Association of Radioactivity from $^{14}$C-Labelled 5-Hydroxytryptamine with Proteins of Rat Liver Mitochondria

S. Kveder, S. Iskrić, and L. Stančić

Tracer Laboratory, Institute »Ruder Bošković«, Zagreb, Croatia, Yugoslavia

Received May 6, 1967

5-Hydroxy[1'-14C]tryptamine was incubated with rat liver mitochondria and nuclei. After centrifuging the incubation mixture with mitochondria, 35.7% of the applied radioactivity was found in the supernatant and 32.2% in the acetone powder of the sediment. With nuclei the corresponding figures were 78.5% and 10.8% respectively. Kinetic studies showed that the radioactivity of crude mitochondrial protein is due to some enzymatic action while that of nuclei to nonspecific binding. Additional experiments performed with 5-hydroxytryptamine labelled in the indole ring, iproniazid and semicarbazide respectively suggest that the association of radioactivity with mitochondrial protein is due to the formation of a Schiff's base between the intermediate 5-hydroxyindolylacetaldehyde and a free amino group of the protein.

We have shown¹ that the 700 X g sediment of rat liver homogenate in Krebs-Ringer phosphate metabolizes 5-hydroxytryptamine in a different way from the whole animal or liver slices. In the case of liver homogenate, although the metabolic events were initiated by monoamine oxidase (MAO), only traces of 5-hydroxyindoleacetic acid were detected. Besides some unidentified metabolites, in the incubation mixture were found 5-hydroxyindole-3-aldehyde and 5-hydroxyindole-3-carboxylic acid — i.e. compounds having one C-atom less in the side chain than the substrate. Other arylethylamines which are substrates of MAO undergo the same splitting of the side chain too². Recently we reported³ that when $^{14}$C-5-hydroxytryptamine was incubated with the above tissue preparation a great part of the radioactivity was found associated with the particulate fraction of the incubation mixture.

The present communication shows that the mitochondrial part of the homogenate is responsible for these metabolic transformations as well as for the active binding of radioactivity. The nuclei proved to be metabolically inactive and it seems that radioactivity associated with them is due to some nonspecific binding.

**EXPERIMENTAL**

**Compounds**

5-Hydroxytryptamine creatinine sulphate labelled either at the terminal carbon atom of the side chain⁴ or at the 3 — position of the indole ring² was used.

**Tissue Preparations**

Nuclei were isolated from albino rat liver following the isotonic sucrose — CaCl₂ procedure of Hogeboom, Schneider and Striebich⁶. The removal of blood was ascertained by previous perfusion of liver with 0.9% NaCl through the portal vein.
To obtain mitochondria the 25% homogenate of liver in 0.3 M sucrose was first centrifuged at 1000 × g for 5 min. and the sediment was washed twice by resuspending in sucrose and centrifuging at 600 × g. To sediment mitochondria the combined supernatant and washings were centrifuged at 9200 × g for 10 min. The mitochondrial pellet was washed twice by resuspending in sucrose and centrifuging.

**Determination of Radioactivity**

Measurements of radioactivity were performed either under the conditions of infinite thinness, or — in parallel experiments — with samples of the same thickness.

**RESULTS AND DISCUSSION**

Electronmicrographs showed that the 700 × g sediment of rat liver homogenate in Krebs-Ringer phosphate consisted mainly of nuclei and mitochondria, and for this reason 5-hydroxytryptamine labelled in the side chain, was incubated with pure preparation of either of these particles. While in the presence of nuclei the substrate was left almost completely unchanged, after the incubation with mitochondria all the metabolites found previously in the experiments with 700 × g sediment were detected in the supernatant of the incubation mixture. Furthermore, on the account of high radioactivity of the supernatant, the amount of radioactivity associated with the acetone powder of nuclei was considerably lower than in the case of mitochondria (Table I).

**TABLE I**

**Distribution of Radioactivity in the Incubation Mixture**

<table>
<thead>
<tr>
<th>Particles</th>
<th>Recovery of applied radioactivity (%)</th>
<th>Supernatant and washings</th>
<th>Acetone extract</th>
<th>Acetone powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclei</td>
<td>78.5</td>
<td>6.2</td>
<td>10.8</td>
<td></td>
</tr>
<tr>
<td>Mitochondria</td>
<td>35.7</td>
<td>13.0</td>
<td>32.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(48.8)</td>
<td>(18.8)</td>
<td>(30.3)</td>
<td></td>
</tr>
</tbody>
</table>

The incubation mixtures (10 ml.) contained as follows: mitochondria from 2.5 g, or nuclei from 5.0 g of rat liver in 6 ml. of 0.3 M sucrose, 2.4 ml. of 0.067 M phosphate buffer pH 8.15, and 2.5 × 10⁻² moles (1.8 µc) of substrate dissolved in 1.5 ml. of 0.9%/ NaCl. After one hour of incubation at 37°C in air the incubation mixture was centrifuged at 3500 × g for 10 min. The sediment was washed three times by re-suspending in isotonic saline and centrifuging, and finally homogenized in a warring blender with 20 volumes of cold acetone.

The numbers in parenthesis denote the values obtained with 5-HT labelled in the indole ring.

To get an insight into the nature of binding of ¹⁴C, the radioactivity of proteins was investigated in relation to changes in the substrate concentration and the duration of incubation. (Fig. 1).

The saturation curve obtained with increasing concentration of 5-HT as well as the linear relationship of radioactivity with time in the case of mitochondria led to the conclusion that in this case the binding is the result of some enzymic action. In addition, when incubation was performed at 0°C, the radioactivity of mitochondrial proteins was drastically reduced. In the case of nuclei, on the other hand, the curves obtained suggest that the radioactivity of proteins is due to some nonspecific binding.
The presumption that a one-carbon fragment from the side chain was responsible for the radioactivity of mitochondrial proteins was disapproved by incubating 5-HT labelled in the indole ring. As in this instance the amount of radioactivity bound to acetone powder was the same as after 5-HT labelled in the side chain (Table I), it is obvious that the whole skeleton of the substrate is held on the mitochondrial proteins. From the difference of the recoveries of radioactivity it follows that about 15—20% of 5-HT underwent a splitting of the side chain. After the incubation of the substrate labelled in the side chain only traces of radioactive CO₂ were found and the further metabolic fate of the detached volatile one-C-atom fragment is at the moment obscure.

In the experiments where 5-HT was incubated in the presence of iproniazid the radioactivity bound was only 3% of the control. This finding, as well as the fact that the binding was also diminished by semicarbazide (25% of the control), suggest that the formation of 5-hydroxyindoleacetaldehyde through the action of MAO is a necessary precedent.

The formation of a Schiff's base between 5-hydroxyindoleacetaldehyde and a free amino group of proteins seems to be a plausible explanation for the binding of ¹⁴C. Block⁸ investigated the incorporation of radioactivity from labelled mescaline and phenylethylamine into proteins of subcellular particles of rat and ox liver and also suggested the formation of Schiff's bases as a possible explanation of this phenomenon. However, there is a disagreement between our findings and those of Block regarding the particles responsible for the active binding of radioactivity. He found that liver nuclei — but not mitochondria — incorporated the radioactivity and he ascribed the inactivity of mitochondria to an inhibitory factor. If, in Block's experiments, the radioactivity of nuclear proteins is ascribed to a nonspecific absorption, which was
considerable also in our experiments, then there is no explanation for the
inactivity of mitochondria which in our experiments proved to be metabolically
very vigorous. While the present communication was in preparation the paper
of Alivisatos et al.9 appeared, and their findings agree with ours regarding
both the mechanism of the labelling of proteins and the active particles.

The possible physiological significance of the described metabolic features
of liver mitochondria — i.e. the splitting of the side chain, the formation
of the hitherto unidentified metabolites, and the binding of some metabolite(s)
of 5-HT on their proteins — is not clear at the moment. However, in investi­
gations of the metabolic effects of 5-HT (and other arylethylamines) by use
of homogenates one has to take into consideration this metabolic pathway
(or pathways) which differs so considerably from the »classical« aminooxidative
route in which 5-hydroxyindoleacetic acid appears as the final product.

REFERENCES
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IZVOD

Vezanje radioaktiviteta iz 14C-markiranog 5-hidroksi-triptamina
na proteine mitohondrija jetre štakora

S. Kveder, S. Iskric i L. Stančić

5-Hidroksi [1-14C] triptamin inkubiran je s mitohondrijama i jezgrama iz štakorske jetre. Nakon centrifugiranja inkubacione smjese s mitohondrijama, u supernatantu je nađeno 35,7% a u acetonskom prahu sedimenta 32,2% od primjenjenog radio­aktiviteta. S jezgrama odgovarajuće vrijednosti su bile 78,5% odnosno 10,8% od primjenjenog radioaktiviteta. Kinetička ispitivanja pokazala su da je radioaktivnost srova mitohondrijskoga proteina uvjetovana nekim enzimatskim djelovanjem, a kod jezgra nespecifičnim vezanjem. Kod mitohondrijskih rezultati eksperimenta provedenih s 5-hidroksitRIPTAMINOM markiranim u indolskom prstenu, iproniazidom, odnosno smekarbazidom govore u prilog stvaranju Schiff-ove baze između intermedijarnog 5-hidroksiindolilacetaldehida i slobodne amino skupine proteina.

INSTITUT »RUDER BOŠKOVIĆ«
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