A Rat Liver Sulphohydrolase Enzyme Acting on Adenylyl Sulphate

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The existence of a PAPS* sulphohydrolase in animal tissues has been demonstrated by several workers (e.g. Suzuki & Strominger, 1960; Adams, 1964; Abraham & Bachhawat, 1964), and the sheep brain enzyme has been studied in some detail by Balasubramanian & Bachhawat (1962). This type of enzyme appears to be extremely widely distributed throughout the various phyla of the animal world (R. Bailey-Wood, K. S. Dodgson & F. A. Rose, unpublished work).

During investigations on the rat liver enzyme it became apparent that a second and distinct enzyme is present in liver that is capable of liberating inorganic SO_4^{2-} ions from APS, the immediate biosynthetic precursor of PAPS. Preliminary sub-cellular distribution studies, employing arbitrary enzyme assay conditions, revealed that both lysosomal and supernatant fractions were active in liberating inorganic SO_4^{2-} ions from APS. The supernatant fraction was chosen for a more detailed study because this particular fraction is known to contain the enzymes responsible for APS and PAPS biosynthesis (Hilz & Lipmann, 1955; Robbins & Lipmann, 1957).

APS was prepared and separated from other products by the method of Cherniak & Davidson (1964), and enzyme activity was assayed by a procedure based on that employed by Balasubramanian & Bachhawat (1962) for the assay of PAPS sulphohydrolase, except that unlabelled substrate was used and liberated inorganic SO_4^{2-} ions were determined by the turbidimetric method of Dodgson (1961).

The enzyme was purified 150-fold from the high-speed supernatant fraction of a 0.25 M-sucrose homogenate of rat liver. Inactive protein was first removed by heat treatment for 10 min. at 50° and pH 6.2, followed by fractionation with $(\text{NH}_4)_2\text{SO}_4$ at the same pH. The active fraction (40–55% saturation) was dissolved in 0.01 M-sodium citrate buffer, pH 6.6, and dialysed overnight against this buffer before absorption of the enzyme on alumina C_g gel (gel/protein ratio 0.5:1). The enzyme was eluted from the gel with 0.25 M- $(\text{NH}_4)_2\text{SO}_4$ solution and precipitated from the eluate by fully saturating with $(\text{NH}_4)_2\text{SO}_4$. The precipitate was redissolved in

citrate buffer, dialysed against a large volume of buffer and applied to a column of DEAE-Sephadex (citrate form). After washing with 0.01 M-citrate buffer until no more protein was removed, the enzyme was eluted employing a linear gradient of the buffer between the limits 0.01 M and 0.1 M. The product represented an overall purification from whole homogenate of about 500-fold. Although it gave six bands on polyacrylamide-gel electrophoresis it was considered to be suitable for establishing some of the characteristic properties of the sulphohydrolase.

The enzyme exhibited maximum activity in 0.125 M-sodium acetate-acetic acid buffer, pH 5.2, and at an APS concentration of 11.1 mM (K_m 5×10^{-4} M) when incubated for 5 min. at 37° . At this pH and temperature, and in the absence of substrate, the enzyme was relatively unstable and lost 40% of its activity after 10 min. and 90% after 40 min. However, at pH 6.6 in the frozen state the enzyme could be stored for several weeks without significant loss of activity.

Paper chromatography and electrophoresis experiments under a variety of conditions established that the products of the enzymic reaction were AMP and inorganic SO_4^{2-} ions. These products were shown to be liberated in equimolar amounts, sulphate being determined as described by Dodgson (1961) and AMP being separated by paper electrophoresis before being eluted and assayed spectrophotometrically. The enzyme is thus established as a sulphohydrolase and no evidence was obtained for the presence in the preparation of other enzymes capable of degrading APS (cf. Lewis & Spencer, 1962).

The rat liver supernatant employed as starting material for the purification of APS sulphohydrolase was also capable of liberating sulphate from PAPS (see also Lewis & Spencer, 1962). However, the purified enzyme preparation was completely devoid of any activity towards that substrate when tested under a wide variety of experimental conditions, including the presence of Co^{2+} or Mn^{2+} ions, which are known to activate strongly the sheep brain PAPS sulphohydrolase (Balasubramanian & Bachhawat, 1962).

The purified APS sulphohydrolase exhibited no activity towards the structural analogue, ATP, which inhibited the enzyme to the extent of approx.

* Abbreviations: PAPS, adenosine 3'-phosphate 5'-sulphatophosphate (3'-phosphoadenylyl sulphate); APS, adenosine 5'-sulphatophosphate (adenylyl sulphate).

70% at a concentration of 0.1 mM and completely at a concentration of 1.0 mM. At this latter concentration ITP, GTP, CTP and ADP also inhibited the enzyme (100%, 100%, 70% and 80% respectively). In contrast, AMP at the same concentration had virtually no effect.

A considerable number of other possible enzyme inhibitors and activators have also been examined and two further points of interest emerge. First, as with many other sulphohydrolases, both phosphate and pyrophosphate inhibit enzyme activity (55% and 72% respectively at 10 mM), and, secondly, the inhibiting effects of heavy-metal ions and of the thiol-group-blocking agents, *p*-hydroxymercuribenzoate and 5,5'-dithiobis-(2-nitrobenzoate), indicate that thiol groups are important to the activity of the enzyme.

The presence in rat liver supernatant fractions of a specific sulphohydrolase acting on APS is thus clearly established. The lysosomal fraction of rat liver is also able to liberate inorganic SO_4^{2-} ions from APS, but it is not yet known whether the same enzyme or type of enzyme is involved in both cases. The occurrence of an APS sulphohydrolase in the supernatant fraction of the liver cell is noteworthy in relation to the fact that the enzymes that are collectively responsible for the production of PAPS

from ATP and inorganic sulphate are also localized in the same fraction. The relationship between APS sulphohydrolase and these biosynthetic enzymes remains to be elucidated, but the fact that both ATP and ADP (a starting product and end product respectively of the biosynthetic system) are good inhibitors of the sulphohydrolase may indicate that, under normal circumstances of PAPS biosynthesis, the activity of the hydrolytic enzyme may be suppressed, allowing biosynthesis to proceed.

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