PARTIAL DELETION OF ASPARTIC ACID FROM DNA-PROTEINS DURING BUTTER YELLOW CARCINOGENESIS

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Received February 19, 1962

Earlier studies from this laboratory (Sahasrabudhe,1958; Narurkar, Kumta, Sahasrabudhe, 1957; Kotnis, Narurkar, Sahasrabudhe,1959) have shown that whenever there is rapid nucleic acid synthesis, there is a corresponding diminution in the pyridine nucleotide levels. This was attributed to a chemical competition between pyridine nucleotide and nucleic acid syntheses for the appropriation of a common precursor,adenine. Similar chemical competition was shown to exist for other purines and pyrimidines also (Aboobaker and Sahasrabudhe 1962). Rapid proliferation, which is accompanied by rapid nucleic acid synthesis, invariably results in augmentation of purine and pyrimidine biosynthesis. In the biosynthesis of pyrimidines, aspartic acid is the starting material and forms carbamyl-aspartate which in turn gives rise to orotate. In view of this it was suggested that aspartic acid may be drained towards nucleic acid synthesis and the proteins synthesized under such circumstances may be deficient in aspartic acid. No data are at present available about the levels of aspartic acid in proteins or peptides of actively growing tissue. Kit (1954) however had shown low levels of free aspartic acid in some malignant tissues. In the present investigation aspartic acid levels in proteins of normal, malignant and embryonic livers have been investigated.

Gross amino acid composition, particularly the levels of aspartic acid, of DNA-proteins was investigated. The reasons for restricting our studies to DNA-proteins only were two-fold.

It is well known that in malignant cells, the availability of energy is limited and therefore the synthesis of proteins 'unessential' for cell duplication is gradually slowed down and in highly anaplastic cells it is probably completely dispensed with. There was therefore no point in investigating the proteins from the cytoplasm. Secondly, since the malignant cells are considered to have undergone some sort of somatic mutation (which maintains an uncontrolled growth through repeated transplantations) some change in genetic material was anticipated. It was for this reason that the composition of DNA-proteins isolated from the nuclei of malignant cells was investigated, and compared with DNA-proteins of normal and embryonic cells. In this communication only the gross amino acid levels are reported. Further work on the sequence of amino acids in the DNA-protein complexes is now in progress and will be reported elsewhere.

EXPERIMENTAL

Wistar albino rats, 8-10 weeks old, were used in the present investigation. Diet containing butter yellow (3:4 dimethyl amino azo benzene) and having the composition - rice powder 75% , casein 15%, olive oil 4%, shark liver oil 2%, common salt 4% and butter yellow 0.06% - was fed to these animals throughout their life span till they were sacrificed.

Normal control animals received the same diet except that butter yellow was omitted.

Groups of animals were sacrificed at intervals of 3, 6, 9, 12 and 16 months after butter yellow feeding was started. The animals were sacrificed by cervical dislocation and the livers were quickly removed and homogenized in 0.25 M sucrose containing 0.002 M $CaCl_2$ in Potter-Elvejhem homogenizer. The homogenates were filtered through a double layer of muslin cloth to remove connective tissue fibres. The filtrate was then gently layered over 0.35 M sucrose containing 0.0002 M $CaCl_2$ and centrifuged for 10 minutes at 2000 r.p.m. (800 g) in International Refri-

gerated centrifuge at 0-5°C. Supernatent was discarded and the sediment in the lower layer was resuspended in 0.25 M sucrose containing 0.002 M $CaCl_2$ and recentrifuged as described above. This process was repeated three times when the sedimented nuclei were found to be fairly free from intact cells and other cellular debris. In the final preparation cellular debris were present only in traces and intact cells were present to the extent of 2-3%.

Livers from normal animals with corresponding age groups and from one day old litters were also processed in similar way.

The DNA-proteins were extracted from the nuclei according to the procedure outlined by Butler,Davison, James and Shooter (1954). For this purpose the isolated nuclei were homogenised in isotonic saline containing 0.25 M HCl. The homogenate was left standing at room temperature for fifteen minutes and then centrifuged at 2500 r.p.m. for fifteen minutes. The supernatent which contained the extracted DNA-proteins was separated. Aliquots of this extract were hydrolysed with perchloric acid (70%) for separation and analysis of purines and pyrimidines. The hydrolysates were chromatographed and checked for absence of uracil. Absence of uracil (i.e. absence of RNA-proteins) was taken as an index of purity of DNA-protein complex. Further aliquots were taken for the hydrolysis of proteins by the formic acid-HCl method (Gurnani, Kumta and Sahasrabudhe,1955). The hydrolysates were evaporated on water bath and the amino acids extracted from the residue with 10% isopropanol in water. Separation of the individual amino acids was carried out with two dimensional chromatography using Whatman No.1 filter paper with butanol: acetic acid : water (4 : 1 : 5) and phenol : water (100 : 20) containing 8-hydroxyquinoline as solvent systems. Amino acid spots were developed with 0.5% ninhydrin in acetone, identified and eluted from paper with 50 : 50 mixture of methanol: water containing 5 mg. copper sulphate per 100 cc. of mixture. Final colourimetry was carried out with Klett-Summerson colorimeter using green

filter. Amino acid concentration was expressed as micrograms per mg. of amino nitrogen.

Table I gives the levels of 14 amino acids in DNA-proteins isolated from livers of normal, one day old (embryonic) and butter yellow fed animals. Butter yellow diet was fed for 16 months and all the animals had frank hepatomas. The levels of most amino acids were comparable in

RESULTS

Table I

Gross amino acid composition of DNA-proteins of livers of normal, new-born (embryonic)and butter yellow fed animals

Amino acid	Amino acid content in ug/mg of amino nitrogen		
	Control	Embryonic	Butter yellow fed
Aspartic acid	1105 (1100 - 1130)	1129 (1117 - 1141)	541 (520 - 549)
Glutamic acid	1155 (1130 - 1160)	1154 (1134 - 1174)	1130 (1115 - 1205)
Glycine	396 (360 - 415)	668 (523 - 703)	383 (310 - 405)
Threonine	770 (724 - 794)	833 (793 - 873)	713 (700 - 730)
Alanine	330 (300 - 352)	286 (266 - 306)	304 (270 - 328)
Tyrosine	656 (610 - 688)	961 (926 - 996)	728 (702 - 740)
Valine	165 (160 - 185)	146 (134 - 158)	328 (302 - 342)
Histidine	656 (634 - 665)	522 (502 - 542)	664 (633 - 674)
Lysine	528 (483 - 560)	344 (307 - 371)	459 (428 - 530)
Methionine	260 (249 - 268)	250 (242 - 257)	248 (230 - 262)
Arginine	2272 (2109 - 2435)	2420 (2371 - 2480)	2489 (2442 - 2537)
Phenylalanine) Leucine) Isoleucine)	528 (480 - 560)	428 (404 - 447)	455 (400 - 524)

Figures in parenthesis give the range.

the three groups. Aspartic acid levels however, were considerably lower (50% of normal) in the butter yellow fed animals. No such deviation from normal values was seen in livers of new born animals.

Table II gives the levels of aspartic acid in DNA-proteins in livers of animals sacrificed at various intervals after butter yellow feeding. In our series frank malignancies do not become apparent till 12 months after butter yellow feeding. It will be seen from the results that aspartic acid levels came down long before frank malignancy became apparent. Normal control animals show a value of 1105 ug/mg. amino N_2. With 3 months of butter yellow feeding this value dropped down to 842; aspartic acid levels went on gradually decreasing thereafter till a value 50% of the control was reached after 12 months of butter yellow feeding (frank malignancy). No further decrease in aspartic acid levels was seen in 16 months group.

Table II

Aspartic acid levels in DNA-proteins of livers of animals fed with butter yellow for various periods of time

	Period of Butter yellow feeding in months					
	0	3	6	9	12	16½
Aspartic acid levels ug/mg of amino N_2	1108 (1100-1130)	842 (830-863)	770 (761-783)	632* -	549 (534-560)	541 (520-549)

Figures in parenthesis give the range.

*Data from only one sample of pooled livers from three animals

Whether this partial deletion of aspartic acid from the DNA-proteins is applicable to carcinogenesis induced by other carcinogens or not is now under investigation. The results presented in this communication certainly seem to support the premise that DNA-proteins may become partially depleted in aspartic acid during carcinogenesis. This observation if substantiated

may open up an entirely new avenue of work in unravelling the mechanism of carcinogenesis. Further work is in progress.

Concomitant with the reduction of aspartic acid levels to half, a two-fold increase in the levels of valine is seen in the DNA-proteins of hepatoma. In absence of data on the position of these two amino acids in the amino acid sequence of DNA-proteins of the normal and malignant cells, it is difficult to evaluate their significance. It is however, tempting to suggest that in hepatoma, valine replaces aspartic acid (mutation?) in the amino acid chains of DNA-proteins. This must remain a mere speculation till concrete evidence in support of such a claim is obtained through studies on sequential analysis of amino acids.

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