Isolation procedure for bovine retinal rod outer segments

M.A. Livrea, C. Nicotra, A. Bongiorno, G. Ciaramitaro and M. Romano

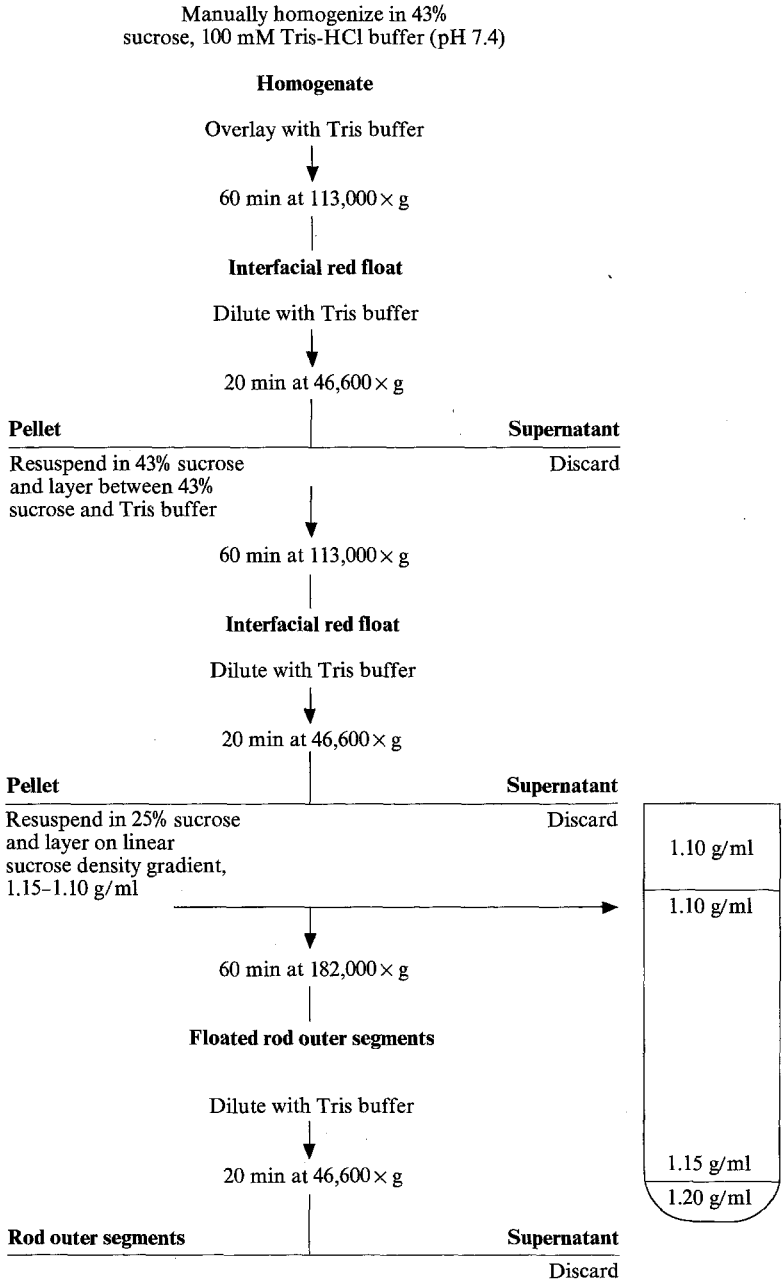
Istituto di Chimica Biologica-Policlinico, I-90127 Palermo (Italy), 5 November 1979

Summary. An isolation procedure to obtain rod outer segments from cattle retinas is reported. Centrifugation of homogenates in discontinuous and continuous sucrose density gradients yields purified photoreceptor cell outer segments. Assay of the final preparation for rhodopsin content gives a ratio of 2.4 for $DO_{280\ nm}/DO_{498\ nm}$.

Retinol dehydrogenase activity of the eye has been extensively investigated in our laboratory[1-3]. For the extraction of the enzyme and its purification from photoreceptor cells, rod outer segments need to be isolated in pure form. These organelles can easily be obtained from dissected retinas. Due to the very fragile ciliary connection to the inner segment the organelles are readily removed from the retina by mild homogenization[4], and the homogenates can be further purified by density gradient centrifugation in a sucrose or a Ficoll medium[5-8]. Although the low density of the rod outer segments permits their easy separation from other particulate matter, simple flotation of rod outer segments may lead to preparations containing impurities.

As very pure rod outer segments were required for the purification of retinol dehydrogenase, a method giving a good yield and purity has been developed in our laboratory by adapting previous studies of other authors[5-8].

Materials and methods. In order to obtain maximal rhodop-



Outline of the procedure for obtaining rod outer segments from a homogenate of 100–120 bovine retinas.

sin content, the following entire isolation procedure has to be carried out in a dim red light to prevent formation of opsin. This method permits the preparation of rod outer segments from 100–120 frozen bovine retinas. The retinas, suspended with 43% sucrose in 100 mM Tris-HCl buffer, pH 7.4, (1 ml/retina) were homogenized manually with a Kontes glass homogenizer. The suspension (180 ml) was divided between the tubes of a Beckman SW 27 rotor and overlaid with Tris buffer, pH 7.4. The discontinuous gradients were centrifuged at 113,000 × g for 60 min. The interfacial material was diluted with an equal volume of Tris buffer, pH 7.4, and centrifuged in a Beckman 35 rotor at 46,600 × g for 20 min to sediment rod outer segments. The sedimented pellet, suspended in 20 ml of 43% sucrose in 100 mM Tris-HCl buffer, pH 7.4, was layered between 15 ml of 43% sucrose buffered solution (bottom) and 15 ml of 100 mM Tris-HCl buffer, pH 7.4 (top). The Beckman SW 27 rotor was operated at 113,000 × g for 60 min. The interfacial red membranes were removed, diluted with Tris buffer and sedimented at 46,600 × g for 20 min. The sedimented red pellet, suspended in 12 ml of 1.10 g/ml density sucrose solution, was pipetted in 3 ml portions onto the tops of 4 gradients containing 1 ml of 1.2 g/ml buffered sucrose and 8 ml of a continuous density sucrose gradient 1.15 to 1.10 g/ml. Gradients were centrifuged in a Beckman SW 36 rotor at 182,000 × g for 60 min. A sharp red band at the top of the gradient was collected, care being taken to discard the superior phase containing synaptosomal particles. Rod outer segments were diluted and sedimented and the last step was repeated.

Membranes were stored at -20 °C and used within 3 weeks of preparation. The figure shows a schematic representation of the above procedure. Spectrophotometric analysis of rhodopsin content has been used as the purity criterium for rod outer segment preparations. As long as the rod disc membranes contain the bulk of the eye's rhodopsin, in pure preparations of rod outer segments, the ratio between absorbance at 280 nm and absorbance at 498 nm should correspond to a rhodopsin content of 65–70% of the total proteins[9]. Considering that cattle retinas are from animals not previously dark-adapted, the observed ratio of 2.4–2.6 is indicative of good disc purification.

1 C. Nicotra, M.A. Livrea, A. Bongiorno and A. Bono, IRCS Med. Sci. *3*, 530 (1975).
2 C. Nicotra and M.A. Livrea, Expl Eye Res. *22*, 367 (1976).
3 C. Nicotra and M.A. Livrea, Ital. J. Biochem. *27*, 2 (1978).
4 F.J.M. Daemen, Biochim. biophys. Acta *300*, 255 (1973).
5 D.G. McConnell, J. Cell Biol. *27*, 459 (1965).
6 D.G. McConnell, G.W. Ozga and D.A. Solze, Biochim. biophys. Acta *184*, 11 (1969).
7 W.J. de Grip, F.J.M. Daemen and S.L. Bonting, Vision Res. *12*, 1697 (1972).
8 D. Bownds, A. Brodie, W.E. Robinson, D. Palmer, J. Miller and A. Shedlovsky, Expl Eye Res. *18*, 253 (1974).
9 F. Siebert, H. Schmid and R.H. Mull, Biochem. biophys. Res. Commun. *75*, 1071 (1977).

Methoxyphenylethylamines as substrates for type A and type B monoamine oxidase

O. Suzuki, T. Matsumoto, Y. Katsumata and M. Oya

Department of Legal Medicine, Hamamatsu University School of Medicine, Hamamatsu 431-31, and Division of Oncology, First Department of Surgery and Department of Legal Medicine, Nagoya University School of Medicine, Nagoya 466 (Japan), 13 November 1979

Summary. 4-Methoxyphenylethylamine was found to be a specific substrate for type B monoamine oxidase (MAO) in rat brain mitochondria, whereas 3,4-dimethoxyphenylethylamine was common for both types of MAO. These results suggest that O-methylation in the para-position increases the preference of the substrate for type B MAO, while a methoxy-group in the meta-position contributes to the substance being a type A substrate.

Mitochondrial monoamine oxidase [amine: oxygen oxidoreductase (deaminating, flavin-containing); EC 1.4.3.4] (MAO) is believed to exist in many animal tissues in 2 functional forms called type A and type B[1–3]. The relationship between the structure of a monoamine and its substrate specificity for type A and type B MAO has been investigated; a hydroxyl-group in the para- or meta-position may contribute to a substance being a type A substrate[4–6]. The β-hydroxylation of a monoamine seems to enhance its preference for type A MAO only slightly[7]; N-methylation causes almost no change in the substrate specificity[8]. In the present paper, we have studied the effect of a methoxy-group on substrate specificity for type A and type B MAO using 4-methoxyphenylethylamine (MPEA) and 3,4-dimethoxyphenylethylamine (DMPEA).

Materials and methods. A crude mitochondrial fraction was isolated from whole brains of male Sprague-Dawley rats weighing 150–250 g as described previously[7]. MPEA and DMPEA were purchased from Sigma Chemical Company, St. Louis, Mo. Clorgyline, a selective inhibitor of type A MAO[1], was generously supplied by May & Baker Ltd, Dagenham, England. Deprenyl, a selective inhibitor of type B MAO[9], was kindly donated by Prof. J. Knoll, Semmelweis University of Medicine, Budapest, Hungary.

MAO activities were determined fluorometrically by the method of Guilbault et al.[10] and Snyder and Hendley[11]. For each assay (final volume, 3.0 ml), 0.35 mg of protein was used. The assays were carried out at pH 7.4 for 30 or 60 min. Under the conditions used, the assays were linear during incubation for 60 min. For kinetic analyses, the concentration ranges of MPEA and DMPEA were 15–1000 µM and 0.3–10 mM, respectively.

For inhibition experiments with clorgyline and deprenyl, the assay mixtures were preincubated with each inhibitor at 37 °C for 10 min to ensure maximal enzyme inhibition. It was confirmed that each inhibitor neither interfered with the formation of the fluorescent compound nor quenched its fluorescence when hydrogen peroxide was added directly. Protein was measured by a modification[12] of the conventional biuret method.

Results. Both MPEA and DMPEA were active substrates for MAO in rat brain mitochondria. Their Michaelis-Menten kinetic constants were determined from Lineweaver-Burk plots as shown in figure 1. The K_m value for MPEA was much lower than that for DMPEA; the V_{max} value for MPEA was higher than that for DMPEA. There was slight substrate inhibition at the highest substrate concentrations for both substrates.