The Intracellular Localization in the Rat Adrenal of Enzymes Which Degrade 3′-Phosphoadenosine-5′-Phosphosulfate[1] (36657)

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(Introduced by I. L. Schwartz)

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Because of the nature of its structure, 3′-phosphoadenosine 5′-phosphosulfate (PAPS) can be degraded by a number of enzymes. Brunngraber (1) demonstrated the presence in rat liver of a 3′ nucleotidase which acted on both PAPS and its desulfated derivative, 3′-phosphoadenosine-5′-phosphate (PAP). Suzuki and Strominger (2) presented evidence for the presence of a PAPS sulfatase in hen oviduct. The mitochondrial and microsomal fractions from rat liver have been shown to be able to degrade PAPS to PAP and to adenosine-5′-phosphosulfate (APS), indicating the presence of sulfatase and 3′-nucleotidase while PAP underwent both 3′ and 5′-dephosphorylations (3). The soluble fraction had much less activity. Similarly, in the pig kidney PAPS sulfatase and phosphatase activity was found in several subcellular fractions with no clear localization of the activities in any fraction (4).

During an investigation of sulfate metabolism in the adrenal gland of the rat we had the occasion to measure the degradation of PAPS. It was found that this process is inhibited by inorganic phosphate and that the enzymes involved are located within the lysosomes.

Methods. PAPS degradation. Incubation mixtures, as described in the tables, were prepared in 20 ml beakers and incubations were carried out in a Dubnoff metabolic shaker, in air at 37° for the times indicated. After the reaction, the beakers were placed on ice and 0.5 ml H_2O was added. The samples were quickly mixed, transferred to centrifuge tubes and placed in a boiling water bath for 3 min. After cooling and centrifuging, 1.0 ml of the supernatant was assayed for PAPS. The enzymatic assay for PAPS, based on the transfer of sulfate to *m*-aminophenol and the chemical determination of the resulting sulfate, is similar to that of Brunngraber (1) and has been described elsewhere (J. M. Fry and S. B. Koritz, unpublished research). PAPS degradation was determined by subtracting the amount of PAPS remaining after incubation from that present in a zero-time control.

Succinic dehydrogenase was measured according to Bachmann *et al.* (5) and acid phosphatase was assayed by the procedure of Schuel and Anderson (6), a modified King–Armstrong procedure (7, 8).

To obtain fractions rich in lysosomes the rat adrenals were homogenized in 0.25 *M* sucrose containing 0.005 *M* EDTA, pH 7.0, using a procedure similar to that of De Duve *et al.* (9). The tissue was first homogenized in one-third the volume required to give a final 10% (w/v) homogenate using one pass of the pestle. After centrifuging for 10 min at 755*g*, the supernatant was removed and the pellet rehomogenized in the second third of the volume. The supernatant from the centrifugation of this homogenate was added to the first supernatant and the pellet was rehomogenized as before in the last third of the volume of sucrose–EDTA: The resulting homogenate was combined with the two supernatant fractions to give the whole homogenate. This was fractionated by a procedure based upon that of De Duve *et al.* (9). The

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TABLE I. Inhibition of PAPS Degradation by Phosphate.[a]

Tissue fractions	Sodium phosphate	Tris-phosphate	Tris-chloride	Tris-acetate
	nmoles PAPS degraded per mg protein			
Nuclei and cell debris	0.76	0.95	5.98	6.36
Mitochondria	0.00	0.00	2.04	1.60
Microsomes	0.38	0.38	3.25	4.01
Supernatant	0.00	0.00	1.78	1.89

[a] One ml incubation mixture contained 40 μmoles of the indicated buffer at pH 7.5, 30 nmoles of PAPS and 0.5 ml of the various fractions. The incubation was carried out at 37° for 20 min.

whole homogenate was centrifuged at 755*g* for 10 min to yield the nuclei and cell debris and the supernatant centrifuged at 3600*g* for 10 min to sediment the heavy mitochondrial fraction. The resulting supernatant was centrifuged at 11,000*g* for 30 min to sediment the light mitochondrial fraction which is enriched with lysosomes. The supernatant was centrifuged at 105,000*g* for 60 min to give the microsomes and the soluble fraction. The heavy mitochondria were washed twice by resuspension in the EDTA–sucrose and centrifugation at 3600*g* for 10 min and the light mitochondria washed once by resuspension and centrifugation at 11,000*g* for 30 min. All centrifugation pellets were resuspended in a volume of 0.25 *M* sucrose equal to that of the original homogenate.

Results and Discussion. In the initial work on PAPS degradation large variations in activity from experiment to experiment were found. This prompted experiments in which the only variable was the type of ion present in the buffer. It was found that inorganic phosphate is an inhibitor of PAPS degradation (Table I). The time course for PAPS degradation in the absence of phosphate is linear for 20 min under the conditions used and subsequent experiments were carried out for 20 min.

The precise changes which take place in the degradation of PAPS in these experiments are not known since the assay method used measures only the ability of the remaining material to sulfate *m*-aminophenol. It has been found that PAPS is acted upon by a sulfatase and by a 3′-nucleotidase (3). The inhibition by phosphate could affect either of these activities.

The distribution of activity among all the fractions, with the highest activity in the microsomes, suggested that the enzymes involved in PAPS degradation are located within the lysosomes. These organelles are known to be distributed between the mitochondrial and microsomal fractions in the conventional fractionation procedure used to obtain the data in Tables I and II. This was further indicated by the effects of freezing and thawing, a procedure which destroys lysosomal structure and makes their enzymatic activity more apparent. The data in Table II show that freezing increases the activity of the mitochondrial and microsomal fractions about threefold while that of the soluble fraction remained the same. These results are consistent with the release of the degradative

TABLE II. Effect of Freezing and Thawing of Subcellular Fractions on PAPS Degradation.[a]

Subcellular fractions	Untreated	Frozen and thawed
	nmoles/mg protein	
Nuclei and cell debris	9.58	11.04
Mitochondria	5.05	18.76
Microsomes	9.98	25.59
Supernatant	4.79	4.79

[a] Frozen fractions were frozen and thawed three times using a dry ice acetone bath for freezing. One ml of incubation medium contained 40 μmoles of Tris-HCl pH 7.5, and 30 nmoles of PAPS and 0.5 ml of the tissue fractions all corresponding to the same amount of original tissue. Incubation was for 20 minutes at 37°.

TABLE III. Evidence for Subcellular Location of PAPS Degradative Enzymes.[a]

	Subcellular fractions[b]					
	W.H.	N., C.D.	H.M.	L.M.	M.	S.
Protein in fractions (mg/ml)	10.5	2.80	1.80	1.22	1.06	3.24
Acid phosphatase						
specific activity (nmoles/mg protein/10 min)	470	750	260	1000	390	250
total activity (nmoles)	4920	2100	470	1220	410	810
PAPS degradation						
specific activity (nmoles/mg protein/20 min)	41.4	28.1	25.4	126	43.1	13.4
total activity (nmoles)	433	78.7	45.7	154	45.7	43.4
Succinic dehydrogenase						
specific activity (nmoles/mg protein/min)			16.6	6.1		
total activity (nmoles)			29.9	7.4		

[a] Incubation mediums contained 0.5 mg/ml triton X-100. Conditions for PAPS degradation were same as in Table I.

[b] W.H. = Whole homogenate; N., C.D. = Nuclei and cell debris; H.M. = Heavy mitochondria; L.M. = Light mitochondria; M. = Microsomes; S. = Supernatant.

enzymes from the lysosomes present in the mitochondrial and microsomal fractions while the free enzymes in the soluble fraction are unaffected.

On the basis of the above results the tissue was then fractionated to obtain a lysosomal-rich fraction (the "light mitochondria"). To release particle-bound enzymes and insure measurement of total enzyme activity, the nonionic detergent, TX-100, was added to the reaction mixture. As can be seen from the data in Table III the PAPS degradative enzymes were concentrated in the "light mitochondrial" fraction. That this fraction is enriched with lysosomes and that the PAPS degradative activity in it is not due to mitochondria is indicated by the following findings. When the four postnuclear plus cell debris fractions are considered, acid phosphatase activity, a marker enzyme for lysosomes, was found to have the greatest activity in the "light mitochondrial" fraction in accord with the results of De Duve *et al.* (9). Data of Schuel and Anderson (6), as well as earlier observations by others (11–18) indicate that more than one enzyme contributes to acid phosphatase activity in the rat liver. The relatively large total activity found in the nuclear plus cell debris fraction in the rat adrenal probably respresents this non-lysosomal "acid phosphate" activity The measurements of succinic dehydrogenase, a marker enzyme for mitochondria, clearly show that the "heavy mitochondrial" fraction contains the bulk of the mitochondria.

Summary. The degradation of phosphoadenosinephosphosulfate in a cell free preparation of the rat adrenal is inhibited by inorganic phosphate. The enzymes responsible for this degradation are found to be localized in the lysosomes of this organ.

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