Short Communications

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Action of arylsulfatase on vitamin K_3 disulfate

In the course of examining the metabolism of vitamin K_3 (2-methyl-1,4-naphthoquinone) it appeared that the excreted sulfate ester of vitamin K_3 was probably resistant to hydrolysis by aryl sulphate sulphohydrolase (arylsulfatase, EC 3.1.6.1)[1]. Recently it was found that the synthetically prepared disulfate ester[2] failed to affect brain metabolism in the manner found when the diphosphate ester (Synkavite) was used[3]. The hydrolysis of vitamin K_3 disulfate by arylsulfatase has therefore been examined and the results are reported in this paper.

Two previous observations[1] were pertinent to the present investigation: first, the ultraviolet-absorption spectrum of vitamin K_3 differs markedly from that of the disulfate or diphosphate esters, especially in the region 245–265 mμ, making spectrophotometry a useful tool for quantitative analysis, and second, the spectra of 2-methyl-1, 4-dihydroxynaphthalene shaken in solution in the presence of air and of vitamin K_3 are identical, indicating that the former is readily oxidized to the latter.

In the present work, vitamin K_3 itself, added to and homogenized with brain, liver, and other biological material, was recovered in yields of 99–109% by the following procedure: 3 ml aq. homogenate containing vitamin K_3 was added dropwise to 5 ml hot (75°) cyclohexane; this mixture was alternately heated and shaken in a glass-stoppered tube for 5 min and then centrifuged; the ultraviolet-absorption spectrum of the upper (cyclohexane) layer was determined, a suitable blank being employed in the reference light path, and special attention being paid to the absorbancy at the 250–251-mμ peak. When 2-methyl-1,4-dihydroxy-naphthalene (reduced vitamin K_3) was liberated into aq. homogenate by the action of acid phosphatase (EC 3.1.3.2) or alkaline phosphatase (EC 3.1.3.1) on Synkavite, vitamin K_3 was identified and measured, again by cyclohexane extraction and spectral examination. Typical results are shown in Fig. 1 A.

Turning now to the sulfate esters, the enzymic hydrolysis of potassium *p*-nitrophenyl sulfate by homogenized rat liver and by a preparation from *Aspergillus oryzae* (Mylase P, Wallerstein Laboratories, New York) was followed at 420 mμ after addition of alkali[4,5] (see Fig. 1 B). When *p*-nitrophenyl sulfate was replaced by vitamin K_3 disulfate these proven sources of arylsulfatase, *i.e.*, rat liver and Mylase P, failed to release vitamin K_3, *i.e.*, reduced vitamin K_3. Even after 24 h incubation, no ultraviolet-absorption spectrum of vitamin K_3 or of anything else was detectable, and especially the absorbancy at 250–251 mμ remained at zero during the entire period. However, during the incubation of vitamin K_3 disulfate with Mylase P, inorganic sulfate was found[6] to be released, reaching, in 24 h, 20–25% of the amount to be expected from the total hydrolysis of vitamin K_3 disulfate, and in no experiment exceeding 50% (see Fig. 1 C).

These results show that vitamin K_3 disulfate is hydrolyzed by arylsulfatase to

a monosulfate ester, 2 (or 3)-methyl-4-hydroxy-1-naphthyl sulfate. This latter compound is not hydrolyzed farther by arylsulfatase. This second conclusion is in agreement with experiments on, and theoretical considerations of, *p*-hydroxyphenyl sulfate[7], wherein V_{max} for the enzymic hydrolysis of this latter compound as well as of many other substituted arylsulfates has been tested as a function of the substitution constant, σ.

For purposes of comparison the disulfate esters of three dihydroxynaphthalenes were prepared[2], namely the 1,6-, 1,7-, and 2,7-members. Although a quinone-like structure can be written for 1,7-dihydroxynaphthalene, there is no evidence that any of them exist in any but the dihydroxy form. All three of the dihydroxynaphthalenes are quantitatively extractable with ether (not cyclohexane) and have absorption maxima and extinction coefficients quite different from those of their

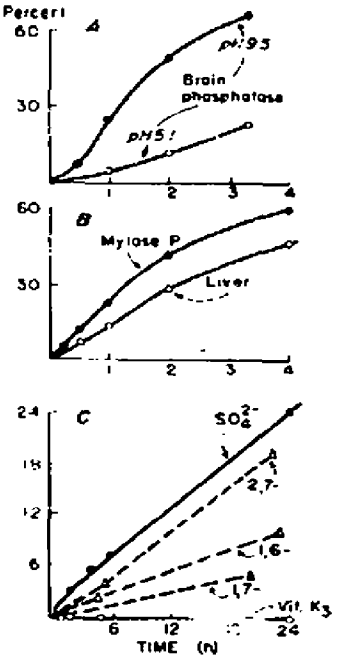

Fig. 1. A, hydrolysis of the diphosphate ester of vitamin K_3 (Synkavite) by rat brain. Synkavite, $1.5 \cdot 10^{-3}$ M; 25 mg homogenized rat brain per ml. Upper curve, 0.1 M Tris; lower curve, 0.1 M acetate. B, hydrolysis of *p*-nitrophenyl sulfate ($1 \cdot 10^{-2}$ M) by Mylase P (6 mg/ml) and by homogenized rat liver (10 mg/ml); 0.1 M acetate (pH 6.1). C, action of arylsulfatase of Mylase P on vitamin K_3 disulfate (●—●) indicating release of inorganic sulfate as a per cent of total possible. Action of arylsulfatase of Mylase P or rat liver on vitamin K_3 disulfate (○—○) indicating per cent of 2-methyl-1,4-dihydroxynaphthalene, *i.e.*, of vitamin K_3, released. Broken lines, action of arylsulfatase of Mylase P on disulfate esters of 2,7-, 1,6-, and 1,7-dihydroxynaphthalene, indicating release of dihydroxy compounds as per cent of total possible.

water-soluble disulfate esters (see Table I). All three of these disulfate esters are hydrolyzed by the arylsulfatase of Mylase P to the free dihydroxynaphthalenes as shown in Fig. 1C. The rates are considerably slower than that for the hydrolysis of *p*-nitrophenyl sulfate (Fig. 1B) and even somewhat slower than that for the hydro-

lysis of vitamin K_3 disulfate to the monosulfate, while the latter, as noted previously, is not hydrolyzed at all.

In this investigation conditions have been chosen arbitrarily to meet, to a large extent at any rate, the various pH optima and K_m values of the several kinds of arylsulfatase[9]. Crude liver homogenate, especially, was employed to include these arylsulfatases. In view of the relatively broad pH activity curves for these enzymes and the degree of enzyme activity still remaining at substrate concentrations of an

TABLE I

SPECTRAL DATA FOR THREE DIHYDROXYNAPHTHALENES IN ETHER AND THEIR DISULFATE ESTERS IN WATER

Compound	λ_{max}	ε_M
1,6-Dihydroxynaphthalene	244	26 000
Disulfate ester	282	4 700
1,7-Dihydroxynaphthalene	240	30 000
Disulfate ester	280	5 000
2,7-Dihydroxynaphthalene	232.5	88 000
Disulfate ester	267.5	3 700

order of magnitude lower than those represented by the K_m values[9], our conclusions appear substantiated by the "all-or-nothing" nature of the observations, the fact that the analytical method has been shown to be adequate and the demonstrable hydrolysis of the three disulfate esters mentioned in the preceding paragraph.

The fact that synthetic vitamin K_3 disulfate is without stimulatory or inhibitory effect in brain-slice metabolism even at $5 \cdot 10^{-4}$ M, and that the disulfate is enzymically hydrolyzed to the monosulfate by the widely distributed arylsulfatases makes it unlikely that vitamin K_3 monosulfate has an effect on brain metabolism similar to that found using the diphosphate ester of vitamin K_3 itself[5,8].

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