

IDENTIFICATION OF SIALIC ACIDS IN HUMAN AND BOVINE FIBRINOGEN

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Sialic acids are a group of biologically important substances which occur widely in the animal kingdom. The recent report by Chandrasekhar, et al., (1962) that fibrin contains less sialic acid than fibrinogen and that sialic acid in fibrinogen influences both the rate and nature of clot formation [Chandrasekhar and Laki (1964)], are the latest roles attributed to sialic acids. Many workers have identified N-acetyl, N-glycolyl, methoxy and ON-diacetyl neuraminic acids in various secretions and tissue extracts from human, equine, bovine and porcine sources (Martensson, et al., 1958; Cabezas, et al., 1964). But there is no report available in the literature about the nature of sialic acids in fibrinogen. This communication reports our work on the isolation and identification of sialic acids in human and bovine fibrinogen.

Pure fibrinogen was prepared from fresh human and bovine plasma according to the method described by Chandrasekhar and Laki (1964). The fibrinogens were homogenous in the ultracentrifuge and were 99-100% clottable. Sialic acids were obtained from fibrinogen by repeated treatment with neuraminidase (E C 3.2.1.18) from Vibrio cholera, 100 units per ml. obtained from Behringwerke A. G., W. Germany). A typical reaction mixture contained 25 ml of a 3%

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fibrinogen solution in 0.3 M KCl, 15.0 ml. tris-maleate buffer (pH 6.5, 0.2 M) containing Ca (0.4M), 25.0 ml. of 0.3 M K Cl and 5.0 ml. of the neuraminidase enzyme. Incubation was carried out at room temperature for 24 hours and the reaction mixture was then dialysed against repeated changes of 0.3 M K Cl. The dialysates were pooled together. The treated fibrinogen was tested for the presence of sialic acid, was recovered by ammonium sulphate precipitation and was again treated with neuraminidase. The whole process was repeated until all the sialic acid from the fibrinogen was collected in the dialysate. The pooled dialysates were then passed through a Dowex 50 (H^+) column (3 cm x 40 cm), the effluent lyophilized and the lyophilized material passed through a Dowex 1 (formate form) column. The sialic acids were then eluted from the column with 6 N formic acid and the formic acid was removed in a flash evaporator at low temperature and pressure. The pure sialic acid obtained was now chromatographed on Schleicher & Schüll No. 598 filter paper; the descending one dimensional technique and the circular paper chromatographic technique were employed. Three solvents were employed for chromatography: (a) n-butanol: acetic acid: water (4:1:5) (b) n-butanol: n-propanol: 0.1 N H Cl (1:2:1), and (c) ethyl alcohol: water: ammonia (80:20:1).

Sialic acids were also identified by the technique of fingerprinting, which consisted of chromatography on Whatman 3 MM paper in butanol-acetic acid-water (4:1:5) in one dimension followed by electrophoresis at pH 3.5 in 0.1 M pyridine-acetate buffer in the second dimension. The electrophoresis was carried out for 90 minutes at 2,400 V and 140 ma.

After developing the chromatogram, the area of the spots was measured by a 'Map measure' (1719B, Dietzgen, Switzerland) to determine the percentage composition of sialic acids. Bovine

fibrinogen contains 47% N-acetyl neuraminic acid and 53% N-glycolyl neuraminic acid. In electrophoresis, the N-glycolyl and N-acetyl neuraminic acids migrated toward the anode a distance of 30 cm and 29.5 cm respectively.

Table I

Sialic Acids from Fibrinogens

Sialic acid	Human fibrinogen	Bovine fibrinogen	RF values *		
			solvent systems		
			a	b	c
N-acetyl neuraminic acid	+	+	0.35	0.67	0.73
N-glycolyl neuraminic acid	-	+	0.31	0.59	0.66

+ Present.

- Not detected.

* RF values calculated on horizontal circular paper chromatography.

As shown in Table I, bovine fibrinogen contains both N-acetyl and N-glycolyl neuraminic acid, whereas the human fibrinogen contains primarily N-acetyl neuraminic acid. Chromatograms of sialic acid in human fibrinogen with solvent (c) revealed two unidentified spots with RF values lower than that of N-glycolyl neuraminic acid. It is interesting to note that N-acetyl and N-glycolyl neuraminic acids were also found to occur in other bovine material; N-acetyl neuraminic acid was the only sialic acid found to occur in human material so far. Sialic acids have been attracting increasing interest of late, due to the discovery that they provide the groupings which attract and bind influenza virus particles to the surface of host cells as a preliminary to infection [Gottschalk (1958)]. Sialic acids are also implicated in the biological activity of gonadotrophins [Brossmer and Walter (1958)]. In the light of this information and the fact that N-acetyl neuraminic acid is the only sialic acid identified in human material, it is

quite likely that the N-acetyl neuraminic acid is the more important member of the sialic acids group.

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