Lack of Sulfate-Activating Enzymes in Human Breast Tumors[1] (34701)

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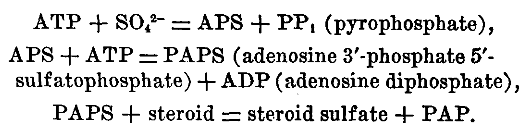
In our earlier studies (1, 2), we found that about 30% of human breast tumor preparations lacked the ability to form steroid sulfate esters when incubated with steroids and radioactive sulfate. Interestingly, it was also observed that tumors that lacked the ability to sulfurylate steroid hormones uniformly failed to regress after bilateral adrenalectomy (2). This correlation between clinical and laboratory findings led to our further investigation of steroid sulfate formation in these tumors.

Three enzymes are necessary for the formation of steroid sulfates: Sulfate adenylyltransferase (ATP:sulfate adenylyltransferase EC 2.7.7.4), adenylylsulfate kinase (ATP:adenylylsulfate 3′-phosphotransferase, EC 2.7.1.25), and one of two sulfotransferases, 3-β-hydroxysteroid sulfotransferase (3′-phosphoadenylylsulfate: 3-β-hydroxysteroid sulfotransferase EC 2.8.2.2.) or estrone sulfotransferase (3′-phosphoadenylylsulfate: estrone sulfotransferase EC 2.8.2.4).



$ATP + SO_4^{2-} = APS + PP_i$ (pyrophosphate),
$APS + ATP = PAPS$ (adenosine 3′-phosphate 5′-sulfatophosphate) + ADP (adenosine diphosphate),
PAPS + steroid = steroid sulfate + PAP.

The first two enzymes are necessary for the formation of "active sulfate," PAPS. The sulfotransferases are probably a family of related enzymes that act on different hydroxyl groups in different steroid molecules used as donors in our previous studies.

Lack of any one of these three enzymes would result in the failure of steroid sulfate formation by the tumor preparations. Results of the present investigation demonstrate that the tumor tissues that fail to synthesize steroid sulfates are lacking in both sulfate adenylyltransferase and adenylylsulfate kinase, but have measurable amounts of steroid sulfotransferases.

Materials and Methods. Steroid sulfate formation was measured as previously described (1), except that 5 μCi of $H_2{}^{35}SO_4$ and 0.1 μmole of K_2SO_4 were used/tube.

A crude sulfate-activating system was purified from yeast by the method of Robbins (3) with slight modification. The sulfate-activating enzymes were precipitated with sodium chloride, 250 g/liter of pH 5 supernatant, rather than with ammonium sulfate, to ensure no contamination with sulfate ions. The specific activity of the preparation was 0.02 μmoles of ATP degraded/mg of protein in 15 min. Five mg of yeast protein were added to each tube in the experiments shown in Table I.

TABLE I. Synthesis of Steroid Sulfate in Inactive Breast Tumor Preparations Following the Addition of Yeast Enzymes.

Tumor preparations	Synthesis of steroid sulfate (μμmole/mg of protein/hr)	
	DHEAS[a]	E_2S[b]
M.O.	35.9	31.4
R.S.	93.8	61.8
K.B.	36.6	37.7
H.O.	26.2	34.7
H.D.	36.5	36.0
A.E.	29.6	35.5
D.Z.	18.2	22.6
J.P.	33.2	28.9
H.B.	53.1	54.3
M.M.	31.2	28.4
C.Z.	4.4	6.6
R.B.	3.6	5.0
G.W.	6.6	3.4

[a] Dehydroepiandrosterone sulfate.
[b] Estradiol sulfate.

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Yeast sulfate adenylyltransferase and yeast adenylylsulfate kinase were purified by the method of Robbins (3). The specific activity of sulfate adenylyltransferase was 424 μmoles/mg/hr. In experiments in which sulfate adenylyltransferase was added, 25 μg of protein was added to each tube. The specific activity of adenylylsulfate kinase was 2.1 μmoles/mg/hr. In the experiments in which adenylylsulfate kinase was added 2.5 mg of enzyme was added to each tube.

None of the yeast preparations showed any steroid sulfotransferase activity.

Results. Addition of yeast enzymes to inactive tumor preparations. When 13 inactive tumor preparations were reassayed after the addition of a crude PAPS-generating system from yeast, it was found that these preparations now catalyzed the formation of significant quantities of steroid sulfates, ranging from 3 to 94 $\mu\mu$moles/mg of protein/hr (Table I). The amounts of steroid sulfates synthesized are comparable to those formed in the presence of tumor preparations that are active in the absence of the yeast enzymes.

These results suggest that the defect in the inactive tumor preparations is a lack of sulfate activation rather than a lack of a transferase, but the data do not indicate which enzyme is missing. In order to answer this question, the yeast preparation was purified further, and the two enzymes, sulfate adenylyltransferase and adenylylsulfate kinase, were separated from one another. When each enzyme was added separately to tumor preparations which showed lack of steroid sulfate formation under standard conditions, no evidence was found for steroid sulfate formation. However, if both yeast enzymes were added together, both steroid sulfotransferase activities being measured became apparent (Table II). Again, these tumor preparations show the same wide range as has previously been found in this series; namely, 6.5 to 126 $\mu\mu$moles/hr.

Addition of yeast enzymes to active tumor preparations. To establish whether the sulfate-activating enzymes or the sulfotransferases were the limiting factor in determining the level of steroid-conjugating activity of the tumor preparations that have the ability

TABLE II. Synthesis of Steroid Sulfate in Inactive Breast Tumor Preparations Following the Addition of Yeast Sulfate Adenylyltransferases and Yeast Adenylylsulfate Kinase.

Tumor preparations	Steroid	+ Both enzymes[c]
M.F.	E_2[a]	54.2
	DHEA[b]	61.0
M.H.	E_2	28.8
	DHEA	17.0
H.D.	E_2	39.9
	DHEA	25.2
H.B.	E_2	51.3
	DHEA	29.8
C.Z.	E_2	7.2
	DHEA	6.5
H.O.	E_2	30.2
	DHEA	16.3
J.C.	E_2	24.0
	DHEA	17.9
N.S.	E_2	126.0
	DHEA	77.8

[a] Estradiol-17β.
[b] Dehydroepiandrosterone.
[c] Steroid sulfate ($\mu\mu$mole/mg of protein/hr).

to form steroid sulfates, three such tumor preparations were tested by the addition of the two yeast enzymes separately and together. The results (Table III) strongly suggest that it is the sulfate-activating enzymes, rather than the steroid sulfotransferases, that limit the formation of sulfate esters in not only the inactive but also the active tumor preparations. When either enzyme is added alone, the activity of the tumor for the production of either DHEA (dehydroepiandrosterone) sulfate or estradiol sulfate is stimulated. A synergistic effect occurs, however, when the two enzymes are added together. From the constancy of the ratio of DHEAS to E_2S after the addition of either enzyme or both (Table III), it is obvious that the relative amounts of the two sulfates formed remain constant upon the addition of the two enzymes separately and together.

Discussion. The present data provide evidence that the defect in the tumors that do not show steroid sulfate synthesis is a lack of both enzymes necessary to form active

TABLE III. Synergestic Effect of Sulfate-Activating Enzymes from Yeast in Active Tumor Preparations.

		Steroid sulfate synthesized ($\mu\mu$moles/mg of protein/hr)			
Tumor preparations	Steroid	Without yeast enzymes	+ Sulfate adenylyltransferase	+ Adenylylsulfate kinase	+ Both enzymes
H.W.	E_2[a]	25.8	33.7	45.5	119.2
	DHEA[b]	7.3	10.1	13.1	23.9
	DHEA:E_2	0.20	0.30	0.29	0.20
E.S.	E_2	20.5	21.8	36.2	69.8
	DHEA	13.1	20.0	32.2	37.8
	DHEA:E_2	0.64	0.92	0.89	0.54
A.M.	E_2	24.8	29.8	37.4	76.7
	DHEA	8.4	12.3	15.8	20.9
	DHEA:E_2	0.34	0.34	0.41	0.27

[a] Estradiol-17β.
[b] Dehydroepiandrosterone.

sulfate. In all such tumor preparations tested, both enzymes were completely absent (Table II). Even so, this finding does not necessarily mean that these enzymes are completely lacking in the tumors *in situ.* These enzymes, as obtained from other mammalian sources, are known to be very easily destroyed by such mild treatment as dialysis (4, 5). It possible that there are present in some or all of the "inactive" tumors either very small amounts of the sulfate-activating enzymes which are destroyed on homogenization, or else variant forms of these enzymes which are much more labile to treatment than are those found in active tumor preparations. The present data cannot distinguish among these three possibilities; *i.e.,* very low level of the enzymes, variant form of the enzymes, or absence of the enzymes. The third possibility, complete absence of sulfate-activating enzymes, is one which is amenable to experimental determination. A comparison of sulfate incorporation by slices of tumor tissue and the formation of steroid sulfate by homogenates of these tumors should show whether sulfate-activating enzymes are completely absent (in this case slices should not be able to incorporate sulfate into macromolecular structures) or are, for one reason or another, easily degraded. Such a study has been started in our laboratory.

It is interesting to note that the ratio of DHEAS to E_2S is unaffected by the addition of an excess of either sulfate adenylyltransferase or adenylylsulfate kinase or both. Presumably the ratio is controlled, in at least these three cases, by the relative amounts of the two sulfotransferases, rather than by an effect of the steroids on the sulfate-activating enzymes.

The possibility that sulfate metabolism in these tumors may be related to polysaccharide metabolism cannot be entirely ruled out. Although human breast tumors have never been investigated in this regard, several tumors are known to form sulfated polysaccharides, such as chondroitin sulfates (5, 6). The possibility that some human breast tumors do not synthesize sulfated polysaccharides, because they lack the sulfate-activating enzymes, or because the level of sulfate-activating enzymes is very low, is interesting, and this possibility is being investigated by this laboratory.

Summary. Preparations from human breast tumors which are inactive in the formation of steroid sulfates are shown to be lacking the two sulfate-activating enzymes, sulfate adenylyltransferase, and adenylylsulfate kinase, and to have measurable amounts of 3-β-hydroxysteroid sulfotransferase and estrone sulfotransferase. The study also suggests that the sulfate-activating enzymes are the limiting factor in determining the level of steroid

conjugating activity of the tumor preparations that have the ability to form steroid sulfates.

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