Roche Applied Science

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Introduction

Hydrolysis of steroid esters and glycosides

The various steroids found in urine may be present in one or more of three forms: (a) the free compound (in minor or trace quantities and of little importance); (b) the sulfate (may be predominant in some cases); (c) the β-Glucuronide (the predominant form in most cases). The relative proportions are given in the following table:

Compound or category	free [%]	sulfate [%]	glucuronide [%]
17-Hydroxycorticosteroids	1	10 – 15	85 – 90
Pregnanediol	0	trace	≈ 100
Pregnanetriol	trace	trace	≈ 100
Estrone (O_1)	1 –3	10 – 15	85 – 89
Estradiols (O_2)	1 – 3	5 – 10	90 – 95
Estriol (O_3)	0 – 2	5 – 10	90 – 95
Androsterone	trace	20	80
Etiocholanolone	trace	10	90
Dehydroepiandrosterone (DHEA)	trace	≈ 100	trace
Epiandrosterone	trace	≈ 100	trace
11 β-Androsterone	trace	10	90
11 β-Etiocholanolone	trace	10	90
11 Ketoandrosterone	trace	trace	≈ 100
11 Ketoetiocholanolone	trace	trace	≈ 100

Several methods of hydrolysing steroid esters and glycosides are in common use. For the sulfates of DHEA and androsterone, solvolysis is suitable; this involves treatment with excess organic solvent (*e.g.*, ethyl acetate, dioxan, or tetrahydrofuran) at a temperature of 38°C for 18 – 24 h. Acid hydrolysis at elevated temperatures is a more general method, but has two disadvantages: it may alter the structures or constitutions of the steroids, and the resinified pigments formed need to be specially removed, since they are taken up in the extract. The third method, enzymatic hydrolysis (with β-Glucuronidase and sulfatase), does not involve these drawbacks.

The enzyme preparation obtained from the Roman snail, *Helix pomatia*, which exhibits very strong β-Glucuronidase and arylsulfatase activity, is widely used for the simultaneous hydrolysis of β-Glucuronides (β-Glucosiduronic acids) and sulfate esters in urine and other biological fluids. (1,2,3,4)

Application

- Enzymatic hydrolysis of steroid β-Glucuronides and sulfates
- Cell biology (removal of cell walls from yeasts in the preparation of protoplasts) (5)
- Enzyme immobilization studies (6)
- Determination of drugs in urine (7)

Hydrolysis of glucuronides and sulfates in urine

Adjust the pH of a portion of the sample (10 ml) to 5.5 by adding dilute acetic acid. Add acetate buffer of pH 5.5 (1 ml) and β-Glucuronidase/arylsulfatase solution (2 ml) and maintain at a temperature of 37°C for 16 h. Cool and extract with an appropriate solvent (*e.g.*, chloroform or dichloromethane) to isolate the hydrolysis products.

Product Description

Composition

The β-Glucuronidase/arylsulfatase obtained from *Helix pomatia* is a crude mixture of enzymes; its preparation does not involve any kind of chromatographic separation. Any attempt to purify it further (*e.g.*, by fractional precipitation with ammonium sulfate, ion-exchange chromatography, or gel exclusion chromatography) tends to lead to a decrease in activity. The preparation is stabilized with 0.01% thiomerosal.

Substrate specificity β-Glucuronidase

The glycosides that β-D-glucuronic acid forms with a variety of compounds containing hydroxyl groups hydrolyse readily in the presence of β-Glucuronidase. Such compounds include sterols, such as estriol (K_m = 0.42 mM, pH 4.5), androsterone, pregnanediol, tetrahydrocortisone, phenols, such as phenolphthalein (K_m = 0.39 mM), 4-nitro-phenol, 4-methylumbelliferone, drugs such as chloramphenicol and tetrahydrocannabinols, and metabolites such as thyroxine and bilirubin. Polysaccharides that contain β-Glucuronic acid residues, such as hyaluronic acid, are also hydrolysed. β-Glucuronidase is highly specific for the carbohydrate part: neither β-Glucosides nor α-Glucosiduronic acids are hydrolysed. However, the nature of the residue linked to the β-glucuronic acid residue is hardly important at all.

Substrate specificity arylsulfatase

Sulfate esters of many phenols are hydrolysed in the presence of arylsulfatase. Examples are steroid sulfates such as estrone sulfate, 4-nitrophenyl hydrogen sulfate (K_m = 1.8 mM, pH 7.3), 4-nitro-pyrocatechol 2-sulfate (K_m = 1.25 mM, pH 7.5), and phenolphthalein disulfate.