CCA-469

547.755:546.26.02:577.1
Original Scientific Paper

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 »Ruđer Bošković«, Zagreb, Croatia, Yugoslavia

Received May 6, 1967

5-Hydroxy [1′-^{14}C] 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 shown[1] that the 700 × 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 too[2]. Recently we reported[3] 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 chain[4] or at the 3 — position of the indole ring[5] was used.

Tissue Preparations

Nuclei were isolated from albino rat liver following the isotonic sucrose — $CaCl_2$ procedure of Hogeboom, Schneider and Striebich[6]. 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 re-suspending 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[1] 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

Particles	Recovery of applied radioactivity (%)		
	Supernatant and washings	Acetone extract	Acetone powder
Nuclei	78.5	6.2	10.8
Mitochondria	35.7	13.0	32.2
	(48.8)	(18.8)	(30.3)

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 *p*H 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 blendor 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 ^{14}C, 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.

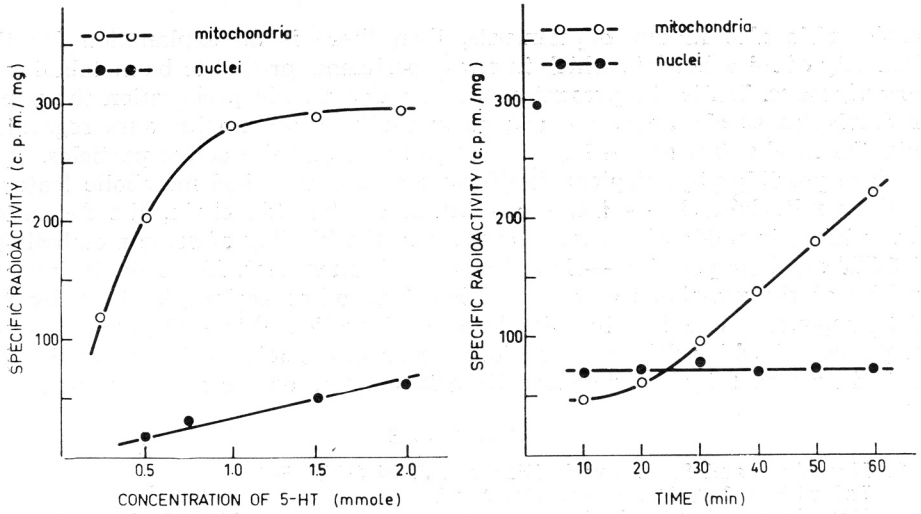

Fig. 1. The binding of ^{14}C by proteins of mitochondria and nuclei.
The incubation mixtures contained as follows: mitochondria from 0.25 g. or nuclei from 0.5 g. of rat liver in 2 ml. of 0.3 *M* sucrose, 1.5 ml. of 0.067 *M* phosphate buffer *p*H 8.15, and the substrate dissolved in 1.5 ml. of 0.9% NaCl. The incubations were stopped by the addition of 5 ml. of 10% trichloroacetic acid and crude proteins further elaborated according to the procedure of Siekewitz[7].
The left hand figure was obtained with incubation time of 2 hours and the right hand one with the substrate concentration of 0.0015 *M*.

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 CO_2 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 ^{14}C. Block[8] 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 investigations 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

1. S. Kveder and S. Iskrić, *Biochem. J.* **94** (1965) 509.
2. S. Iskrić and S. Kveder, *Croat. Chem. Acta* **37** (1965) 233.
3. S. Kveder and S. Iskrić, *3rd FEBS meeting*, Abst. No. F53 Warsaw, 1966.
4. D. Keglević-Brovet, S. Kveder, and S. Iskrić, *Croat. Chem. Acta* **29** (1957) 351.
5. D. Keglević and L. Stančić, *J. Labelled Compds.* **3** (1967) 144.
6. G. H. Hogeboom, W. C. Schneider, and M. J. Striebich, *J. Biol. Chem.* **196** (1952) 111.
7. P. Siekewitz, *J. Biol. Chem.* **195** (1952) 549.
8. W. Block, *Z. Physiol. Chem.* **296** (1964) 108.
9. F. G. A. Alivisatos, F. Ungarn, and F. F. Parmar, *Biochem. Biophys. Res. Commun.* **25** (1966) 495.

IZVOD

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

S. Kveder, S. Iskrić i L. Stančić

5-Hidroksi [1'-^{14}C] 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%. Kinetička ispitivanja pokazala su da je radioaktivnost sirova mitohondrijskoga proteina uvjetovana nekim enzimatskim djelovanjem, a kod jezgara nespecifičnim vezanjem. Kod mitohondrija rezultati eksperimenata provedenih s 5-hidroksitriptaminom markiranim u indolskom prstenu, iproniazidom, odnosno semikarbazidom govore u prilog stvaranju Schiff-ove baze između intermedijarnog 5-hidroksiindolilacetaldehida i slobodne amino skupine proteina.

INSTITUT »RUĐER BOŠKOVIĆ«
ZAGREB

Primljeno 6. svibnja 1967.