DIFFERENCES OF MITOCHONDRIAL PROTEIN SYNTHESIS IN VITRO BETWEEN TUMOUR AND NORMAL TISSUES

A. Graffi, G. Butschak and E. J. Schneider
Institute for Cancer Research, Experimental Section,
German Academy of Sciences, Berlin-Buch

Received October 15, 1965

During the last years, data have frequently reported on mitochondrial protein and nucleic acid synthesis, and on the presence of DNA in mitochondria of various organisms (Simpson, 1962; Roodyn, 1962; Kalf, 1964; Kroon, 1963; Nass and Nass, 1963; Schatz et al., 1964; Wintersberger, 1964; Luck and Reich, 1964; Neubert and Helge, 1965). Recently we also published some results concerning this problem (Graffi et al., 1965). In the present paper experiments are described on differences between mitochondrial protein synthesis in tumour and normal tissues. We were particularly interested in the problem, since our previous studies had shown that cancerogenic hydrocarbons are preferably accumulated in these organelles, and it appeared possible that the malignization might be based upon a mutation of mitochondria (Graffi, 1940).

Methods

The methods used are largely those described in our earlier publications. Particular care was taken to observe sterility during isolation and incubation of mitochondria. The mitochondria were isolated and washed twice at $\sim$ 4500 g. They were incubated at 37° C, while being shaken in single dosages of

1 ml of mitochondrial suspension each (approx. 0.5 - 1 mg of protein) using the incubation medium of Wintersberger (1964) with 0.2 µC of ^{14}C-labeled amino acid for each single sample. A hot extraction (15 min, 90° C) with 5 per cent PCA was made of each sample.

Results

Table 1 presents the incorporation of ^{14}C-leucine and -arginine (guanidine label), and of uniformly ^{14}C-labeled algal protein hydrolysate into mitochondrial proteins of different tissues. This constitutes an average of a great number (about 150) of different mitochondrial preparations. The tumour mitochondria showed a low incorporation rate for leucine and, in general, a markedly increased incorporation rate for arginine. The latter, moreover, applies to mitochondria from the kidneys of mice, rats and cows, as well as from bovine liver (in contrast to mouse and rat liver). Mitochondria from embryonic tissues are characterized mainly by high incorporation rates of leucine and algal protein hydrolysate. The low leucine- and at the same time high arginine-incorporation for almost each tumour tested results in a very low leucine/arginine ratio as compared with embryonic and postnatal normal tissues, with the exception of renal tissues and bovine liver. For the remaining ^{14}C-labeled amino acid used so far in fewer single experiments (alanine, valine, lysine, glycocoll, methionine, citrulline) and for ^{35}S-labeled cysteine and ^{3}H-labeled ornithine, no specific pattern of incorporation of the different tissues could be demonstrated as yet. The specific activities here were generally lower than for leucine with the exception of cysteine, which yielded extremely high incorporation rates in the mitochondria of all normal and tumour tissues so far investigated,

Incorporation of ^{14}C-labeled algal protein hydrolysate, leucine and arginine into the proteins of mitochondria of different tissues (c/10 min/mg protein)

tissue	protein hydrolysate	leucine	arginine	leucine/arginine
isologous mouse sarcoma (methylchol., UV light, plastics)	1481 (16)	122 (30)	2198 (20)	0,06
epithelial mouse tumours (mamma, lung)	1813 (4)	222 (5)	809 (5)	0,27
mouse: virus-induced chloroleukemia	603 (1)	222 (1)	2070 (1)	0,11
Ehrlich Ca, S 37	237 (3)	73 (3)	1520 (3)	0,05
mouse: primary methylcholanthrene sarcoma	2010 (1)	96 (1)	927 (1)	0,10
rat tumours (Jensen Sa, Walker Ca)	1128 (7)	60 (6)	549 (5)	0,11
embryonic tissue of mouse and rat	3670 (12)	1950 (20)	648 (10)	3,0
embryonic tissue of chicken	3130 (7)	1076 (15)	359 (7)	3,0
mouse: normal organs (liver, heart, brain)	542 (4)	217 (3)	90 (4)	2,4
rat: normal organs (liver, heart, testicle)	1570 (13)	559 (17)	272 (9)	2,0
kidney (mouse, rat, cow)	1120 (11)	222 (10)	4084 (12)	0,054
bovine liver	1414 (5)	64 (3)	11438 (6)	0,006

( ) number of mitochondrial preparations

experiments: mitochondrial suspension in incubation medium according to Wintersberger: saccharose (0.25 m), versene (10^{-3} M), $MgCl_2$ (0.01 M), KCl (0.04 M), tris (0.02 M), pH 7,4 + 0,2 µC of radioactive amino acid/ml, 10 min, 37° C

as well as also in the nuclear and microsomal fraction.

The mitochondrial origin of protein synthesis as found in our studies resulted from the fact that admixture of microsomes (isolated at 100000 g) to the mitochondrial fraction did not increase the incorporation of protein hydrolysate, leucine and arginine. Moreover, the microsomal and nuclear fraction showed nearly without exception and very strongly for arginine a lower incorporation than the mitochondrial fraction, by using the same conditions as for the mitochondria.

Incorporation of amino acids into proteins of mitochondria in vitro is absolutely insensitive to RNase, and can be inhibited by actinomycin D to a certain extent only and irregularly. A more pronounced, but also irregular inhibitory effect is exerted by chloramphenicol and puromycin, in contrast to the strong effect of these substances on the protein synthesis in microsomes and nuclei. Addition of substrates of respiration such as α-ketoglutaric acid, ß-oxibutyric acid, succinic acid, pyruvate, citrate etc. caused very seldom an appreciable increase of leucine- and arginine-incorporation, whereas protein hydrolysate was even weakly inhibited by the addition of α-ketoglutaric acid.

KCN ($4 \cdot 10^{-2}$ M) strongly inhibited the arginine incorporation, especially in the presence of high incorporation rates (tumours, kidneys, bovine liver), in contrast to the incorporation of leucine and protein hydrolysate. Addition of dinitrophenol, however, had practically no effect regarding all three active substances. This leads to the conclusion that arginine incorporation is connected with an oxidative step, which, however, in all probability does not become effective via ATP formation.

In contrast to leucine and protein hydrolysate incorporation arginine incorporation is inhibited by the following additives: strong inhibition by ornithine (0.1 mM), glycine (from 0.1 mM); moderate inhibition by citrulline and lysine (from 1.0 mM); weak inhibition by histidine (from 1.0 mM). The remaining amino acids tested had no effect, neither did guanidine $NH_4^{\cdot}$ and $CO_3^{\prime\prime}$ ions.

Only arginine labeled at the guanidine C atom, shows such high incorporation into the protein of the mitochondria of tumour kidney and bovine liver, while C_5-labeled arginine, ^{14}C-citrulline and ornithine caused only a low incorporation. This suggests that not predominantly the unchanged arginine molecule is incorporated into the mitochondrial protein, but that one or more amino acids are newly snythetized from the guanidine portion in the mitochondria, which, in a second step, are included into the mitochondrial protein synthesis. It is, furthermore, improbable that arginine in mitochondria is utilized to a greater extent to build up basic proteins of the histone or protamine type, as the activity bound to mitochondrial protein is extractable neither by HCl/alcohol, nor by citric acid. After decomposition of the mitochondrial protein according to Sanger, there are found, following incorporation of protein hydrolysate, leucine and arginine, approximately 80 to 90 per cent of the activity in the residual chains of peptides, and only a minor portion in the end groups (thus demonstrating this incorporation to be a real protein synthesis).

We regard as essential result of our studies the high arginine incorporation into the mitochondrial protein of malignant tumours, compared with a low leucine incorporation. The former is probably related to malignancy in such a manner that

owing to the increased arginine incorporation due to the high nitrogen content in the guanidine portion, the prerequisite conditions for an increase of intracellular production of amino acids and possibly of nucleic acid bases are created in the tumour cell, thus enabling an autonomic growth. In these findings we see a further evidence for our views concerning the possible role of the mitochondria in the process of malignization (Graffi, 1940).

The high arginine incorporation into mitochondria of kidney and bovine liver is obviously connected with the urea metabolism, in that the valuable nitrogen is being economically retained by incorporation into the proteins instead of excreting it as urea.

REFERENCES

Graffi, A., Schneider E. J., and G. Butschak, Dtsch.Gesundheitswes. 20, 99 (1965).
Graffi, A., Z.Krebsforsch. 50, 196 and 501 (1940).
Kalf, G. F., Biochemistry 3, 1702 (1964).
Kroon, A. M., Biochim.Biophys.Acta 72, 391 (1963).
Luck, D. J. L., and Reich, E., Proc.Nat.Acad.Sci. 52, 931 (1964).
Nass, M. M. K., and Nass, S., J.Cell Biol. 19, 593 (1963).
Neubert, D., and Helge, H., Biochem.biophys.Res.Commun. 18, 600 (1965).
Roodyn, D. B., Biochem.J. 85, 177 (1962).
Schatz, G., Haslbrunner, E., and Tuppy, H., Mh.Chem. 95, 1135 (1964).
Simpson, M. V.: Ann.Rev.Biochem. 31, 333 (1962).
Wintersberger, E., Hoppe-Seyler's Z.physiol.Chem. 336, 285 (1964).