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SULFATION IN ISOLATED KIDNEY TUBULE FRAGMENTS OF RATS

DEPENDENCE ON INORGANIC SULFATE

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Abstract—Uptake and conjugation of sulfate was studied in isolated kidney tubule fragments of rats: Sulfate is rapidly taken up, and maximal cellular concentrations are attained after 5–10 min; intracellular steady-state levels depend on the sulfate concentrations of the medium and attain a maximal value at 2 mM. At 1 mM sulfate in the medium the ratio of intracellular/extracellular concentrations of the inorganic anion amount to about 0.25.

Formation of 7-hydroxycoumarin sulfate increases almost linearly up to an extracellular sulfate concentration of 500 μM. Thereafter, rates of sulfation increase more slowly and maximal sulfation rates are attained at 2 mM sulfate.

The data indicate that sulfation of 7-hydroxycoumarin proceeds at almost maximal rates in kidney tubule fragments at physiological serum sulfate concentrations.

Sulfate conjugation plays an important role in the detoxication of various endogenous and exogenous compounds [1, 2]. The sulfate ions which take part in the conjugation reaction after conversion to adenosine 3′-phosphate 5′-sulfatophosphate (PAPS) are supplied either via S-oxidation of cysteine [3, 4] or via dietary sulfate [4, 5]. In general the liver is the organ with the highest capacity to sulfate. However, extrahepatic organs such as the kidney also possess an appreciable activity to sulfate compounds [1, 6–9]. Drug metabolism in the kidney may become especially important when a compound enters the body via a route other than oral (e.g. intravenous, dermal) or in cases where the metabolic capacity of the liver is overwhelmed (e.g. high drug concentrations, liver dysfunction).

In the past several years the relationship between sulfate availability and sulfate conjugation of phenols has been studied intensively [4]. In rat serum, sulfate concentrations fluctuate between 0.6 and 0.9 mM according to the circadian rhythm [10], they may decrease due to a high dose of a drug that becomes sulfated [11] or increase due to orally administered sulfate [5].

Hepatic sulfation has been shown to depend on extracellular sulfate concentrations in isolated liver cells, in the isolated perfused liver and in the conscious rat [11, 12–17]. However, using different phenolic substrates various apparent K_m values for sulfate have been found, ranging from 0.1 to 3 mM [12, 13, 15, 16]; the reason for this variation is not yet understood.

In the gut recent data suggest that intestinal sulfation is restricted due to a limited availability of sulfate. Sulfation of orally administered tyramine or salicylamide was increased when the compounds were co-administered with sodium sulfate or L-cysteine [18, 19]. Furthermore, sulfation of 1-naphthol in isolated mucosa cells of the jejunum was not yet saturated at the physiological serum sulfate concentration [20].

Recently it has been shown that kidney tubule fragments are well suited to study sulfate conjugation of phenolic compounds [7, 21] and that sulfate is a precursor for sulfation in isolated kidney cells [9]. However, the effect of extracellular sulfate concentrations on intracellular sulfate levels and on sulfate conjugation is not known. We therefore studied the dependence of cellular sulfate uptake and sulfation of 7-hydroxycoumarin on different sulfate concentrations in isolated kidney tubule fragments.

MATERIALS AND METHODS

Chemicals. The radiochemicals $Na_2[^{35}S]O_4$ (542 mCi/mmole), [Carboxyl-^{14}C] dextran, mol. wt. 75000, 1.3 mCi/g and $[^3H]_2O$ (250 μCi/ml) were from NEN Chemicals (Dreieich, FRG). 7-Hydroxycoumarin was obtained from Sigma (München, FRG), bovine liver β-glucuronidase from Serva (Heidelberg, FRG), arylsulfatase (Helix pomatia) and collagenase from Boehringer (Mannheim, FRG). Silicon oils AR 20 and AR 200 for centrifugal filtration were from Wacker Chemie (München, FRG). All other chemicals were commercially obtained, and of analytical grade.

Preparation and incubation of kidney tubule fragments. Sprague–Dawley rats of about 180 g body weight (inbred strain, Neuherberg) served as kidney donors. Kidney tubules were prepared according

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to Guder [22] except that $MgSO_4$ in the Krebs–Henseleit bicarbonate buffer (KHB) was replaced by $MgCl_2$ in order to obtain sulfate depleted tubules; Pfeifer and Guder demonstrated that their preparations consist to about 90% of proximal kidney tubule fragments [23]. Viability of the preparations was checked determining their gluconeogenic activity in unsupplemented KHB. Glucose production from pyruvate (10 mM) was almost linear for 2 hr and amounted to about 10 nmole/mg cellular prot. × min when animals were fasted overnight. This value is in good agreement with published data amounting to 503–620 μmole/g protein/hr [22]. Tubule fragments were incubated in KHB, containing varying amounts of sulfate. The medium was enriched with the amino acids mixture of Leibowitz L-15 medium except that the S-containing amino acids methionine and cysteine were omitted and that the concentration of L-glutamine was 5 mM. Incubations were performed under a gas phase of 95% O_2/5% CO_2 at 37°C.

Sulfate uptake. To study uptake of inorganic sulfate, tubule fragments (3 mg cellular prot./ml) were incubated with various concentrations of sulfate and 4 μCi/ml $Na_2[^{35}S]O_4$. The reactions were terminated and tubule fragments were separated from the medium by centrifugal filtration [16]. Incubation medium adherent to the tubule fragments was separately estimated from the amount of [^{14}C]dextran (0.5 μCi/ml) precipitating with the cells; it amounted to about 3.9 μl/mg cellular protein. Intracellular concentrations were estimated on the basis of an aqueous cellular volume of 4.7 μl/mg cellular protein. This value was obtained subtracting the [carboxyl-^{14}C] dextran space of the cell pellet (i.e. fluid adherent to the cells) from the $[^3H]_2O$ space (i.e. fluid adherent to the cells + aqueous cellular volume). To determine the total aqueous volume cells were incubated with 2.5 μCi $[^3H]_2O$.

Sulfation of 7-hydroxycoumarin. To study sulfation of 7-hydroxycoumarin, tubule fragments (3 mg cellular prot./ml) were incubated with various concentrations of sulfate for 15 min before 7-hydroxycoumarin (100 μM) was added. After 30 min 0.9 ml samples were withdrawn and mixed with 3 ml of ice cold ether and 1.5% isoamyl alcohol. 7-Hydroxycoumarin conjugates were determined according to Dawson and Bridges [24] and Greenlee and Poland [25]. After removal of the parent compound by repeated extractions with the ether/isoamyl alcohol mixture (3 × 3 ml), 0.2 ml of the aqueous layer was incubated with 0.6 ml of citrate buffer (0.25 M, pH 4.8), 0.2 ml arylsulfatase (0.25 mg/ml) or β-glucuronidase (10 mg/ml). In case of the arylsulfatase treatment 5 mM saccharo-1,4-lacton was added to inhibit β-glucuronidase activity which is an impurity of the enzyme. The reaction was terminated with 0.125 ml of ice-cold 15% trichloroacetic acid. After the addition of 2 ml chloroform, stirring and centrifugation, 1 ml of the chloroform phase was mixed with 1.5 ml of 0.01 M NaOH and 1 M NaCl. The fluorescence was determined in the aqueous layer at an excitation wavelength of 368 nm and an emission wavelength of 456 nm.

Cellular protein was determined by the method of Lowry *et al.* [26]. The experiments were performed with 5 different cell preparations.

RESULTS

The time course of the uptake of sulfate (500 μM) into sulfate-depleted kidney tubule fragments is shown in Fig. 1. After a short rapid phase of influx the curve attains a plateau by about 10 min, indicating an equilibrium between the transport of the inorganic anion into and out of the cells. In Fig. 2 the intra-

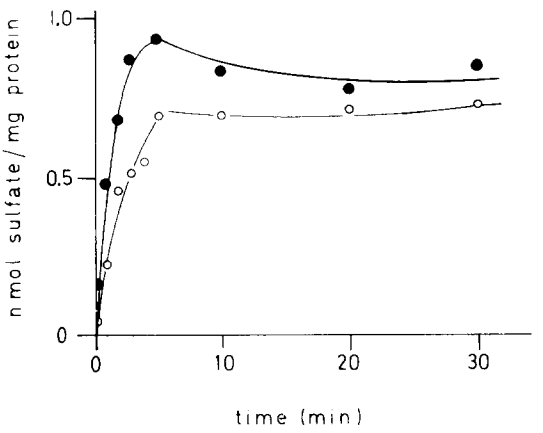

Fig. 1. Time course of sulfate uptake into kidney tubule fragments. Sulfate-depleted tubules were incubated with 500 μM Na_2SO_4 and 4 μCi $Na_2[^{35}S]O_4$ at 37°. At times indicated the reaction was stopped by centrifugal filtration. The open and closed circles show data of two independent experiments.

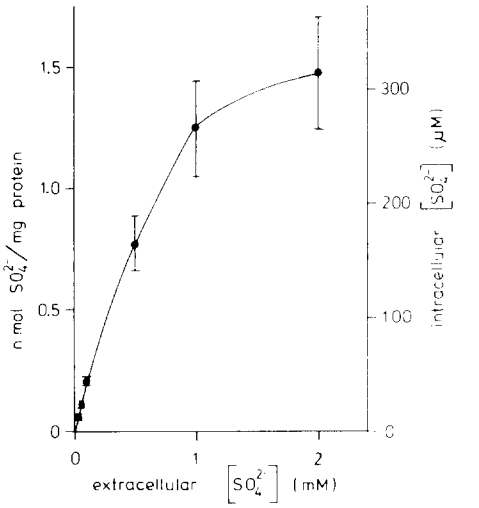

Fig. 2. Dependence of intracellular sulfate concentrations on the extracellular concentrations of the inorganic anion at steady-state conditions. Tubules were incubated with various concentrations of Na_2SO_4 and $Na_2[^{35}S]O_4$ as described in Fig. 1. The curve represents the intracellular sulfate concentrations after 15 min when intracellular sulfate levels are constant. Intracellular concentrations were calculated on the basis of a cellular volume of 4.7 μl/mg cellular protein. Data represent mean ± S.E. of experiments with 4 separate cell preparations.

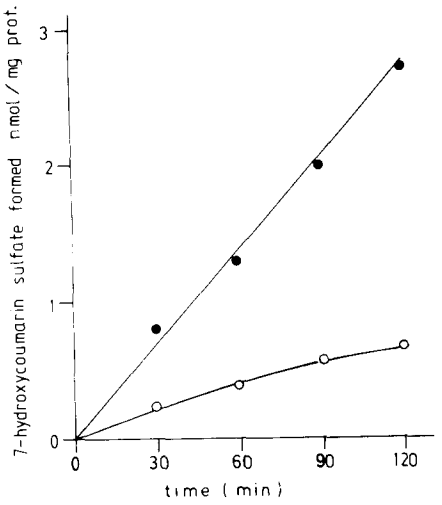

Fig. 3. Time course of 7-hydroxycoumarin sulfation in kidney tubule fragments. Tubules were incubated for 15 min at 37° before the addition of 100 μM 7-hydroxycoumarin. At times indicated the formation of 7-hydroxycoumarin sulfate was determined. The medium contained no sulfate (○) or 1 mM sulfate (●). The data represent duplicate determinations of one out of two similar experiments. The duplicates as well as the two experiments differed by not more than 10%.

cellular steady-state concentrations of sulfate are plotted as a function of the extracellular sulfate concentrations (0.025–2 mM); at a sulfate concentration of 1 mM in the medium the curve bends markedly. The cellular steady state sulfate concentrations were calculated on the basis of a cellular water space of 4.7 μl/mg protein; at 0.1 and 1 mM the ratios of intracellular/extracellular sulfate amount to about 0.5 and 0.25, respectively.

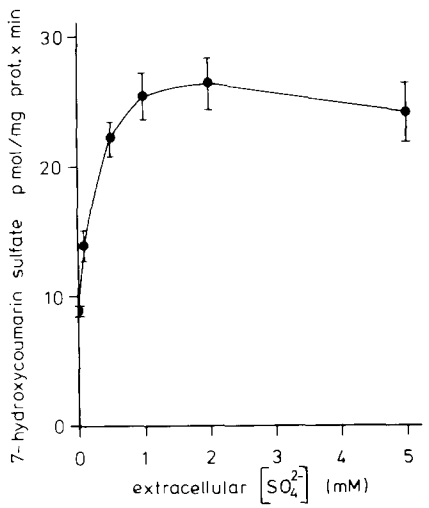

Fig. 4. Dependence of 7-hydroxycoumarin sulfation on extracellular sulfate concentrations. Tubules were incubated in medium containing various sulfate concentrations as described in Fig. 3. Rates of the formation of 7-hydroxycoumarin sulfate were determined after 30 min incubation. Data represent mean ± S.E. of experiments with 4 separate cell preparations.

Formation of 7-hydroxycoumarin sulfate by rat kidney tubule fragments proceeds linearly for two hours in the presence of 1 mM sulfate. In the absence of sulfate conjugation rates amount only to about 30% of the value obtained at 1 mM (Fig. 3). The dependence of 7-hydroxycoumarin sulfation on the sulfate concentrations of the medium has been analysed in detail (Fig. 4). Sulfate conjugation increases up to a concentration of 2 mM extracellular sulfate; at higher levels of sulfate the conjugation rates begin to decrease. In two experiments we also determined glucuronidation of 7-hydroxycoumarin by the tubule fragments which amounted to about 110 pmoles/mg protein × min in sulfate free medium and which did not differ by more than 10% in the presence of 1 mM sulfate.

To determine the apparent K_m for intracellular sulfate in the overall sulfation of 7-hydroxycoumarin conjugation rates were plotted according to Hanes against the cellular concentrations of sulfate (Fig. 5). The apparent K_m can be directly determined from the intercept on the abscissa and amounts to about 50 μM.

DISCUSSION

In the mammalian kidney inorganic sulfate is freely filtered and subsequently reabsorbed to a large extent by the renal tubule [27]. In the present investigation we determined the steady-state distribution between intracellular and extracellular sulfate in tubule fragments. The inorganic anion was not concentrated in the cells and while intracellular sulfate levels were greatly affected up to an extracellular sulfate concentration of 1 mM a further increase of the medium concentration to 2 mM did only raise intracellular levels by 18%. As the intracellular concentrations represent the equilibrium between influx and efflux of sulfate our data suggest that the systems catalysing the uptake are saturated prior to the systems catalysing the efflux. An important site for efflux may be the postulated carrier at the basolateral membrane [28].

Rates of 7-hydroxycoumarin sulfation depend on the extracellular sulfate concentration. The curve which is obtained (Fig. 4) closely correlates with that for intracellular sulfate concentrations (Fig. 2). Interestingly, sulfation also occurs at considerable rates in sulfate-free medium which does not contain methionine or cysteine (Figs. 3 and 4). Possibly there are still remnants of the inorganic anion in the tubule fragments despite their preparation in the absence of sulfate or there is a continuous generation of sulfate from S-containing amino acids which are liberated by proteolysis. Despite a low sulfate concentration under this condition, the rate of formation of 7-hydroxycoumarin sulfate still amounts to one third of the maximal rate obtained at 2 mM sulfate in the medium. This suggests a low apparent K_m value for intracellular sulfate in the sulfation reaction. Indeed, an apparent K_m for intracellular sulfate has been determined which amounts to only 50 μM (Fig. 5). This value is significantly lower than the 500 μM which we recently determined in rat liver

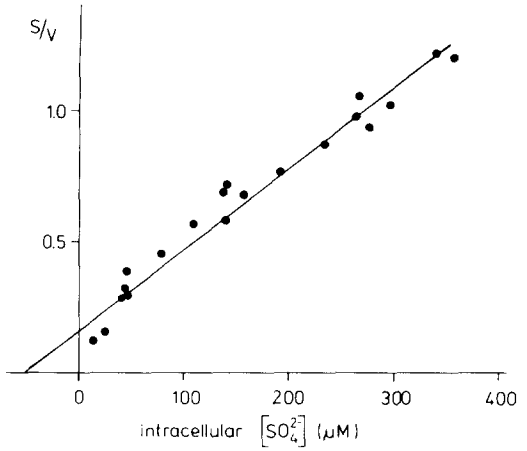

Fig. 5. Hanes plot showing the dependence of 7-hydroxycoumarin sulfation on intracellular sulfate levels. The rates of 7-hydroxycoumarin sulfation (v) are expressed as pmol × min^{-1} × mg $protein^{-1}$ and the intracellular sulfate concentrations (S) as μM. The intercept on the abscissa gives an apparent K_m value for the intracellular sulfate of about 50 μM. The data are derived from four experiments carried out with different cell preparations.

cells using 1-naphthol as substrate [16]. At present it cannot be decided whether the apparent Michaelis-Menten constant reflects the apparent K_m for sulfate in the formation of PAPS or the dependence of the phenolsulfotransferase reaction on PAPS.

The present data suggest that sulfation of phenolic compounds such as 7-hydroxycoumarin proceeds at almost maximal rates in the kidney at the physiological serum sulfate concentration of about 0.8 mM. Thus, raising the serum and urinary concentrations above the physiological levels by means of oral administration of sulfate will have only little effect on renal sulfation rates.

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REFERENCES

1. G. M. Powell and A. B. Roy, in *Extrahepatic Metabolism of Drugs and other Foreign Compounds* (Ed. Th. E. Gram), pp. 389–425. MTP-Press, Lancaster (1980).
2. G. J. Mulder, in *Sulfation of Drugs and Related Compounds* (Ed. G. J. Mulder), pp. 131–185. CRC Press Inc., Boca Raton, Florida (1981).
3. T. P. Singer, in *Metabolic Pathways, Metabolism of Sulfur Compounds* (Ed. D. M. Greenberg), pp. 535–546. Academic Press, New York (1975).
4. G. J. Mulder, in *Sulfation of Drugs and Related Compounds* (Ed. G. J. Mulder), pp. 31–52. CRC Press Inc., Boca Raton, Florida (1981).
5. K. R. Krijgsheld, H. Frankena, E. Scholtens, J. Zweens and G. J. Mulder, *Biochim. biophys. Acta* **586**, 492 (1979).
6. M. W. Anders, *Kidney Int.* **18**, 636 (1980).
7. J. R. Fry, P. Wiebkin, J. Kao, C. A. Jones, J. Gwynn and J. W. Bridges, *Xenobiotica* **8**, 113 (1978).
8. D. P. Jones, G.-B. Sundby, K. Ormstad and S. Orrenius, *Biochem. Pharmac.* **28**, 929 (1979).
9. J. R. Dawson, K. Norbeck and P. Moldéus, *Biochem. Pharmac.* **32**, 1789 (1983).
10. K. R. Krijgsheld, E. Scholtens and G. J. Mulder, *Comp. Biochem. Physiol.* **67A**, 683 (1980).
11. K. R. Krijgsheld, E. Scholtens and G. J. Mulder, *Biochem. Pharmac.* **30**, 1973 (1981).
12. K. R. Krijgsheld, E. Scholtens and G. J. Mulder, *Biochem. Pharmac.* **31**, 3997 (1982).
13. P. Moldéus, B. Andersson and V. Gergely, *Drug Metab. Dispos.* **7**, 416 (1979).
14. L. R. Schwarz, *Arch. Toxic.* **44**, 137 (1980).
15. M. Koike, K. Sugeno and M. Hirata, *J. pharm. Sci.* **70**, 308 (1981).
16. L. R. Schwarz, *Hoppe-Seyler's Z. physiol. Chem.* **365**, 43 (1984).
17. G. J. Mulder and K. Keulemans, *Biochem. J.* **176**, 959 (1978).
18. I. Smith and P. D. Mitchell, *Biochem. J.* **142**, 189 (1974).
19. G. Levy and T. J. Matsuzawa *J. Pharmac. exp. Ther.* **156**, 285 (1967).
20. L. R. Schwarz and M. Schwenk, *Biochem. Pharmac.* **33**, 3353 (1984).
21. J. R. Fry and N. K. Perry, *Biochem. Pharmac.* **30**, 1197 (1981).
22. W. G. Guder, *Biochim. biophys. Acta* **584**, 507 (1979).
23. U. Pfeifer and W. G. Guder, *Virchow's Arch. B. Cell. Path.* **19**, 51 (1975).
24. J. R. Dawson and J. W. Bidges, *Biochem. Pharmac.* **30**, 2409 (1981).
25. W. C. Greenlee and A. Poland, *J. Pharmac. exp. Ther.* **205**, 596 (1978).
26. O. Lowry, J. H. Roseborough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
27. G. H. Mudge, W. O. Berndt and H. Valtin, in *Handbook of Physiology*, Chap. 19. American Physiological Society, Bethesda, MD (1973).
28. J. B. Pritchard and J. L. Renfro, *Proc. natn. Acad. Sci.* **80**, 2603 (1983).