BUTANOL EXTRACTS FROM MYELIN FRAGMENTS-V.
5-HYDROXYTRYPTAMINE BINDING TO THE
MITOCHONDRIAL BUTANOL EXTRACTS AND THE INTER-
RELATIONSHIP WITH MYELIN BUTANOL EXTRACTS

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In previous studies (1-4), we demonstrated that the butanol extracts from myelin
fragments possessed high selectivity and specificity for 5-HT binding, and that these extracts
contained basic proteins, DM-20 and proteolipid protein. We also found that the recon-
stituted system with crude basic proteins and lipids from myelin showed a saturable binding
capacity for 5-HT (3). Basic proteins are one of the main structural proteins of myelin
and induce an autoimmune disease, i.e., experimental allergic encephalomyelitis. For the
subcellular distribution of basic proteins, it has been reported that these proteins are mainly
located in myelin rather than other subcellular organelles, e.g., mitochondria, microsomes,
synaptosomes, etc. (5). To obtain more detailed information on the interaction between
basic proteins and 5-HT binding affinity, we planned experiments in which 5-HT bound to
the butanol extracts from mitochondria.

Male Wistar rats (150-200 g) were decapitated and the brain stems removed. The
myelin- and mitochondria-rich fractions were isolated from the homogenate (10% in 0.32 M
sucrose) by the method of Whittaker et al. (6) and purity of both fractions was examined by
electron microscopy (7). The resultant pellet of mitochondria fraction was suspended in
50% sucrose (2 ml/g stem) and extracted with 10 vol. of water-saturated butanol for 2 hr
at room temperature. The butanol phase thus obtained was concentrated under N₂ at 38°C

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to about 1/20 of its original volume (total extracts = TE). Binding experiments of C\textsuperscript{14}-5-HT (48.54 mCi/mmol, New England Nuclear) and displacement studies with an excess of cold 5-HT were performed by Sephadex LH\textsubscript{20} column chromatography as described previously (2). Protein and lipid phosphorus was assayed using the method of Lees and Paxman (8) and Chen et al. (9), respectively. Radioactivity was counted in a toluene/Triton X-100 emulsion phosphor. Extracts of myelin and mitochondria with 0.1 N HCl were prepared by the method of Martenson et al. (5). SDS-urea polyacrylamide gel electrophoresis was carried out according to the method of Chan and Lees (10). Amino acid analysis was carried out using a Hitachi 835 automatic amino acid analyzer. Samples of protein were hydrolysed in 6 N HCl at 110°C for 18 hr (11).

Chromatographic pattern of TE is shown in Fig. 1. Total recovery of protein and lipid phosphorus from the column was approx. 100 and 95%, respectively. Initial protein peaks (58%) were eluted with chloroform together with 73% of the lipid phosphorus. Other peaks of both components were eluted with the solvents of CM 6:1 and 4:1. The yield of hydrophobic proteins from mitochondria was 1.46 mg/g particle. C\textsuperscript{14}-5-HT radioactivity appeared as a single peak at the solvent system of CM 4:1 and a relative binding value of 5-HT was 82 nmoles /mg protein at 5 × 10\textsuperscript{-7} M. Furthermore, an interesting finding was that 1,000 fold excess of unlabeled 5-HT completely displaced the bound 5-HT. These results suggest that the 5-HT binding macromolecules present in TE are composed of saturable components alone. For the myelin butanol extracts, we found that 5-HT binding components of those were a composite constituted with the saturable and non-saturable groups (2).

From the studies on the acid extracts of subcellular fractions isolated from rat brain
(5), it has been reported that only those of myelin contained significant amounts of highly basic proteins, but detailed information was not presented. In a preliminary series, we undertook the SDS-urea gel electrophoresis and amino acid analysis of acid extracts of myelin and mitochondria. Gel electrophoresis revealed that the extracts of mitochondria also contained a light band (band B) which has the same migration as that of myelin basic proteins (band A). However, the results of amino acid analysis (Table 1) indicated that band A contained approx. 23 moles of total basic residues and 14 moles of total acidic residues, whereas value of band B was 15 and 21 moles, respectively. Both values of total basic and acidic residues of band A are in good agreement with the report of Martenson et al. (12). These results clearly demonstrate the lack of encephalitogenic basic proteins in mitochondria.

Finally, the present results suggest that butanol extracts from mitochondria also have a 5-HT binding capacity, but the binding components differ from those of the myelin. In the myelin butanol extracts, basic proteins may be implicated in the binding of 5-HT under the participation of lipids as reported previously (3). To our knowledge, acid extracts from mitochondria did not induce experimental autoimmune encephalomyelitis which is characterized by paralysis and infiltration of lymphocytes into the brain and spinal cord. From these observations it is plausible that the 5-HT binding affinity may play a role in the encephalitogenic activity of myelin basic proteins. Carnegie (13) also proposed specific interaction of myelin basic proteins with 5-HT as receptor and encephalitogenic proteins.

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<table>
<thead>
<tr>
<th>Amino acid</th>
<th>A</th>
<th>B</th>
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<tbody>
<tr>
<td>Lysine</td>
<td>6.52</td>
<td>7.36</td>
</tr>
<tr>
<td>Histidine</td>
<td>5.39</td>
<td>2.29</td>
</tr>
<tr>
<td>Arginine</td>
<td>11.56</td>
<td>5.36</td>
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<tr>
<td>Aspartic acid</td>
<td>7.43</td>
<td>8.74</td>
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<tr>
<td>Threonine</td>
<td>6.51</td>
<td>4.99</td>
</tr>
<tr>
<td>Serine</td>
<td>13.21</td>
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</tr>
<tr>
<td>Glutamic acid</td>
<td>6.40</td>
<td>11.80</td>
</tr>
<tr>
<td>Proline</td>
<td>7.51</td>
<td>4.51</td>
</tr>
<tr>
<td>Glycine</td>
<td>13.41</td>
<td>11.16</td>
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<tr>
<td>Alanine</td>
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<tr>
<td>Cystine</td>
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<tr>
<td>Valine</td>
<td>1.48</td>
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<tr>
<td>Methionine</td>
<td>1.43</td>
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<tr>
<td>Isoleucine</td>
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<tr>
<td>Tyrosine</td>
<td>1.57</td>
<td>3.54</td>
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<tr>
<td>Phenylalanine</td>
<td>4.51</td>
<td>3.84</td>
</tr>
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</table>

Results are expressed as moles/100 moles of total amino acids (mean of 2 preparations). Notations of bands A and B are given in the text.
REFERENCES


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EFFECTS OF CORIOLAN, AN ANTITUMOR POLYSACCHARIDE, PRODUCED BY CORIOLUS VERSICOLOR IWADE

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Mechanisms involved in the antitumor action of basidiomycete polysaccharides against various implantable tumor cells have not been elucidated. This activity is generally considered to be host-mediated (1-3) and not directly cytotoxic, unlike that of bacterial lipopolysaccharides.

Antitumor polysaccharides (ATSO) from the mycelium of Coriolus versicolor Iwade