COMMUNICATIONS TO THE EDITOR

THE INCORPORATION OF VALINE-1-C^{14} INTO CYTOCHROME c BY RAT LIVER MITOCHONDRIA[1]

Sir:

Protein biosynthesis in cell-free systems has been investigated by studying the incorporation of labeled amino acids into the total protein of subcellular structures (*e.g.*, microsomes,[2] mitochondria,[3] nuclei,[4]) and by measuring increases in enzymic activity.[5] However, no cell-free system has been available for the study of the biosynthesis of a discrete protein that can be isolated in purified form. This report describes the incorporation of labeled amino acids into cytochrome c by rat liver mitochondria. Studies on the incorporation of labeled amino acids into the cytochrome c of rat liver slices have been reported.[6]

After incubation of mitochondria in the presence of an ATP-generating system and DL-valine-1-C^{14} cytochrome c was isolated by salt fractionation[7] and column chromatography.[8] The eluted material was washed with trichloroacetic acid containing unlabeled DL-valine. The specific radioactivity of the preparation remained constant after extensive washing with trichloroacetic acid, exhaustive dialysis, paper electrophoresis, and after removal of the porphyrin group.[9] The porphyrin fraction was not radioactive. The isolated cytochrome showed one band on paper electrophoresis and contained 0.442% Fe; its absorption spectra and extinction coefficients were identical to those reported for highly purified preparations[10] and for the crystalline protein.[11] However, the present state of uncertainty about the purity of cytochrome c preparations,[12] makes it difficult to assess unequivocally the homogeneity of our preparation.

Table I shows the dependence of valine-1-C^{14} incorporation on the presence of ATP generated either by oxidative phosphorylation or by creatine phosphate (CP). Replacement of valine by DL-lysine-1-C^{14} or of CP by D-3-phosphoglycerate gave similar results. The relatively small extent of valine incorporation in the presence of 2,4-dinitrophenol (DNP) or in the absence of added CP may be ascribed to endogenous substrate level phosphorylation. Conditions known to inhibit such phosphorylation completely suppressed incorporation (expt. 2). Valine incorporation also depends on the presence of a soluble liver fraction (the supernatant fluid obtained after centrifuging a 1:3 homogenate at 100,000 × *g* for 1 hour).

TABLE I

INCORPORATION OF VALINE-1-C^{14} INTO CYTOCHROME c

The reaction mixtures for experiments 1 and 2 consisted of potassium succinate (250 μmoles), L-histidine buffer of *p*H 7.4 (1.0 mmole), $MgCl_2$ (125 μmoles), cytochrome c (0.09 μmole), adenosine 5′-phosphate (AMP) (200 μmoles), DL-valine-1-C^{14} (10.8 μmoles containing 19 × 10^6 c.p.m.), orthophosphate (250 μmoles), soluble fraction (5 ml.), and the 3 to 6 × washed mitochondria obtained from 24 g. of rat liver, in a final volume of 25 ml. Incubated at 37° for 20 min. In the zero time experiment, the reaction mixture was treated immediately with acid. The final concentrations of the inhibitors used were: DNP (3.0×10^{-4} *M*), KF (0.01 *M*), sodium arsenate (0.002 *M*). In experiment 3, CP (313 μmoles), and adenosine 5′-diphosphate (ADP) (50 μmoles), were substituted for succinate, AMP, cytochrome c, and orthophosphate. The amount of cytochrome c isolated from each flask was about 0.15 μmole, corrected for cytochrome c additions where these were made.

Expt.	Conditions	Sp. activity, c.p.m./mg. cy.c.
1	Complete system	875
	+ DNP	78
	anaerobiosis	66
	zero time experiment	0
2	Complete system	509
	+ F^-, arsenate, DNP, anaerobiosis	0
	− soluble fraction	125
3	Complete system	295
	− CP	7

The presence of labeled valine in the peptide chain of cytochrome c is suggested by the retention of the label throughout the purification procedures employed, and by the ATP dependence of the incorporation process. Further evidence for this view was obtained by treating a partial acid hydrolysate of the labeled cytochrome with 1-fluoro-2,4-dinitrobenzene and fractionating the products by a differential extraction procedure[13] which separates N(α)-(2,4-dinitrophenyl)-amino acids from 2,4-dinitrophenylpeptides, and by paper chromatography. The major portion of the radioactivity appeared in fractions usually associated with dinitrophenylpeptides, and distinct from dinitrophenylvaline.

Although the evidence presented strongly favors the view that isolated mitochrondria can incorporate labeled amino acids into cytochrome c, the immediate role of ATP in the process is unknown; *e.g.*, the possibility that energy is needed only for amino acid transport into mitochondria has not been excluded. It is hoped that further studies will yield information on this point and on the question of the relationship between amino acid incorporation and the *de novo* synthesis of cytochrome c.

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RECEIVED DECEMBER 27, 1957

(1) Aided by a grant (A-428-C4) from the U. S. Public Health Service and the Muscular Dystrophy Associations of America.

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