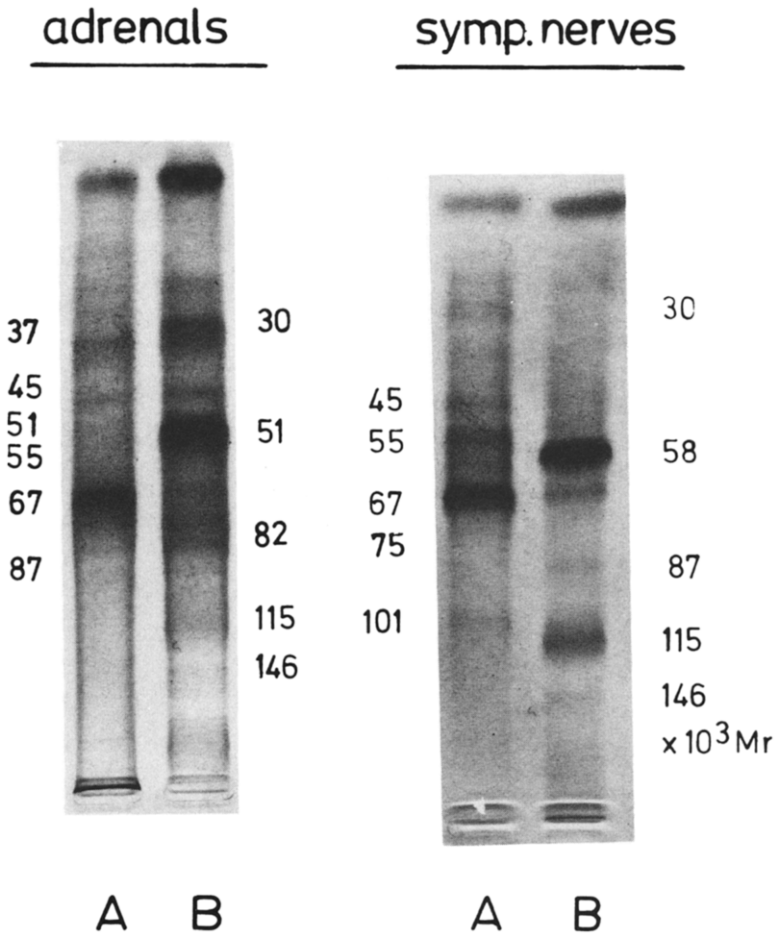

Fig. 7. Comparison between the total soluble protein fractions isolated from the nerve vesicles and from the chromaffin granules of the adrenals by SDS–PAGE with (A) and without (B) 2-mercaptoethanol.

5). Fig. 6 shows that the fractions found by SDS–PAGE can be accounted for by both proteins of the DOPA decarboxylase and DBH subunits, with exception of the M_r 18 000 protein.

Comparative studies of the soluble proteins of the sympathetic nerves and the adrenal chromaffin granules showed that similar fractions were present in both preparations. The main protein was M_r 67 000 in both, but the M_r variation differs in the absence of ME. In the chromaffin granules the molecular weight varied from 67 000 to 51 000, whereas in the sympathetic nerves the range was 67 000 to 58 000. The variation in the chromaffin granules was accompanied by an increased amount of the 82 000 and 30 000 fractions (Fig. 7).

The DOPA decarboxylase and the DBH-protein subunits, as estimated by SDS–PAGE with and without ME, were all identified in the soluble proteins of the chromaffin granules of the adrenals, as accounted for in the sympathetic nerve vesicles.

REFERENCES

1 H. O. Balzer and H. S. Husseini, *J. Chromatogr.*, 259 (1983) 87.
2 H. S. Husseini and H. O. Balzer, *J. Chromatogr.*, 297 (1984) 375.
3 J. G. Christenson, W. Dairman and S. Udenfriend, *Arch. Biochem. Biophys.*, 141 (1970) 356.
4 H. Fehrnström and U. Moberg, *L.K.B. Application Note 306*, Bromma, Sweden, 1977.
5 O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, 193 (1951) 256.
6 M. S. Gasparis, W. H. Yang and R. L. Klein, *Neurochem. Res.*, 8 (1983) 1417.
7 H. J. Schümann and A. Burger, *Arch. Pharmacol.*, 266 (1970) 185.
8 I. Hufnagel and H. O. Balzer, *Seventh International Congress on Pharmacology, Paris, July 16–21, 1978*, Pergamon, Oxford, 1978, p. 594.
9 H. S. Husseini and H. O. Balzer, *Arch. Pharmacol.*, 319 (1982) 242.
10 A. D. Smith and H. Winkler, *Biochem. J.*, 103 (1967) 480.
11 H. O. Balzer and A. R. Khan, *Arch. Pharmacol.*, 291 (1975) 319.
12 H. O. Balzer, A. R. Khan and S. Ristic-Radivojević, *Biochem. Pharmacol.*, 33 (1984) 21.