PN 1202

Effect of diphosphopyridine nucleotide on the formation of ^{35}S-labeled substances in a particle-free supernatant of mouse mast-cell tumor

The mast-cell tumor has proved to be a valuable system for the study of heparin biosynthesis. The incorporation *in vitro* of [^{35}S]sulfate into heparin in mouse mast-cell tumor homogenates and soluble fractions has been demonstrated by a number of investigators[1-3]. This incorporation in Dunn-Potter tumor homogenates was found to be stimulated by DPN*. The present note describes the enhancement by DPN of sulfate incorporation into a low-molecular-weight sulfate-containing fraction as well as into heparin in a high-speed supernatant of the Furth mastocytoma.

The Furth mast-cell tumor was carried in either the subcutaneous or intramuscular form in LAF_1J mice[6]. The tumor was homogenized at 0° in a solution (1:1, w/v) containing 0.1 M Tris buffer (pH 7.4), 0.003 M phosphate buffer (pH 7.4), 2.5 mM cysteine, and 6 mM $MgCl_2$. The homogenate was centrifuged at 105000 × *g* for 1 h in a Spinco ultracentrifuge. The clear supernatant fluid was incubated under the conditions described in Fig. 1. The incubations were terminated by the addition of an equal volume of saturated ammonium sulfate. Inorganic [^{35}S]sulfate was removed from aliquots of each incubation sample by two different methods.

Method 1. An aliquot of the sample was heated at 100° for 5 min and then dialyzed against cold running water for 48 h. Aliquots of the non-dialyzable fraction

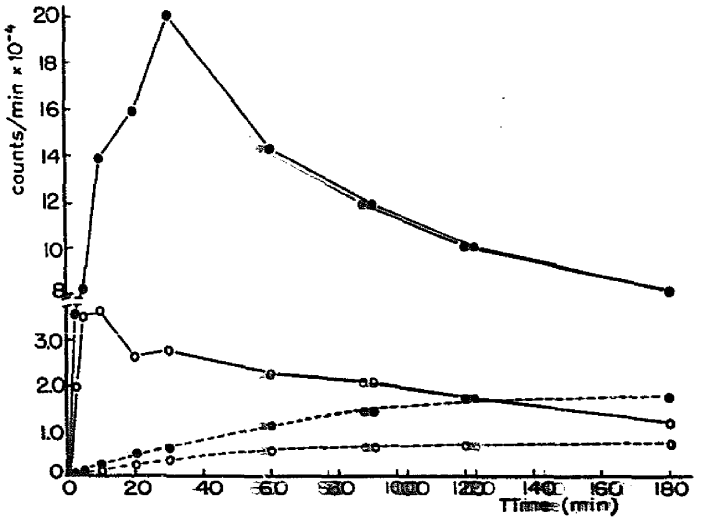

Fig. 1. The time course of [^{35}S]sulfate incorporation into Fraction I (heparin fraction) and Fraction II (paper-fixed fraction) of Furth tumor high-speed supernatant. ●---●, Fraction I, DPN added; ○---○, Fraction I, no DPN; ●—●, Fraction II, DPN added; ○—○, Fraction II, no DPN. Each tube contained the following materials in a volume of 0.5 ml: High-speed supernatant from 50% Furth-tumor homogenate, 0.13 ml; [^{35}S]sulfate, carrier-free, $5.4 \cdot 10^6$ counts/min; Tyrode's solution (pH 7.4), 125 µl; and the following substances in µmoles: Tris buffer (pH 7.4), 26; phosphate buffer (pH 7.4), 4.0; ATP, 1.00; $MgCl_2$, 0.81; cysteine, 0.41; nicotinamide, 0.5; L-glutamine, 0.5; and DPN, 0.05. The incubation was carried out at 37° in a Dubnoff metabolic shaker.

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were plated and counted in a micromil end-window gas-flow counter. It was shown by paper chromatography that the bulk of the radioactivity in the non-dialyzable fraction resides in a material which moves with the same R_F as that of the major metachromatic component of commercial beef heparin[7]. This material was designated "Fraction I".

Method 2. Appropriate aliquots of the incubation mixture were spotted on Whatman No. 1 filter paper. The paper was then dried for 30 min at 90–100°, fixed with ethanol–formalin (4:1), and stained with Alcian blue. The unbound [^{35}S]sulfate was washed from the paper with 5 alternating washes of tap water and glacial acetic acid. After drying, the stained spots were cut out, placed on planchets, and counted. The values obtained with this technique represent the bound sulfate remaining on the paper. This paper-fixed material was designated "Fraction II"; it included the heparin fraction and one or more as yet uncharacterized sulfate-containing substances. Since the latter compounds are removed during the manipulations of Method 1 (Fig. 1), it would appear that they are dialyzable and, therefore, of relatively low molecular weight.

The effect of DPN on the time course of [^{35}S]sulfate incorporation into both fractions is shown in Fig. 1. Comparison of the broken lines shows that DPN caused a significant stimulation of sulfate uptake into Fraction I (heparin fraction). The solid lines show a very rapid uptake of labeled sulfate into Fraction II (paper-fixed material); this incorporation was much more strongly stimulated by DPN. This experiment has been repeated twice using different high-speed supernatant preparations.

The stimulatory effect of DPN on sulfate incorporation into the heparin fraction has been repeatedly demonstrated in this laboratory in homogenates and in a soluble fraction of the Furth mast-cell tumor. The mechanism of this DPN effect is not clear at this time. It is unlikely that the dialyzable sulfate-containing material formed during the early part of the incubation as a result of the addition of DPN is identical with 3'-phosphoadenosine 5'-phosphosulfate, since the synthesis of the latter substance does not appear to require DPN as long as ATP is available[8]. The possibility is considered that the low-molecular-weight material is a heparin precursor or an unknown intermediate involved in the transfer of sulfate to heparin. An attempt to characterize this fraction is now underway.

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