# Selective chemisorbents
# IV.* Cleavable binding of thiols to *S*-alkylthiosulfate derivatives of cellulose

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Received 6 July 1983

It has been found that bead and powder *O*-(3-thiosulfato-2-hydroxypropyl)celluloses adsorb low- and high-molecular-mass thiols. The efficiency of sorption of low-molecular-mass thiols was controlled by mass ratio of thiol and $S_2O_3^-$. In removing cysteine from aqueous medium it was necessary to use large excess of *S*-alkylthiosulfate groups of the sorbent. Chemisorption of papain was inhibited by side reactions. Desorption of papain and cysteine was accomplished by addition of thiols in weak alkali medium. The purity of papain increased after desorption. One of the sorbents, bead *O*-(3-thiosulfato-2-hydroxypropyl)cellulose, was prepared by the reaction of bead *O*-(3-iodo-2-hydroxypropyl)cellulose with sodium thiosulfate.

Найдено, что сферическая и порошкообразная *O*-(3-тиосульфо--2-гидроксипропил)целлюлозы адсорбируют низко- и высокомолекулярные тиолы. Эффективность сорбции низкомолекулярных тиолов зависела от весового отношения тиола и тиосульфата. При удалении цистеина из водной среды было необходимо использовать большой избыток *S*-алкилтиосульфатных групп сорбента. Хемосорбция папаина подавлялась побочными реакциями. Десорбция папаина и цистеина завершалась прибавлением тиолов в слабощелочной среде. Степень чистоты папаина повышалась после десорбции. Один из сорбентов, гранулированная *O*-(3-тиосульфо-2-гидроксипропил)целлюлоза была приготовлена реакцией гранулированной *O*-(3-иод-2-гидроксипропил)целлюлозы с тиосульфатом натрия.

Sorbent of thiols are used to isolate thiols or remove them when they are undesirable [1]. These processes are based mostly on the principle of selective chemisorption.

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* For Part III see *React. Polym., Ion Exch., Sorbents 2*, 189 (1984).

*S*-Alkylthiosulfates belong to reagents selective for thiols [2, 3]. Their preparation is simple [2, 3] and their reaction products with thiols can be easily decomposed [4]. Preparation of polymeric *S*-alkylthiosulfates and decomposition of their reaction products with thiols to polymeric thiols are governed by the same principles, as confirmed in the experiments with *S*-alkylthiosulfate derivatives of cellulose [5]. In spite of the advantages mentioned above, polymeric *S*-alkylthiosulfates have not been utilized as sorbents of thiols so far.

The present work is focused on the use of *S*-alkylthiosulfate derivatives of cellulose as chemisorbents of thiols, namely of biological origin. Moreover, problems concerning the preparation of *O*-(3-thiosulfato-2-hydroxypropyl)cellulose from bead cellulose by a nondestructive procedure ensuring high conversion are dealt with.

## Experimental

L-Cysteine, pure grade, and L-cysteine hydrochloride, anal. grade, were purchased from Lachema, Brno, DL-[$^{35}$S]cysteine hydrochloride (546 MBq mol$^{-1}$) from Isocommerz, Berlin. Papain from papaya juice (EC 3.4.22.2) was a product of BDH, Poole, S-Test Protease Universal in tablet form (batches No. 0480 and 0982) was obtained from CHZJD, Bratislava. The SH group content in papain (0.57 mol thiol/(mol papain)) was determined spectrophotometrically after the reaction with 2,2′-dithiopyridine [6], relative molecular mass of papain 23 400 was taken from the literature [7].

Bead *O*-(3-thiosulfato-2-hydroxypropyl)cellulose THPC *Ia* (4.62 % S) and THPC *Ib* (5.37 % S) were prepared by 2 h reaction of bead *O*-(3-iodo-2-hydroxypropyl)cellulose IHPC (10.68 % I) [8] with sodium thiosulfate at 100 °C and 80 °C, respectively. Bead IHPC was obtained by the reaction of bead *O*-(3-chloro-2-hydroxypropyl)cellulose CHPC (4.26 % Cl) with sodium iodide in 2,4-pentanedione [8]. Powder THPC *IIa* (2.97 % S) and THPC *IIb* (5.77 % S) were prepared by 15 h reaction of powder CHPC (3.12 % Cl and 4.57 % Cl, respectively) with sodium thiosulfate at 100 °C [5]. Powder CHPC was synthesized by acid-catalyzed etherification of powder Whatman cellulose (standard grade) with chloromethyloxirane [5].

### *Sorption of cysteine on THP derivative of cellulose*

Suspension of dry THPC (10 mg) in 0.2 M phosphate buffer (1 cm$^3$) of pH = 7 containing [$^{35}$S]cysteine (30—40 mmol dm$^{-3}$) was stirred at room temperature for 2 h. The solid phase was washed with water, 0.1 M acetic acid containing NaCl ($c$ = 0.1 mol dm$^{-3}$), water, ethanol, and acetone. The product was dried, weighed, dissolved in concentrated sulfuric acid, and further worked up [9] to measure its radioactivity.

The effect of concentration of hydrogen ions (pH = 4—9, citrate—phosphate, phosphate, and borate buffers) and cysteine ($n$(cys)/$n$(—$S_2O_3^-$) = 0.1 to 10), reaction time

(10—240 min), and presence of further reagents (glycine, D-glucitol, D-glucose) on the course of sorption was followed by the procedure mentioned above.

### *Desorption of cysteine from THP derivative of cellulose*

Dry THPC (10 mg) containing sorbed [$^{35}$S]cysteine was suspended in 0.5 M borate buffer (1 cm$^3$, pH = 8 or 9) and thiol (L-cysteine, 2-mercaptoethanol, mercaptoacetic acid, and sodium sulfide) was added in concentration ensuring the mole ratio $n(\text{thiol})/n(-S_2O_3^-) = 10$, followed by addition of tributylphosphine (10 mm$^3$). After 30 min stirring the solid phase was washed and worked up to measure the radioactivity [9]. In desorption brought about with mercaptoacetic acid, the effect of concentration of hydrogen ions (pH = 5—8) and mercaptoacetic acid ($n(\text{thiol})/n(-S_2O_3^-) = 0.01—10$) on the amount of the desorbed thiol was followed.

### *Sorption of papain on THP derivative of cellulose*

The solution (1 cm$^3$) of papain (10 mg) in 50 mM phosphate buffer containing NaCl ($c = 0.3$ mol dm$^{-3}$) and EDTA ($c = 1$ mmol dm$^{-3}$) was added to THPC. After 30 min mild stirring at room temperature, the concentration and proteolytic activity of papain in the solution were measured. Control experiments with and without unmodified cellulose were performed similarly. The amount of THPC sorbing about 50 % of papain ($S_{50}$, mg cm$^{-3}$) or bringing about 50 % decrease in proteolytic activity of papain in the solution ($I_{50}$, mg cm$^{-3}$) under the given conditions was determined by interpolation of the relationship $w$ *vs.* log $\{S\}$ or $f_I$ *vs.* log $\{S\}$, where $w$ (%) is the relative concentration of papain and $f_I$ the relative proteolytic activity of papain in the solution after removal of the sorbent and $S$ (mg cm$^{-3}$) is the amount of the sorbent in a unit volume. The amount of the sorbed papain was determined from the difference of protein concentrations in the solution prior to and after sorption. The concentration of papain was determined either spectrophotometrically at $\lambda = 280$ nm using $A(w' = 1\ \%, l = 1\ \text{cm}) = 25.0$ [9] or colorimetrically [10]. The proteolytic activity of papain was determined by using a chromolytic substrate in tablet form [11]. Proteolysis proceeded at 25 °C for 20 min in 50 mM phosphate buffer (pH = 7.5) containing EDTA ($c = 1$ mmol dm$^{-3}$).

### *Desorption of papain from THP derivative of celullose*

THPC with bonded papain was suspended in 50 mM phosphate buffer (1 cm$^3$, pH = 7.5) containing NaCl ($c = 0.3$ mol dm$^{-3}$) and EDTA ($c = 1$ mmol dm$^{-3}$) and the suspension was shaken for 30 min at room temperature. Then the sorbent was separated and the concentration and proteolytic activity of papain in the solution were measured. The experiment was repeated (3—5 times) until no more activity was found in the solution. The sorbent was suspended in 50 mM phosphate buffer (pH = 7.5) containing cysteine ($c =$

40—210 mmol $dm^{-3}$) and EDTA ($c = 1$ mmol $dm^{-3}$) and further worked up as in the previous case.

## Results and discussion

Bead THPC was prepared by the known sequence of reactions [5] completed with conversion of CHPC to the more active IHPC. Preparation of CHPC by "wet procedure" and replacement of chlorine with iodine were described in our previous paper [8]. The chosen way for the preparation of bead THPC has the following advantages over the previous procedure [5]:

i) in the preparation of bead CHPC, irreversible changes in the porous structure of the product did not occur,

ii) conversion of chlorine to *S*-alkylthiosulfate groups increased,

iii) the reaction conditions of alkylation of thiosulfate with IHPC were milder than with CHPC.

The course of chemisorption of cysteine on THPC in dependence on pH, concentration of cysteine, and time is illustrated in Figs. 1—3. The agreement with the data published on the reactions of *S*-alkylthiosulfates with thiols [2, 3] was only partial. The formation of asymmetric disulfide derivative of cellulose was preferred in weak acid and neutral media (Fig. 1) as well as by excess of THP groups of cellulose (Fig. 2). Surprisingly, only a small portion of THP groups (Table 1) took part in chemisorption of cysteine even under the conditions when maximum sorption capacity was achieved (Fig. 2). The presence of amino acid (glycine) and nonreducing (glucitol) or reducing (glucose) saccharides, namely in 1 to 10-fold excess per thiol, increased the sorption of cysteine by 50 to 100 %.

THPC was more effective as an inhibitor than as a sorbent of papain (Fig. 4). At 50 % decrease of proteolytic activity of papain in the solution, brought about for

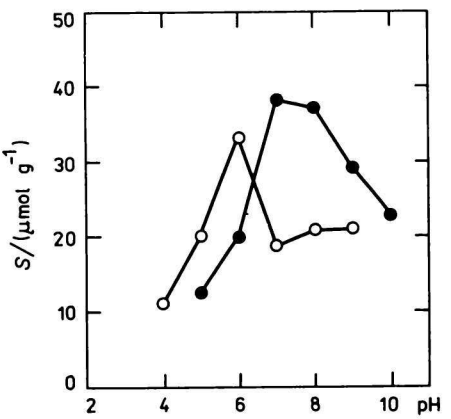


*Fig. 1.* pH Dependence of sorption of [$^{35}$S]cysteine on THPC *Ia* (○) and THPC *IIa* (●). ($c$(Cysteine) = 21.6 mmol $dm^{-3}$ and 23.0 mmol $dm^{-3}$.)

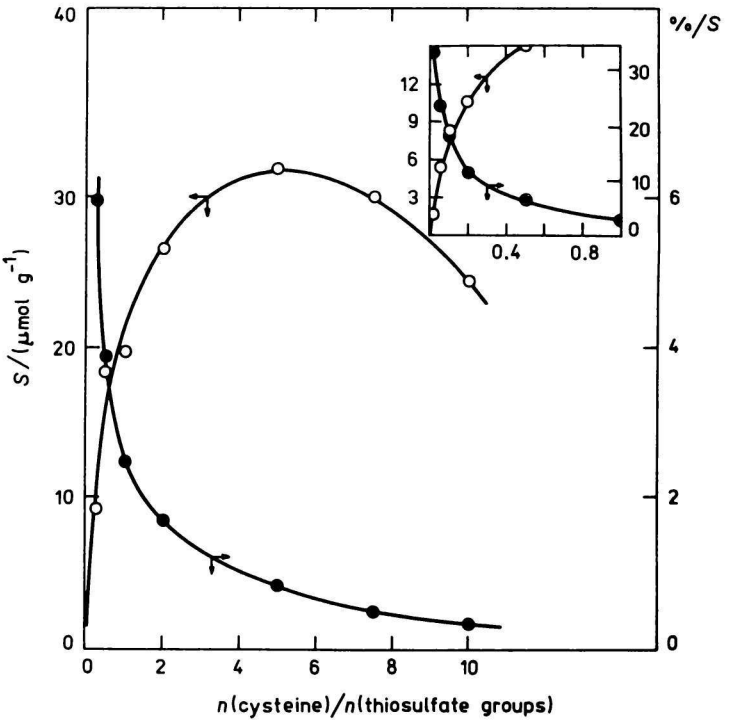


*Fig. 2.* Concentration dependence of sorption of [$^{35}$S]cysteine on THPC *Ib*. The highest concentrations of cysteine used were 80.0 mmol dm$^{-3}$ and 4.6 mmol dm$^{-3}$ (fig. in the upper right corner), pH = 6. For both figures the same variables are valid.

*Table 1*

Sorption of [$^{35}$S]cysteine and sorption/inhibition of papain by THP derivatives of cellulose

| Process | THPC | | | |
|---|---|---|---|---|
| | *Ia* | *Ib* | *IIa* | *IIb* |
| Sorption of [$^{35}$S]cysteine/(μmol g$^{-1}$) | 36.7 | 33.3 | 38.6[b] | 23.6[b] |
| Conversion of *S*-alkylthiosulfate groups/%[a] | 5.1 | 4.2 | 8.3 | 2.6 |
| Sorption of papain, $S_{50}$/(mg g$^{-1}$) | 28.2 | 34.1 | — | 2.3[c] |
| Inhibition of papain, $I_{50}$(mg cm$^{-3}$)[d] | 6.6 | 5.4 | — | 5.25 |
| Inhibition of papain, $I_{50}$/(mmol dm$^{-3}$) | 4.8 | 4.5 | — | 4.7 |

*a*) Portion of the THP groups of cellulose forming mixed disulfide with [$^{35}$S]cysteine; the amount of the THP groups was calculated from sulfur content. *b*) pH = 7. *c*) Could not be determined by the chosen method. *d*) Concentration of papain $c = 1.4$—$1.6$ mg cm$^{-3}$.

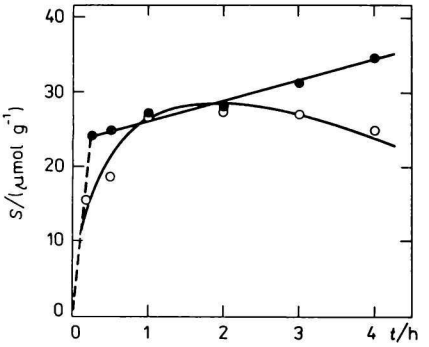


*Fig. 3.* Time dependence of sorption of [$^{35}$S]cysteine on THPC *Ib* (○) and THPC *IIa* (●). pH = 6, $c$(cysteine) = 21.6 mmol dm$^{-3}$; pH = 7.5, $c$(cysteine) = 23.0 mmol dm$^{-3}$.

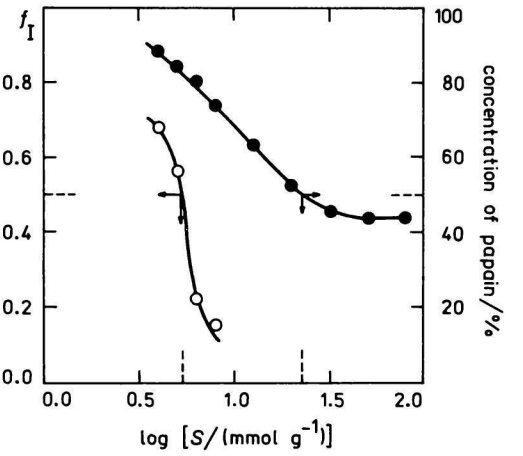


*Fig. 4.* Dependence of relative proteolytic activity of papain $f_I$ and mass % of papain, respectively, on log [$S$/(mmol g$^{-1}$)].

example by THPC *Ib* and THPC *IIb*, only 17 % and 2 % of papain, respectively, were sorbed. Based on the knowledge of the reactions of *S*-alkylthiosulfate with thiols [2, 3], the inhibition of papain may be connected with its chemical modification. In the experiment, we failed to achieve complete sorption of papain (Fig. 4) also due to the quality of the enzyme preparation [12]. Control experiments of sorption of papain on unmodified cellulose showed that powder cellulose did not influence the $S_{50}$ and $I_{50}$ values determined with THPC *IIa* and THPC *IIb* (Table 1). Bead cellulose contributed to the $S_{50}$ values for THPC *Ia* and THPC *Ib* by 5—10 %, however, it did not influence the $I_{50}$ values. The results mentioned above were in numerical agreement with the portion of nonspecific sorptions* (determined by desorption with buffer solutions) in the total sorption of papain on powder and bead THPC.

Desorption of papain and cysteine from THPC after short treatment with thiol in weak alkali medium proceeded differently. While complete desorption of cysteine was achieved in a single desorption with excess thiol (Fig. 5, Table 2), repeated desorptions of papain with excess thiol resulted in 40—55 % of catalytically active papain only (Table 3).

* Nonspecific sorption means binding of papain noncovalently.

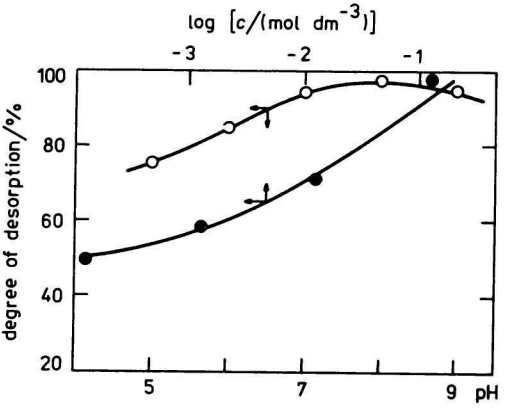


*Fig. 5.* Dependence of degree of desorption on pH ($c$(mercaptoacetic acid) = 122.5 mmol dm$^{-3}$) (○). Dependence of degree of desorption on concentration of mercaptoacetic acid (pH = 8) (●).

*Table 2*

Desorption of [$^{35}$S]cysteine from THPC *Ib* with thiols

| Thiol ($c$ = 80 mmol dm$^{-3}$) | Desorption/% | |
|---|---|---|
| | pH = 8 | pH = 9 |
| Sodium sulfide | 87.1 | — |
| L-Cysteine | 94.2 | 94.25 |
| 2-Mercaptoethanol | 98.3 | 99.3 |
| Mercaptoacetic acid | 97.6 | 94.7 |

*Table 3*

Desorption of papain from THP derivatives of bead cellulose with L-cysteine

| Number of cycles | Proteolytic activity $U \cdot 10^3$/(mg cm$^{-3}$ min$^{-1}$) THPC | | Proteins $\varrho$/(mg cm$^{-3}$) THPC | | Degree of purification THPC | |
|---|---|---|---|---|---|---|
| | *Ia* | *Ib* | *Ia* | *Ib* | *Ia* | *Ib* |
| *1* | 45.8 | 4.2 | 0.055 | — | 1.84 | — |
| *2* | 13.9 | 20.2 | 0.031 | 0.050 | 0.99 | 1.41 |
| *3* | 3.0 | 38.0 | 0.011 | 0.045 | 0.60 | 1.32 |
| *4* | — | 14.9 | — | 0.028 | — | 0.83 |
| *5* | — | 6.1 | — | — | — | — |

On 51.6 mg THPC *Ia* 0.67 mg papain with proteolytic activity of $116.0 \times 10^{-3}$ mg cm$^{-3}$ min$^{-1}$ (measured in 200 mM cysteine) was adsorbed, desorption was accomplished with 200 mM cysteine. On 50.0 mg THPC *Ib* 0.74 mg papain with proteolytic activity of $177.4 \times 10^{-3}$ mg cm$^{-3}$ min$^{-1}$ (measured in 42 mM cysteine) and $199.6 \times 10^{-3}$ mg cm$^{-3}$ min$^{-1}$ (measured in 210 mM cysteine), respectively, was adsorbed; in the first two cycles desorption was brought about with 42 mM cysteine, in further cycles with 210 mM cysteine.

Under the conditions of desorption the *S*-alkylthiosulfate groups of cellulose are reduced to thiol groups [5] which can be reconverted [13] by sequence of reactions known in chemistry of *S*-alkylthiosulfates [3].

Combination of covalent affinity chromatography on *S*-alkylthiosulfate derivatives of cellulose with ion-exchange and gel chromatography was utilized in isolation of low-molecular-mass thiols from yeast extracts [13]. THPC and polymeric *S*-alkylthiosulfates generally may be regarded as a suitable material for isolation and purification of thiol biopolymers by covalent affinity chromatography.

*Acknowledgements. We thank Dr. M. Beneš for inspiring discussion and critical remarks on the manuscript, Dr. J. Talová, Dr. H. Kertészová, and L. Barteltová for helpful technical assistance.*

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Translated by A. Kardošová