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REACTIONS OF CELLULOSE ISOTHIOCYANATES WITH THIOL AND AMINO COMPOUNDS

P. GEMEINER*, J. AUGUSTÍN, AND Ľ. DROBNICA
Department of Microbiology and Biochemistry, Slovak Institute of Technology, 880 37 Bratislava (Czechoslovakia)

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ABSTRACT

A cellulose isothiocyanate has been prepared by treatment of cellulose with 2,4-di-isocyanatotoluene followed by hydrolysis and reaction of the resulting amine with thiophosgene. The cellulose isothiocyanate was characterized by its binding capacity with respect to [^{14}C]-glycine, [^{131}I]-human serum albumin, and 2-mercaptoethanol. An analytical method for binding capacity, based on reaction with [^{35}S]-α-toluenethiol, was developed. Because of the aromatic character of the NCS group of the cellulose isothiocyanate, the covalently bonded thiol can be quantitatively liberated.

INTRODUCTION

We have been concerned with analytical methods for determining the binding capacity of cellulose isothiocyanates[1]. The elaboration of these methods was based on classical reaction kinetics, but the methods for determining the binding capacity for thiols were not satisfactory. Information on the reactions between macromolecular polyisothiocyanates and thiols may be utilised[2] in enzymology[3], covalent chromatography[3], and for the immobilization of enzymes[4]. Moreover, macromolecular polyisothiocyanates have been used as matrices for the immobilization of enzymes[5-8] and for the solid-phase sequence analysis of peptides and proteins[9]. We now report on the development of a reliable method for determining the binding capacity of polyisothiocyanates for thiols using [^{35}S]-α-toluenethiol and a new procedure for preparing cellulose isothiocyanates containing the aromatic NCS group.

EXPERIMENTAL

General. — The following cellulose isothiocyanates were used: cellulose-OCH_2CH_2NCS (**1**), cellulose-$OCH_2CONHC_6H_4C_6H_4NHCSNHC_6H_4NCS$ (**2**), cellulose-$OCH_2CH_2NHCSNHC_6H_4NCS$ (**3**), cellulose-$OCONH(CH_2)_5NCS$ (**4**),

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*Present address: Institute of Chemistry, Slovak Academy of Sciences, 809 33 Bratislava, Czechoslovakia.

cellulose-$OCONHC_6H_3(CH_3)NCS$ (5); 1–4 were prepared and characterized by the methods previously described[1,10].

[^{35}S]-Sulphur with an initial specific activity of 180 Ci/mol and [2-^{14}C]-glycine (50 Ci/mol) were obtained from UVVVR Prague, and [^{131}I]-human serum albumin (initial specific activity, 50 Ci/g) was a product of the Institute for Research, Radioisotope Production and Distribution Centre, Otwock (Poland). Organic solvents were purified by distillation before use.

U.v. spectrophotometry was performed with a Zeiss VSU-1 instrument. I.r. spectra were measured for KBr pellets using a Zeiss UR-20 double-beam spectrophotometer. The radioactivity of solid samples was measured on a Frieseke and Hoepfner FH 41-T methane-flow counter. The pH of buffer solutions was adjusted by means of an OP-205 pH-meter (Radelkis, Hungary).

Synthesis of cellulose isothiocyanate 5. — A mixture of *N*,*N*-dimethylformamide (100 ml), triethylamine (1 ml), 2,4-di-isocyanatotoluene (12 ml), and dried powdered cellulose (Whatman, standard grade, 25 g) was stirred for 4 h at 60°, then cooled, diluted with cyclohexane, and filtered. The product was washed with *p*-dioxane, then treated with boiling water (100 ml) for 1 h, cooled, collected, and washed with water. A suspension of the product in chloroform (120 ml) and thiophosgene (5 ml) was stirred with 10% aqueous sodium hydrogen carbonate (50 ml) at 25° for 3.5 h and then centrifuged. The sediment was washed with chloroform (70 ml), and dried *in vacuo*, and the pale-yellow product was ground, washed with chloroform, and dried *in vacuo*, to give 5 as fibrous material, ν_{max} 2100 ± 50 cm^{-1} (NCS) [Found: C, 45.4; H, 5.4; N, 4.4; S, 0.9. $C_6H_9O_5\cdot OCONHC_6H_3(CH_3)NCS$ calc.: C, 49.3; H, 4.4; N, 7.7; S, 8.8%].

The binding capacities of 5 were as follows: [^{14}C]-glycine, 38 µmol/g; 2-mercaptoethanol, 94 µmol/g; [^{131}I]-human serum albumin, 11.2 mg/g.

[^{35}*S*]-*α-Toluenethiol.* — [^{35}S]-α-Toluenethiol (2.9 Ci/mol) was prepared from benzylmagnesium chloride and elemental [^{35}S]-sulphur as described earlier[11,12]. An analytical purity of 97% was determined from the radiochromatogram of the product formed by reaction with $AgNO_3$ on a thin-layer of silica gel. The concentration of α-toluenethiol was determined spectrophotometrically[1].

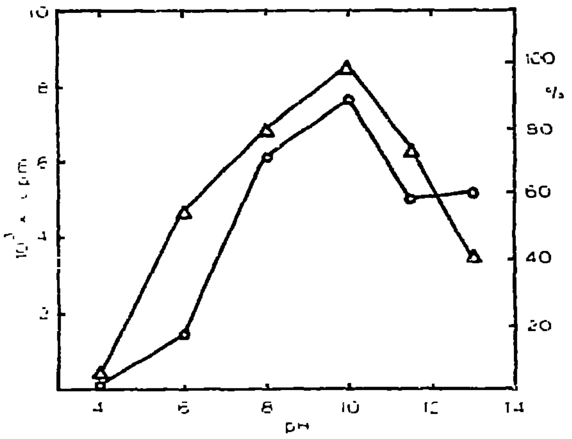
Determination of the binding capacity of cellulose isothiocyanates using [^{35}*S*]-*α-toluenethiol.* — Samples of polyisothiocyanates (20 mg) suspended in 200mM KH_2PO_4–100mM NaOH (1 ml, pH 8) were stirred with 2M methanolic [^{35}S]-α-toluenethiol (10 µl) at 25° for 1 h, and then centrifuged. The sediment was repeatedly suspended in acetone containing 1% of acetic acid, then filtered, washed with acidified acetone, dried, and assessed for radioactivity (see Table I).

TABLE I

BINDING CAPACITIES OF CELLULOSE ISOTHIOCYANATES DETERMINED WITH [^{35}S]-α-TOLUENETHIOL

Sample	1	2	3	4	5
Binding capacity (µmol of NCS/g)	360	0	10	185	196

Reaction of [^{35}*S*]-*α-toluenethiol with cellulose isothiocyanate* (**5**). — (*a*) *Effect of pH*. A suspension of **5** in a 100mM buffer solution (citrate pH 4, phosphate pH 6 and 8, carbonate pH 10 and 11.5, and 100mM NaOH) containing 20% of ethanol and 20mM α-toluenethiol (1 ml) was stirred at 25° for 15 and 60 min. Unreacted thiol was removed from the cellulose isothiocyanate by washing with acidified acetone, and the radioactivity was then measured and expressed as c.p.m./20 mg. The results are shown in Fig. 1.



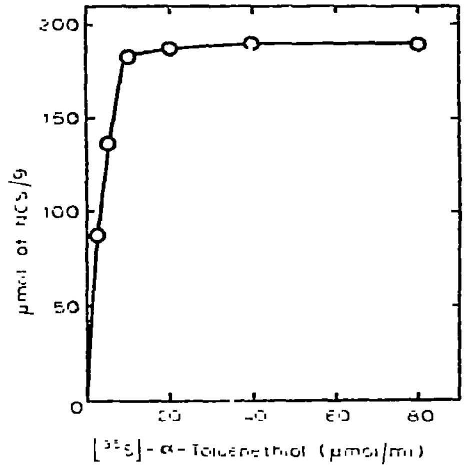


Fig. 1. Effect of pH on the amount of [^{35}S]-α-toluenethiol bound to cellulose isothiocyanate **5**; reaction times: 15 (—○—) and 60 min (—△—)

Fig. 2. Dependence of the binding capacity of cellulose isothiocyanate **5** on the initial concentration of [^{35}S]-α-toluenethiol.

(*b*) *Effect of initial concentration*. A suspension of **5** (20 mg/ml) in 200mM KH_2PO_4-200mM NaOH (pH 8) containing [^{35}S]-α-toluenethiol (0–80mM) and 1% v/v ethanol was stirred at 25° for 60 min. The samples were then treated as described above for determination of bound thiol. The results are shown in Fig. 2.

Release of [^{35}*S*]-*α-toluenethiol from the* [^{35}*S*]-*α-toluenethiol-cellulose isothiocyanate* **5** *product*. — (*a*) After twice washing with acidified acetone, modified **5** was dried and then stirred at 25° for 30 min with a buffer solution [200mM phosphate (pH 7.5), 200mM carbonate (pH 9 and 10.5), and 100mM NaOH] or the buffer containing 100mM sodium sulphide adjusted to the original pH value. The suspension was then treated as described above for the determination of the binding capacity. The [^{35}S]-α-toluenethiol bonded to **5** corresponded to 6250 c.p.m. The results are shown in Fig. 3.

(*b*) After two washings with acidified acetone and drying, modified **5** was stirred at 25° for 30 min with 200mM carbonate buffer (pH 10.5) containing 20% v/v of ethanol or 100mM sodium sulphide. Each suspension was then treated as described above

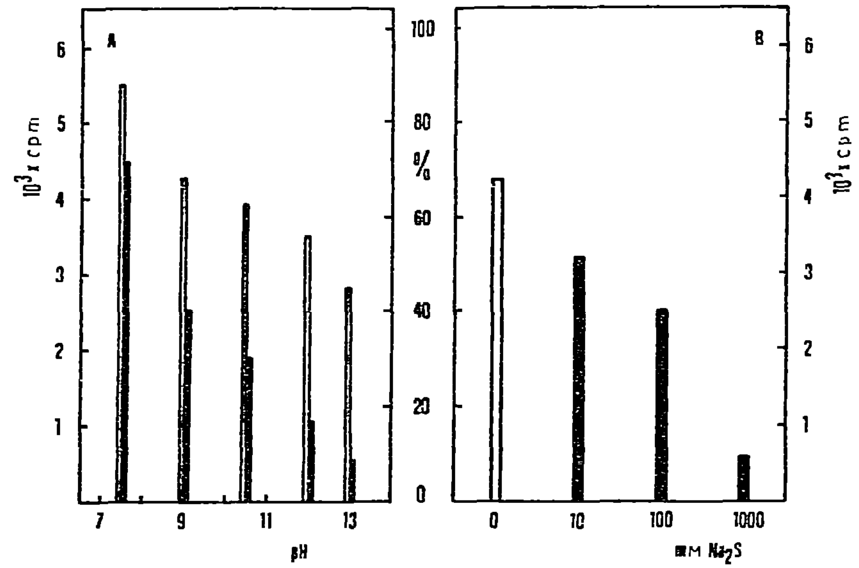


Fig. 3. Release of [^{35}S]-α-toluenethiol from the [^{35}S]-α-toluenethiol–cellulose isothiocyanate 5 product; *A*, effect of pH (white columns, release effected by buffers and NaOH; black columns, release effected by the same solutions containing sodium sulphide); B, effect of concentration of sodium sulphide (white column, release by buffer pH 10.5; black columns, release by the same buffer containing sodium sulphide).

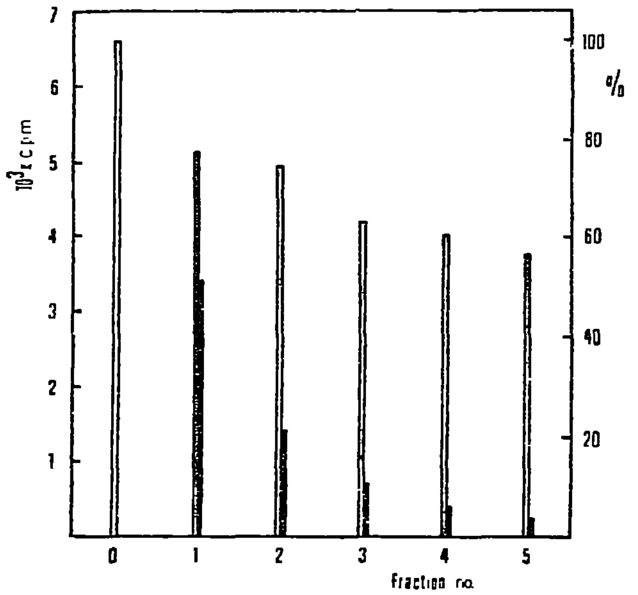


Fig. 4. Repeated release of [^{35}S]-α-toluenethiol from cellulose isothiocyanate 5. White columns, release by means of a buffer pH 10.5; black columns, the same buffer containing sodium sulphide.

for the determination of binding capacity by measuring residual radioactivity. The results are shown in Fig. 4.

DISCUSSION

The cellulose isothiocyanates **1**–**4** have been described previously[1]. Cellulose isothiocyanate **5**, which contains an aromatic NCS group and exhibits substantial thiol-binding capacity, was prepared by treatment of cellulose with 2,4-di-isocyanatotoluene in a water-free medium followed by hydrolysis of the free NCO groups and reaction of the resulting NH_2 groups with thiophosgene. The isothiocyanate group in **5** is linked to the cellulose skeleton through a urea bond (confirmed by the i.r. spectrum). However, the elemental analysis of **5** reveals a high N:S ratio and indicates that the conversion into isothiocyanate was poor. Loss of NCO groups occur could by crosslinking of the cellulose on reaction with 2,4-di-isocyanatotoluene and/or the conversion of adjacent pairs of NCO groups into -NHCONH- linkages. Since the reactions were carried out under non-swelling conditions, modification may be limited to the cellulose-fibre surfaces.

The free NCS group in **5** can react with amines and thiols, as do isothiocyanates of low molecular weight[13,14], to give the corresponding substituted thioureas or dithiocarbamates[1,10]. On reaction of **5** with α-toluenethiol, the i.r. band ($2100 \pm 50\,cm^{-1}$) for NCS groups disappeared. Cellulose isothiocyanate **5** retains the hydrodynamic properties (bed volume, flow rates) characteristic of the original cellulose. The binding capacity of **5** for glycine (38 μmol/g) and 2-mercaptoethanol (94 μmol/g) accords with the series of cellulose isothiocyanates **1**–**4** previously tested[1]. The correlation between the values for the binding capacities for glycine and 2-mercaptoethanol in this series was good ($r = 0.98$).

The reaction of **5** with [^{35}S]-α-toluenethiol is relatively rapid at pH 6–10 and is complete in 60 min. The reaction rate and the amount of thiol bound is a function of pH with a sharp maximum at pH 10 (Fig. 1). Since the reaction between NCS groups and the hydroxide ions occurs at pH > 10, it is not possible to obtain a complete ionization curve with the point of inflexion at pH 9.4 corresponding to the pK_a value of α-toluenethiol[15]. At pH 13, there was a partial release of the bound thiol after 10 min

At least a 10-fold molar excess of thiol is necessary to maximise the reaction of the NCS group of **5** (Fig. 2). If the concentration ratios used for determining the binding capacity for 2-mercaptoethanol[1,10] are applied to α-toluenethiol, the binding capacity should be ~ 100 μmol of NCS/g, whereas the value obtained by the spectrophotometric method is 94 μmol of NCS/g. These differences also remain for the cellulose isothiocyanates **1**–**4**.

The release of [^{35}S]-α-toluenethiol bound to **5** on treatment with buffered solutions of sodium sulphide was also investigated in relation to the equilibrium character and mechanism of the reaction between isothiocyanates and thiols[16]. Thus, treatment of the α-toluenethiol-**5** product with 100mM Na_2S effects ~30%

release of α-toluenethiol at pH 9 and 40% release at pH 12 or 13 (Fig. 3A). A 10-fold increase in the concentration of Na$_2$S causes (Fig. 3B) release of an additional amount (30%) of α-toluenethiol, leaving only 10% of that originally bound to **5**. The effect of the Na$_2$S concentration on the rate of release of bound thiol is significant; M Na$_2$S has the same effect in 5 min as 100mM Na$_2$S in 30 min, so that three of the latter treatments are necessary to effect 90% release of bound thiol (Fig. 4). The presence of organic solvents (up to 75% by volume) such as ethanol, *p*-dioxane, methyl sulphoxide, pyridine, *N,N*-dimethylformamide, and triethylamine buffer containing 50% of acetone affects the release of bound thiol. No significant release of α-toluenethiol bound to cellulose isothiocyanate **4** was observed after treatment for 30 min with M Na$_2$S; the NCS group of **4** is linked to the cellulose molecule by an alkyl chain.

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REFERENCES

1 P. Gemeiner, J. Augustín, and Ľ. Drobnica, *Chem. Zvesti*, 30 (1976) 254–260.

2 Ľ. Drobnica and P. Gemeiner, *Abstr. Commun. Intern. Congr. Biochem., 9th*, Stockholm, 1973, p. 119.

3 Ľ. Drobnica and P. Gemeiner, in J. L. Fox, Z. Deyl, and A. Blažej (Eds.), *Protein Structure and Evolution*, Dekker, New York, 1976, pp. 105–115.

4 P. Gemeiner and Ľ. Drobnica, *Collect. Commun. Slovak Institute of Technology 1975*, Alfa, Bratislava, in press.

5 R. Axén and J. Porath, *Nature (London)*, 210 (1966) 367–369.

6 S. A. Barker, P. J. Somers, and R. Epton, *Carbohydr. Res.*, 9 (1969) 257–263.

7 S. A. Barker, P. J. Somers, and R. Epton, *Carbohydr. Res.*, 14 (1970) 323–326.

8 G. Manecke, *Chimia*, 28 (1974) 467–474

9 G. R. Stark, *Methods Enzymol.*, 25 (1972) 360–368.

10 J. Augustín, Ľ. Drobnica, and P. Gemeiner, *Chem. Zvesti*, 30 (1976) 246–253

11 R. C. Thomas and L. J Reed, *J Amer. Chem Soc* , 77 (1955) 5446–5448

12 J. L. Wood, J. R. Rachele, C. M. Stevens, F. H. Carpenter, and V. du Vigneaud, *J. Amer. Chem. Soc.*, 70 (1948) 2547–2550.

13 Ľ. Drobnica and J. Augustín, *Collect. Czech. Chem. Commun.*, 30 (1965) 1221–1228.

14 Ľ. Drobnica, D. Podhradský, and P. Gemeiner, *Collect. Czech. Chem. Commun.*, 40 (1975) 3688–3697.

15 M. M. Kreevoy, E. T. Harper, R. E. Duvall, H. S. Wilgus, and L. T. Ditsch, *J. Amer. Chem. Soc.*, 82 (1960) 4899–4902.

16 Ľ. Drobnica and P. Gemeiner, *Collect. Czech. Chem. Commun* , 40 (1975) 3346–3356.