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# Autophagy-Related Changes of Arylsulphatases A and B in Rat Liver Lysosomes

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The total arylsulphatase activity and the relative activities of lysosomal arylsulphatases A and B were measured in the liver of control rats and rats subjected to treatments that provoke hepatic autophagocytosis. The total liver arylsulphatase activities were increased in starved and starved glucagon-treated rats, but not in sham-operated and hepatectomized rats. Arylsulphatases A and B in the mitochondrial–lysosomal (M–L) fraction were separated by polyacrylamide-gel electrophoresis at pH 8.8; they were made visible by incubating the gels with *p*-nitrocatechol sulphate as substrate, and measured by quantitative densitometry. In untreated controls, arylsulphatases A and B comprised 41.4±0.5% and 58.6±0.5% of the total arylsulphatase activity respectively; the arylsulphatase A/arylsulphatase B activity ratio was 0.71. All experimental treatments produced a significant decrease in the percentage of lysosomal arylsulphatase present as the A form and an increase in that present as the B form, and the activity ratio of arylsulphatase A/arylsulphatase B declined. The magnitude of these changes increased in the following direction: starvation for 24h = sham hepatectomy < glucagon + starvation < subtotal hepatectomy. These results indicate that the arylsulphatase A/arylsulphatase B activity ratio in liver lysosomes of normal rats is maintained within rather narrow limits, and this ratio declines during enhanced autophagocytosis. These findings, together with observations that suggest that arylsulphatase B may be a partially degraded form of arylsulphatase A, are consistent with the view that the A form is more rapidly converted into the B form during autophagy, owing to the digestive activity of other lysosomal hydrolases present in autophagic vacuoles.

The role of lysosomal hydrolases in cellular autophagy and heterophagy has been extensively documented (de Duve & Wattiaux, 1966; Ericsson, 1969; Gordon, 1973). Biochemical studies of these processes have generally focused on the digestive capacity of lysosomes and the degradative pathways of macromolecular substrates undergoing lysosomal digestion (Gordon, 1973; Vaes, 1973). However, little is known about the effects of autophagy and heterophagy on the lysosomal enzymes themselves.

Lysosomal hydrolases are relatively resistant to inactivation during incubation *in vitro* (de Duve & Beaufay, 1959; Aronson & de Duve, 1968) owing to the glycoprotein nature of these enzymes (Goldstone & Koenig, 1970, 1974*a*; Goldstone *et al.*, 1973). We have shown that newly synthesized acid hydrolases are packaged in lysosomes in the form of acidic sialoglycoproteins (Goldstone & Koenig, 1974*a*,*b*; Sanghavi & Koenig, 1975). During incubation *in vitro* at an acid pH these glycoprotein enzymes undergo autolytic cleavage of their *N*-acetylneuraminic acid, sugar and peptide residues and they are converted into more basic forms (Goldstone *et al.*, 1971; Goldstone & Koenig, 1974*a*; Needleman & Koenig, 1975). Further, lysosomal sialoglycoproteins become more basic during biodegradation *in vivo* owing to the fact that *N*-acetylneuraminic acid residues turn over more rapidly than the polypeptide portions of these molecules (Goldstone & Koenig, 1974*b*; Sanghavi & Koenig, 1975). It seems reasonable to infer, therefore, that, when lysosomal hydrolases are actively engaged in digestion within autophagic and phagocytic vacuoles, they are subject to accelerated autolytic degradation, with the consequence that the basic forms of these enzymes increase at the expense of the corresponding acidic forms.

This report describes experiments designed to test this hypothesis with respect to arylsulphatase A and arylsulphatase B. The results indicate that, when intense autophagocytosis was induced in rat liver by the administration of glucagon (Ashford & Porter, 1962; Deter & de Duve, 1967; Deter, 1971), partial hepatectomy (Becker & Lane, 1965) or starvation (Novikoff *et al.*, 1964; Swift & Hruban, 1964), the percentage of lysosomal arylsulphatase present as the A form decreased and that present as the B form increased, and the activity ratio of arylsulphatase A to arylsulphatase B declined.

## Experimental

### *Animal experiments*

Young adult female Sprague–Dawley rats (Holtzman Co., Madison, WI, U.S.A.), weighing 180–240g, were used. Untreated control, starved, glucagon-treated, hepatectomized and sham-hepatectomized rats were studied. The starved animals were deprived of food, but not water, for a period of 24h. Some starved animals received glucagon (200$\mu$g/100g body weight, intraperitoneally), and were killed 90min later. Subtotal hepatectomy was performed in fed rats under light ether anaesthesia; approx. 70% of the liver was removed (Becker & Lane, 1965). Sham-operated rats served as controls. These were subjected to the same anaesthetic and surgical treatment except that the liver was manipulated but not resected. Animals were killed 6h after surgery.

### *Preparation of subcellular fractions*

Animals were decapitated and the livers rapidly removed. These were weighed, rinsed with ice-cold 0.3M-sucrose, and homogenized in 9vol. of 0.3M-sucrose with a Potter–Elvehjem-type homogenizer. All manipulations were at 0–4°C. A portion of the homogenates was saved; the remainder was centrifuged at 800*g* for 10min, and the pellet was discarded. The supernatant was centrifuged at 16500*g* for 20min to deposit a mitochondrial–lysosomal (M–L) fraction, which served as the source of arylsulphatases A and B.

### *Biochemical assays*

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. Total arylsulphatase (arylsulphate sulphohydrolase, EC 3.1.6.1) was assayed with *p*-nitrocatechol sulphate as substrate, as previously described (Goldstone & Koenig, 1974*b*). Enzyme units are $\mu$mol of substrate cleaved per h.

There are a number of problems associated with the independent assays of arylsulphatases A and B when both enzymes are present in rat tissue extracts (Worwood *et al.*, 1973). In the present study these enzymes were separated by polyacrylamide-gel electrophoresis and their relative activities were measured in the stained gels by quantitative densitometry. The enzyme was extracted from the M–L fractions by sonication in ice-cold 0.2% Triton X-100 in 0.1M-Tris/HCl buffer, pH8.0, with a Branson Sonifier model W185D (Branson Sonic Power Co., Plainview, Long Island, NJ, U.S.A.). The sonicates were clarified by centrifuging at 100000*g* for 30min at 4°C in a no. 40 rotor in a Spinco model L ultracentrifuge. This procedure solubilized 95–99% of the total arylsulphatase in the M–L fraction. Portions of freshly prepared extracts, containing approx. 0.5mg of protein, were electrophoresed in 5% (w/v) polyacrylamide gels at pH8.8 for about 90min at 2.5mA/tube at 4°C (Davis, 1964). Arylsulphatase activity was demonstrated by incubating the gels in *p*-nitrocatechol sulphate as substrate (Roy, 1958) at pH5.5 for 30min at 37°C. The incubation was terminated and the colour developed by adding 0.5M-NaOH to a pH of about 10.5. The stained gels were immediately scanned at 515nm with a Gilford 2410 linear transport and a Gilford 2000 absorbance recorder. This system resolves a rapidly migrating component ($R_F$0.65), arylsulphatase A, and a slowly migrating component ($R_F$0.45), arylsulphatase B. The identification of these components was confirmed by polyacrylamide-gel electrophoresis of preparations of arylsulphatases A and B obtained from the rat liver M–L fraction by DEAE-cellulose column chromatography and isoelectric focusing (A. Patel & H. Koenig, unpublished work). The areas under the two peaks in the densitometric scans were measured with an OTT Universal Planimeter (F. Weber Co., Philadelphia, PA, U.S.A.), and the activities of arylsulphatases A and B expressed as percentages of the total area in each peak. The significance of the differences between means was assessed by Student's *t* test.

## Results and Discussion

The total arylsulphatase activity of the liver homogenates and M–L fractions of the control and experimental animals are given in Table 1. Starvation for 24h produced a 49% elevation in the specific arylsulphatase activity of the liver homogenate. No further increase occurred after the administration of

Table 1. *Effects of starvation, glucagon and hepatectomy on the total arylsulphatase activity of rat liver*

Homogenates and M–L fractions were prepared from livers from the following groups of rats: untreated controls; 24h-starved; 24h-starved plus glucagon (200$\mu$g/100g, 90min); sham-hepatectomized (6h); subtotal hepatectomized (6h). Other experimental details are given in the text. The results are means±S.E. of three experiments.

| | Arylsulphatase ($\mu$mol/h per mg of protein) | |
|---|---|---|
| Treatment | Homogenate | M–L fraction |
| Control | 0.49±0.03 | 1.47±0.15 |
| Starvation | 0.73±0.07* | 1.89±0.06 |
| Starvation+glucagon | 0.73±0.04* | 1.87±0.10 |
| Sham hepatectomy | 0.55±0.02 | 1.61±0.11 |
| Subtotal hepatectomy | 0.56±0.04 | 1.20±0.06 |

* $P<0.05$ compared with the untreated control.

Table 2. *Effects of starvation, glucagon and hepatectomy on the ratio of arylsulphatase A to arylsulphatase B in rat liver lysosomes*

Arylsulphatases A and B in soluble extracts of the M–L fraction were separated by polyacrylamide-gel electrophoresis and measured by quantitative densitometry. Additional experimental details are given in the legend to Table 1 and in the text. The results are expressed as means±S.E. The numbers of experiments are given in parentheses. *$P<0.05$; **$P<0.01$; ***$P<0.001$ compared with untreated and treated controls.

| Treatment | % of total arylsulphatase | | Ratio of arylsulphatase A/ arylsulphatase B |
|---|---|---|---|
| | Arylsulphatase A | Arylsulphatase B | |
| Untreated control | 41.4±0.5 (5) | 58.6±0.5 (5) | 0.71 |
| Starvation | 39.0±0.2** (7) | 61.0±0.2** (7) | 0.64 |
| Starvation+glucagon | 35.1±0.6*** (6) | 64.9±0.6*** (6) | 0.54 |
| Sham hepatectomy | 39.0±0.6* (6) | 61.0±0.6* (6) | 0.64 |
| Hepatectomy | 32.2±0.5*** (7) | 67.8±0.5*** (7) | 0.47 |

glucagon. A smaller increase (29%) in arylsulphatase activity occurred in the M–L fraction of the starved animals, which just missed statistical significance. Sham hepatectomy and subtotal hepatectomy produced minor statistically insignificant changes in arylsulphatase activities. Filkins (1970) found a similar increase in the total activities of four other lysosomal hydrolases in rat liver after 24h of starvation.

Table 2 presents the relative activities of arylsulphatases A and B and the activity ratios of the enzymes in the M–L fraction of control and experimental livers. It is significant that the percentage of the total lysosomal arylsulphatase occurring as arylsulphatases A and B showed a very narrow range of variation within each group of animals, as indicated by the small standard errors of the mean, which were 0.3–1.6% of the mean values. Starvation produced a small but statistically significant ($P<0.01$) decrease in the activity ratio, and the administration of glucagon to starved animals produced a still greater decrease ($P<0.001$) in this ratio. Sham hepatectomy also elicited a modest decrease ($P<0.05$) in the arylsulphatase A/arylsulphatase B activity ratio, but subtotal hepatectomy produced a far greater decrease ($P<0.001$) in this ratio.

The present experiments indicate that the arylsulphatase A/arylsulphatase B activity ratio in lysosomes of rat liver is normally maintained within rather narrow limits at a value of 0.71. When rats were subjected to several different treatments known to intensify autophagocytosis in the liver, the activity ratio decreased significantly, It can be assumed that the arylsulphatase in the M–L fraction was largely present in autophagic vacuoles (autolysosomes), as their appearance in liver cells is associated with a progressive disappearance of pericanalicular dense-body lysosomes (heterolysosomes, secondary lysosomes) after starvation (Novikoff *et al.*, 1964), glucagon administration (Deter, 1971) and subtotal hepatectomy (Becker & Lane, 1965). Although arylsulphatases A and B share many properties, including a capacity to hydrolyse *p*-nitrocatechol sulphate and certain other aryl sulphates, they nevertheless differ in a number of important respects and they are generally considered to be different enzymes (Nicholls & Roy, 1971). Therefore it would be quite reasonable to suppose that the changes in the relative activities of arylsulphatases A and B during autophagy are unrelated and represent a decrease in arylsulphatase A and/or an independent increase in arylsulphatase B.

On the other hand, we have previously presented evidence suggesting that arylsulphatase B may be a partially degraded enzyme which is derived from arylsulphatase A during its turnover (Goldstone *et al.*, 1971; Goldstone & Koenig, 1974*a*; Needleman & Koenig, 1975; Koenig & Patel, 1974; Patel & Koenig, 1976). Therefore we suggest that the autophagy-related decline in the arylsulphatase A/arylsulphatase B activity ratio is due to an increase in the rate of degradation of the A form and a consequent increase in the rate of formation of the B form, and reflects increased lysosomal digestion within autophagic vacuoles. It will be important in future studies to measure the rate constants of synthesis and degradation of arylsulphatases A and B during autophagy and to relate these rate constants to the pool sizes of these enzymes to fully assess their interrelationships.

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