PN 10074

Sialic acid in the Keilin–Hartree cytochrome c preparation from beef-heart muscle

Cytochrome *c* as prepared by the well known KEILIN AND HARTREE trichloroacetic acid extraction procedure[1] contains a fraction (X) which may be separated electrophoretically[2] or chromatographically[3,4]. Removal of this almost colourless material increases the iron content of the cytochrome *c* from approx. 0.3% to 0.43–0.45%. In a recent study of the amino acid composition of Fraction X, HENDERSON AND PALÉUS[5] reported the presence of unspecified carbohydrate. Determined as glucose by the thymol–sulphuric acid method[6] a value of 8% was obtained. The presence of further non-protein material which was not reacting in this estimation method was indicated, however, by the low nitrogen content of 8.9%.

We have now found that Fraction X contains sialic acid (as determined by the thiobarbituric acid method[7] after acid hydrolysis). Two additional observations confirmed the identity of the liberated substance as sialic acid; first, its absorption spectrum corresponded closely to that of authentic NANA (L. Light and Co., Colnbrook, Great Britain) and second, over 90% was released by the specific action of purified neuraminidase (EC 3.2.1.18). As previous work had shown that Fraction X itself consisted of three components (a, b and c)[5] it was decided to examine the distribution of the sialic acid in terms of its concentration in those components.

A 6% (w/v) solution of beef-heart cytochrome *c* (KEILIN–HARTREE preparation, 0.3% Fe, salt free, freeze-dried) was made in 0.02 M phosphate buffer (pH 7.3), containing 0.15 M KCl, *I* 0.2. This was run in the 2-ml cell of the Perkin-Elmer Model 38 Tiselius electrophoresis apparatus at 110 V, 20 mA. Cytochrome *c* moved toward the cathode and the three components of Fraction X toward the anode. After 4.5 h the ascending limb of the cell contained only the three components of

TABLE I

PROTEIN AND SIALIC ACID CONTENTS OF SAMPLES CONTAINING COMPONENTS a, b AND c OF FRACTION X OBTAINED ELECTROPHORETICALLY FROM BEEF-HEART CYTOCHROME *c*

Sample (in order of withdrawal from ascending limb of cell)	*Components of Fraction X present*	*Protein (μg)*	*Sialic acid calculated as NANA (μg)*			
			Method of hydrolysis			
			Acid		*Neuraminidase*	
			(0.05 N H_2SO_4, 80°, 1 h)	*Control (heated in absence of acid)*	*	*Control (boiled neuraminidase)*
1	c	526	12	11	—	—
2	b + c	1816	117	11	115	11
3	a + b + c	4023	168	12	157	12

* Neuraminidase hydrolysis was carried out with 10 000 units[9] per 0.1 ml final volume purified neuraminidase[10] in 0.05 M Tris–maleic acid buffer (pH 5.7), containing 0.5 mM $CaCl_2$, incubated 1 h at 37°.

Abbreviation: NANA, *N*-acetylneuraminic acid.

Fraction X. A micro-pipette was inserted into this limb and three samples which corresponded to the three peaks present were withdrawn. In this way the fastest component (c) was obtained pure in the first sample withdrawn; the second and third samples, however, were mixtures consisting of components b + c and a + b + c, respectively. The limited amounts obtained so far have hindered attempts to purify components a and b electrophoretically.

Protein (estimated with the Folin–Ciocalteu reagent[8] using bovine serum albumin as the protein standard) and sialic acid contents of the three samples are given in Table I. From calculation of areas under the peaks and from the relative protein content of each sample, the protein and sialic acid contents of components a, b and c have been estimated and are given in Table II. These results show that

TABLE II

CALCULATED PROTEIN AND SIALIC ACID CONTENTS OF COMPONENTS a, b AND c OF FRACTION X

Component	*Protein (μg)*	*Sialic acid (μg)*
c	526	1
b	1290	105
a	2207	50

components a and b which constitute the main portion of Fraction X contain virtually all of the sialic acid. In view of the result with neuraminidase (Table I), it follows that the sialic acid is probably linked *O*-glycosidically to either *N*-acetylgalactosamine or to galactose, and also that components a and b probably contain in addition to sialic acid, stoichiometric amounts of one or the other of these carbohydrates[11].

NANA was compared with glucose in the thymol–sulphuric acid carbohydrate estimation method[6]. The coloured complex formed from glucose gave an absorption peak at 507–509 mμ whereas with NANA the low absorption produced a curve which was almost flat over the wavelength range 600–420 mμ. The absorption at 508 mμ with comparable quantities of NANA was only approx. 10% of that developed with glucose, and appeared in fact to reach a maximum (Table III).

It is clear therefore that the carbohydrate content of Fraction X is higher

TABLE III

COMPARISON OF COLOUR DEVELOPMENT BETWEEN GLUCOSE AND NANA IN THE THYMOL–SULPHURIC ACID METHOD OF CARBOHYDRATE ESTIMATION

NANA (μg)	$A_{508\ m\mu}$	*Glucose (μg)*	$A_{508\ m\mu}$
11.5	0.01_4		
23	0.03	20	0.23
57.5	0.06	40	0.46
115	0.06		

than that obtained by the thymol–sulphuric acid method of estimation. Furthermore Fraction X of which there is 20–25% present in the KEILIN–HARTREE beef-heart cytochrome *c* preparation[5] consists mainly of glycoprotein. Whether or not this material is present with cytochrome *c* in the mitochondrion is not yet known; an attempt is now being made to elucidate this point.

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Alterations of tissue lactate dehydrogenase in human neoplasms*

Conflicting views have recently appeared regarding the presence or absence of qualitative changes of lactate dehydrogenase (L-lactate:NAD oxidoreductase, EC 1.1.1.27) in tumor tissues. STARKWEATHER AND SCHOCH[1] have pointed to a shift towards Fraction III (H_2M_2) in neoplasms as well as a consistent alteration of the Michaelis constant in this fraction, suggesting that this lactate dehydrogenase moiety may represent "a structurally different protein characteristic of neoplastic tissue". NISSELBAUM AND BODANSKY[2], on the other hand, have purified the M_4 subunit from normal human liver and a hepatocellular carcinoma and report no significant differences between these two fractions either catalytically or immunologically. It is the purpose of this brief report to present evidence suggesting that the differences

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