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ISONITRILE DERIVATIVES OF POLYSACCHARIDES AS SUPPORTS FOR THE COVALENT FIXATION OF PROTEINS AND OTHER LIGANDS

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Summary

A method for the introduction of side chains containing isonitrile (isocyanide, —N≡C functional group) on the backbone of polysaccharides and other hydroxylic polymers was developed. The method was based on (a) ionization of some of the hydroxyl groups on the polymer by treatment with a strong base (*tert*-butoxide) in a polar aprotic solvent (dimethylsulfoxide), and (b) introduction of side chains containing isonitrile groups by nucleophilic attack of the polymeric alkoxide ions on a low molecular weight isonitrile containing a good leaving group in the ω-position, (1-tosyl-3-isocyanopropane).

By this method, the side chains containing the -NC functional groups are attached to the polymeric backbone via stable ether bonds.

The isonitrile derivatives of cellulose, linear and cross-linked dextran and cross-linked agarose were utilized for the covalent fixation of high and low molecular weight ligands by four-component reactions carried out in aqueous medium, at neutral pH.

Introduction

Water-insoluble polymers containing isonitrile (isocyanide, —N≡C) functional groups can serve, as recently shown, as versatile supports for the immobilization of enzymes and other biologically active molecules [1—5].

Such materials could be coupled to enzymes and other ligands by four-component condensation reactions [1,4,6—10] carried out in an aqueous

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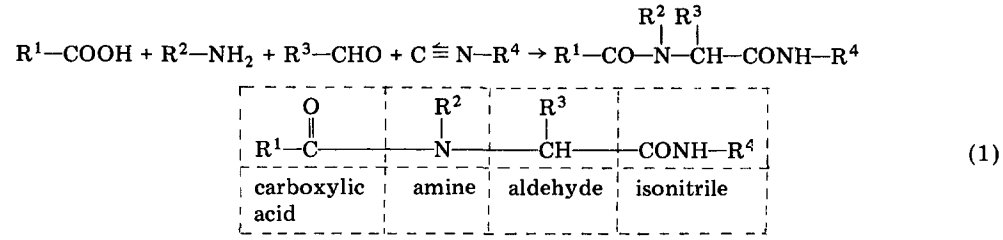
Abbreviations: Bz-Arg-OEt, *N*-benzoyl-L-arginine ethylester; Gly-LeuNH$_2$, glycyl-L-leucine amide.

medium at neutral pH in the presence of a water-soluble aldehyde (Eqn. 1):



(1)

In these reactions the polymeric support supplies the isonitrile function, the ligand supplies either the amino or the carboxyl component, the aldehyde and the missing fourth component being added to the reaction medium; alternatively, supports carrying isonitrile functional groups could serve as 'parent polymers' for further chemical modification, since the —N≡C group can be easyly converted into other types of chemically reactive species, e.g. aminoaryl, aminoalkyl, dibromoisocyanide, acylhydrazide, etc. according to the envisaged application [1—5]. It should be mentioned that immobilization of proteins by four-component reactions has been described earlier by Axén and coworkers [8,10], who, however, used low molecular weight, water-soluble, isonitriles to effect the coupling of enzymes to polymers containing carboxyl, amino or aldehyde functional groups.

The present communication describes a method for the preparation of polyfunctional derivatives of polysaccharides and other hydroxylic polymers containing pendant isonitrile groups. In these materials the 1-oxa-3-isocyanopropane derivatives (-O-$CH_2 \cdot CH_2 \cdot CH_2 \cdot NC$) of cellulose, linear and cross-linked dextran and cross-linked agarose, the side chains are attached to the polysaccharide backbone through stable ether bonds.

The method is based on a displacement reaction involving 1-tosyl-3-isocyanopropane (p-$CH_3 \cdot C_6H_4 \cdot SO_2 \cdot O \cdot (CH_2)_3 \cdot NC$) and the alkoxide ions generated on the polysaccharide backbone by treatment with a strong base in an organic solvent (Eqns. 2 and 3).

The isonitrile derivatives of the various polysaccharides could be used for covalent coupling of proteins and low molecular weight ligands through four-component reactions utilizing the -NC groups on the support.

Materials and Methods

Cellulose, microcrystalline powder, 230—270 mesh (56—63 μm diameter) was a product of Merck (Darmstad, F.R.G.). Sephadex of various grades, Sepharose-CL and linear dextran (M_r 250 000) were purchased from Pharmacia (Uppsala, Sweden). Trypsin (EC 3.4.21.4), type III, twice crystallized, urease (EC 3.5.1.5), type III, (a partially purified preparation), benzoyl-L-arginine ethylester (Bz-Arg-OEt) and glycyl-L-leucine amide hydrochloride were obtained from Sigma (St. Louis, MO, U.S.A.)

Sodium tert-butoxide

Sodium *tert*-butoxide was prepared from freshly cut sodium metal and redistilled *tert*-butanol, and diluted with dimethylsulfoxide (1 : 10) to a final concentration of 0.05 M.

1-Tosyl-3-isocyanopropane

1-Tosyl-3-isocyanopropane was prepared from 3-aminopropanol via the *N*-formylaminopropanol derivative by a modification of the procedure described by Matteson and Bailey [11]:

(a) N-Formylaminopropanol. An equimolar amount of ethyl formate was added dropwise to strongly stirred 3-aminopropanol. Stirring was continued for 1 h at room temperature. The ethanol formed in the reaction was removed by evaporation and the residue was vacuum distilled. The yield was 70%.

(b) 1-Tosyl-3-isocyanopropane. A 4 M pyridine solution of *p*-toluene sulfonylchloride was added dropwise in the course of 30 min to an equal volume of vigorously stirred ice-cooled 2 M *N*-formylaminopropanol in pyridine. The reaction mixture was stirred over ice for 1 h. Cold water was then added and the mixture was extracted with three portions of diethylether/hexane (5 : 1, v/v). The combined extract was washed with cold water and dried over Na_2SO_4. The solvent was removed by evaporation and the residue was dissolved in diethylether/hexane (3.5 : 1, v/v). The solution was left at −18° C. The white crystalline precipitate was separated on a filter, washed with ice-cold hexane and air-dried. The yield was 25%.

Isonitrile derivatives of polysaccharides

Isonitrile derivatives of water-insoluble polysaccharides were prepared by the following general procedure: (1) Preswelling of the polymer; (b) ionization of polysaccharide hydroxyl groups with *tert*-butoxide; and (c) reaction with 1-tosyl-3-isocyanopropane (see Eqns. 2 and 3).

The appropriate polysaccharide was suspended with stirring in anhydrous dimethylsulfoxide for 30 min at 30° C. A 0.05 M solution of sodium *tert*-butoxide in dimethylsulfoxide was added dropwise with stirring to the swollen polymer and the mixture stirred for 15 min to ensure equilibrium ionization of polysaccharide hydroxyl groups.

1-Tosyl-3-isocyanopropane dissolved in dimethylsulfoxide was added to a suspension of powdered potassium hydroxide or anhydrous potassium carbonate in the same solvent and the mixture stirred for 10 min at 30° C (to remove acid impurities). The KOH (or K_2CO_3) powder was removed by filtration (glass wool); the filter was washed with dimethylsulfoxide and the combined filtrate added to the ionized polysaccharide suspension. The reaction was allowed to proceed with stirring for 4 h at 30° C. The powder was separated by centrifugation, resuspended in CH_3OH and washed on a filter with CH_3OH, cold H_2O, CH_3OH, diethylether and air-dried. In the case of linear dextrane, the modified polymer was precipitated from solution by the addition of CH_3OH. The isonitrile polysaccharide derivatives were stored over silica gel in the cold. The experimental details for the various polysaccharides are summarized in Table I.

Coupling of enzymes and peptides to isonitrile derivatives of polysaccharides

(A) Four-component coupling involving ligand amino groups. Dried beads of the appropriate isonitrile polysaccharide derivative (50 mg) were preswollen in dimethylsulfoxide, washed with cold water and suspended in 2 ml of a cold solution of enzyme (5 mg/ml) or peptide (0.1 M) in 0.1 M sodium phosphate, 0.5 M sodium acetate, pH 8, 50—200 μl of cold 1.8 M acetaldehyde (10%, v/v)

in the same buffer were then added and the reaction allowed to proceed overnight with stirring at 4°C. The insoluble enzyme derivative was separated by filtration, washed with water, 1 M KCl and again with water, resuspended in water (4 ml) and stored at 4°C (see Table II). Isonitrile derivatives of linear dextran were dissolved directly in the aqueous buffer.

(B) Four-component coupling involving ligand carboxyl groups. Isonitrile polysaccharide derivative (20—50 mg) pretreated as described in (A), was suspended in cold 0.1 M Tris buffer, pH 7. Enzyme (5 mg/ml) or peptide (final concentration 0.1 M) was then added followed by 25—100 µl/ml of a cold 1.8 M solution of acetaldehyde in the same buffer.

Assay methods

Protein. Bound protein was determined by the Lowry method [13,14] and confirmed by total amino acid anaysis of acid hydrolyzates of the appropriate polysaccharide-protein conjugates.

Linear dextran. Dextran was determined by a modification of the cysteine-sulfuric acid method described by Dische for glucose [15].

Isonitrile groups. (i) The isocyanide content of polyisonitrile cellulose powders was determined titrimetrically as described previously [2,16]. (ii) The isocyanide content of the isonitrile derivatives of all polysaccharides investigated was estimated from the amounts of bound glycyl-L-leucine amide.

Enzymic activities

The enzymic activity of trypsin was determined at 25°C by the pH-stat method [17,18] using 0.1 M NaOH as titrant. The substrate solution (5 ml) was $1.5 \cdot 10^{-2}$ M Bz-Arg-OEt, 0.05 M in KCl. The assays were carried out at the appropriate pH optima: pH 8.0 for the native enzyme and at pH 9.0 for polysaccharide-trypsin conjugates.

The enzymic activity of native and polysaccharide-bound urease was determined by the nitroprusside method according to Chaney and Marbach [19] and Weatherborn [20].

Results and Discussion

Preparation and characterization of isonitrile derivatives of polysaccharides

Side chains containing isonitrile functional groups could be attached to the backbone of some common polysaccharides (cellulose, dextran, agarose) via ether bonds. The method consisted of: (i) ionization of some of the hydroxyl groups on the polymer by a strong base in a non-aqueous solvent (*tert*-butoxide in dimethylsulfoxide); (ii) displacement reaction via nucleophilic attack of the alkoxide ions generated on the polymer in the first step, on a low molecular weight isonitrile containing a good leaving group in the ω-position: 1-tosyl-3-isocyanopropane (p-$CH_3 \cdot C_6H_4 \cdot SO_2 \cdot O \cdot (CH_2)_3 \cdot NC$).

The reaction sequence leading to polyfunctional 1-oxa-3-isocyanopropane ($-O-CH_2 \cdot CH_2 \cdot CH_2 \cdot NC$) derivatives of polysaccharides is summarized in Eqns. 2 and 3:

$$|-OH + O^-Bu^t \xrightarrow{Me_2SO} |-O^- + HOBu^t \qquad (2)$$

$$\text{Cell}\text{—O}^- + \text{CH}_3 \cdot \text{C}_6\text{H}_4 \cdot \text{SO}_2 \cdot \text{O} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{N} \cong \text{C} \xrightarrow[30^\circ\text{C}]{\text{Me}_2\text{SO}} \text{Cell}\text{—O}\text{—CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{N} \cong \text{C}$$

$$+ \text{CH}_3 \cdot \text{C}_6\text{H}_4 \cdot \text{SO}_3^- \qquad (3)$$

Support for the reaction scheme of Eqns. 2 and 3 was obtained by the synthesis of a low molecular weight analogue 1-methoxy-3-isocyanopropane ($CH_3 \cdot O \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CN$), prepared under conditions identical to those employed with polysaccharides and its characterization by NMR.

The choice of experimental conditions was guided by the following considerations:

Dimethylsulfoxide is a good solvent for linear polysaccharides such as linear dextrans and agaroses, and effects the swelling of cross-linked dextran, cross-linked agarose and cellulose. Moreover, dimethylsulfoxide, a highly polar solvent, is known to facilitate ionization and to accelerate displacement reactions, particularly those involving alkoxide ions, by several orders of magnitude [21—25].

1-Tosyl-3-isocyanopropane was chosen for the following reasons: (a) Tosyl (p-$CH_3 \cdot C_6H_4 \cdot SO_2$-) is a good leaving group easily displaced by alkoxide ion in dimethylsulfoxide [26]. (b) The three carbon atom side chain (-$(CH_2)_3$-) is the minimum length required to forestall elimination reactions in a basic medium (e.g. the formation of vinylisocyanide, $CH_2 = CH \cdot NC$, in the case of Tos-$(CH_2)_2 \cdot NC$ [11].

Tert-Butoxide ion in dimethylsulfoxide is among the strongest organic bases known ($pK_a \approx 30$) [21—25]. Bu^tO^- is hence a suitable reagent for the quantitative ionization of secondary as well as primary OH groups.

Model experiments were carried out with cellulose powder preswollen in dimethylsulfoxide and exposed to different amounts of *tert*-butoxide and 1-tosyl-3-isocyanopropane. The -NC content of the isonitrile derivatives of cellulose as determined titrimetrically, varied linearly with the amount of *tert*-butoxide (at a fixed concentration of 1-tosyl-3-isocyanopropane: 0.05 M) as well as with the concentration of 1-tosyl-3-isocyanopropane (at fixed amount of *tert*-butoxide/g polysaccharide; 500 μequiv.). The data are summarized in

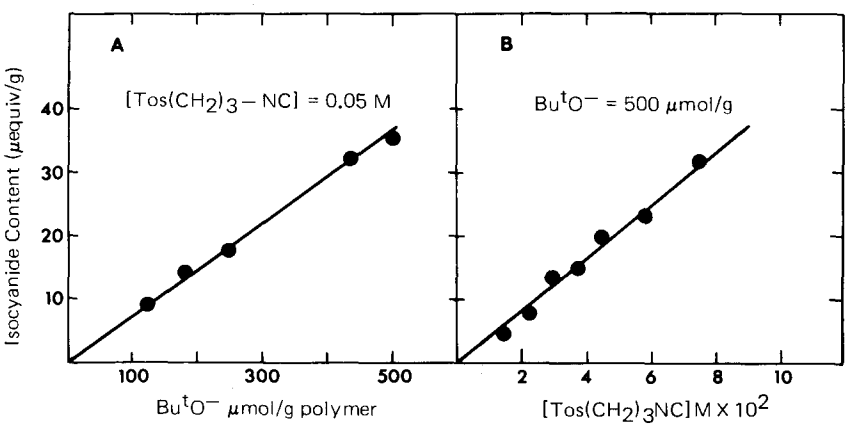


Fig. 1. Dependence of isonitrile content of cellulose powders on reaction conditions. (A) At (Tos-$(CH_2)_3 \cdot NC$) = 0.05 M and varying amounts of sodium *tert*-butoxide. (B) At sodium *tert*-butoxide 500 μmol/g cellulose and varying concentrations of Tos$(CH_2)_3 \cdot NC$.

Fig. 1. The highest amount of *tert*-butoxide employed in our study (500 μequiv. Bu^tO^-/g polysaccharide) was chosen on the basis of control experiments with cross-linked dextran beads (when exposed to *tert*-butoxide at ratios higher than 750 μequiv./g Sephadex G-75 beads were found to undergo partial disintegration). The -NC content of polyisonitrile derivatives of polysaccharides can thus be controlled, by regulating the amount of *tert*-butoxide and/or the concentration of 1-tosyl-3-isocyanopropane.

The conditions employed for the preparation of isocyanide derivatives of cellulose, linear and cross-linked dextran, and cross-linked agarose are summarized in Table I.

Cross-linked agarose beads and cellulose powders exhibited higher isocyanide contents as compared to similarly prepared cross-linked dextran derivatives (Table I). The monomeric units of agarose, alternating 1,3-linked β-D-galactose and 1,4-linked, 3,6-anhydro-α-L-galactose residues, contain both primary and secondary hydroxyl groups; the same is true of cellulose which consists of 1,4-linked β-D-glucose residues. Dextran, on the other hand, is composed of 1,6-linked α-D-glucose units, and hence contains only secondary hydroxyls [27,28]. The higher -NC content of cellulose powder and cross-linked agarose beads relative to cross-linked dextran beads could be possible attributed to the fact that primary alkoxide ions are more reactive than secondary alkoxide.

TABLE I

PREPARATION OF ISONITRILE DERIVATIVES OF POLYSACCHARIDES

All amounts given are for 1 g polysaccharide. Isocyanide content of modified polysaccharide was estimated from the leucine content of polysaccharide-Gly-LeuNH_2 conjugates.

Poly-saccharides	Preswelling in dimethyl-sulfoxide (ml)	Sodium *tert*-butoxide (μmol)	1-Tosyl-3-isocyanopropane			Total volume or reaction mixture (ml)	Isocyanide content of modified poly-saccharide (μmol/g)
			g	Dissolved in dimethyl-sulfoxide (ml)	Final concen-tration (M)		
Cellulose powder 230—270 mesh (56—63 μm diameter)							
	15	500	0.48	20	0.05	45	31
Cross-linked dextran (Sephadex)							
G-75	40	500	0.78	15	0.05	65	19
	40	500	1.56	15	0.10	65	30
G-150	80	500	1.38	25	0.05	115	12
	80	500	3.12	40	0.10	130	33
G-200	120	500	1.92	30	0.05	160	12
	120	500	4.32	50	0.10	180	15
Cross-linked agarose (Sepharose-CL) *							
2B	70	500	1.20	20	0.05	100	64
4B	70	500	1.20	20	0.05	100	63
	70	500	2.51	25	0.10	105	91
Linear dextran (M_r 250 000)							
	20 **	500	0.54	15	0.05	45	88

* Dry powder; prepared from commercial aqueous suspension by washing with a graded series of water/dioxane mixtures and finally with pure dioxane followed by lyophilization (see Pharmacia booklet on Sepharose-CL and Ref. 12.

** Linear dextran is dissolved in dimethylsulfoxide.

All isonitrile polysaccharide derivatives exhibited, when kept in the form of dry powders in the cold, high storage stabilities (no significant decrease could be detected in the binding capacity of isonitrile, Sephadex and Sepharose-CL samples refrigerated, over silica gel, for up to 8 months).

Coupling of proteins and low molecular weight ligands

Proteins and peptides can be coupled to polymers containing isonitrile functional groups via four-component condensation reactions involving amine carboxyl, aldehyde and isonitrile (Eqn. 1) [1,4,6—10]. In this reaction, carried out at neutral pH, the polymeric support (R^4 in Eqn. 1) supplies the isonitrile component; the ligand (protein or peptide) supplies either the amine or the carboxyl component; a water-soluble aldehyde, e.g. acetaldehyde or glyceraldehyde, and the missing fourth component are added to the aqueous medium [1]. The isonitrile group on the support can be steered towards one type of ligand functional group by increasing the relative concentration of one of the complementary components in the aqueous buffer [1,2,4]. Ligands can therefore be bound through their amino groups in the presence of aldehyde (acetaldehyde or glyceraldehyde) and excess carboxylate or alternatively through

TABLE II

COUPLING OF GLYCYL-L-LEUCINE AMIDE AND TRYPSIN TO ISONITRILE DERIVATIVES OF POLYSACCHARIDES

Polysaccharide ionized by the addition of sodium-*tert*-butoxide (500 μmol/g polymer), followed by 1-tosyl-3-isocyanopropane solution at the specified concentration (see * and **). For details, see Table I). Bound Gly-LeuNH$_2$ was estimated from the leucine content of polysaccharide-Gly-LeuNH$_2$ conjugates. Bound trypsin indicates coupling via -NH$_2$ groups on enzyme. Buffer composition: 0.1 M phosphate, 0.5 M acetate, 0.45 M acetaldehyde, pH 8. Total bound trypsin was determined by amino acid analysis of acid hydrolyzates of the appropriate polysaccharide-trypsin conjugate. Active bound trypsin was determined by rate assay.

Polysaccharide	Bound Gly-LeuNH$_2$ (μmol/g)	Bound trypsin		
		Total (mg/g conjugate)	Active (mg/g conjugate)	Percentage of total
Cellulose powder (230—270 mesh; 56—63 μm diameter)				
	31 *	12.2	8.2	67
Cross-linked dextran (Sephadex)				
G-75	19 *	8.8	8.8	100
	30 **	15.1	10.5	70
G-150	12 *	12.7	8.8	70
	33 **	16.5	11.0	66
G-200	12 *	15.7	12.3	78
	25 **	15.4	11.6	75
Cross-linked agarose (Sepharose-CL)				
4B	68 *	61.6	24.3	40
	91 **	91.3	28.5	30
Linear dextran (M_r 250 000)				
	88 *	264	186	70

* Ionized polysaccharide treated with 0.05 M 1-tosyl-3-isocyanopropane.

** Ionized polysaccharide treated with 0.10 M 1-tosyl-3-isocyanopropane.

their carboxyls in the presence of aldehyde and an amine (e.g. tris(hydroxymethyl)aminomethane (Tris)).

Table II summarizes experiments on the coupling of glycyl-L-leucine amide and trypsin to isonitrile polysaccharide derivatives via the amino groups on the ligands. The various supports were prepared with two concentrations of 1-tosyl-3-isocyanopropane (0.05 and 0.10 M). The data show that derivatives prepared with the higher 1-tosyl-3-isocyanopropane concentration exhibited higher binding capacities for both peptide and protein. This finding is in good agreement with the data of Fig. 1.

The specific activity of trypsin bound to cellulose powder, and to linear and cross-linked dextran was 70—80% of that of the native enzyme. In the case of trypsin bound to cross-linked agarose beads, despite the considerably higher protein content (4—6 times, as compared to cross-linked dextrans) the specific activity of the bound enzyme was lower. This is most probably due to diffusional constraints on the penetration of substrate, often encountered with porous beads which are heavily loaded with enzyme [29—31].

Urease, an -SH enzyme known to be sensitive to aldehydes was almost completely inactivated under conditions where the buffers required for the appropriate four-component reactions contained the relatively high concentrations of acetyldehyde normally employed (0.45 M; see Table II). The deleterious effects of aldehyde on the activity of urease bound through the amino and through the carboxyl groups on the protein were essentially eliminated by lowering the concentration of acetaldehyde in the coupling mixture (0.045—0.09 M). Under these conditions the coupling yields and specific activities of immobilized urease were similar to those recorded for trypsin in Table II.

Comparison of the peptide and protein-binding capacities of the isonitrile derivatives of cross-linked agarose with literature values for the binding of peptide (Gly-Leu) and protein (chymotrypsin) to cyanogen bromide-activated cross-linked agarose [32] shows that similar amounts of peptide can be bound by the two methods (Table III). The amounts of protein bound to the isonitrile derivative of agarose (2.6—3.8 μmol/g) are, however, considerably higher than those recorded for CNBr-activated agaroses yielding derivatives of comparable peptide content (0.35—1.7 μmol/g [42]; Table III).

The higher (bound protein)/(bound peptide) ratio obtained with isonitrile

TABLE III

BINDING CAPACITIES OF CROSS-LINKED AGAROSE DERIVATIVES

Method of activation *	Bound ligand (μmol/g)		Bound protein/bound peptide ($\times 10^2$)
	Peptide	Protein	
Cyanogen bromide **	25	0.13	0.5
	70	0.35	0.5
	120	1.7	1.4
Isonitrile derivative ***	68	2.59	3.8
	91	3.84	4.2

* Support: Sepharose-CL, 4B.

** Data of Porath et al. [32]; peptide, Gly-Leu; protein, chymotrypsin.

*** This communication. Data taken from Table II. Peptide, Gly-Gly-LeuNH$_2$; protein, trypsin.

TABLE IV

STABILITY OF CELLULOSE-GLY-LEUNH$_2$ CONJUGATES

Conditions	Peptide content of derivatives			
	Prepared by four component reaction with isonitrile-cellulose		Prepared by CNBr activation of cellulose	
	μmol/g	Percent of control	μmol/g	Percent of control
Control	27.8	100	80.5	100
0.1 M carbonate, pH 9 (48 h, 30°C)	28.2	100	53.5	66.5
0.2 M propylamine, pH 9 (48 h, 30°C)	26.9	98	50.4	62.6

agarose derivatives (last column of Table III) is most probably related to the fact that the -NC group does not undergo competing hydrolytic reactions with the aqueous medium in the pH range employed for coupling (pH 7—8) [1,4,33]; competing hydrolysis of the active intermediate in the CNBr activation method is known to occur at pH values above neutrality [34].

In view of the wide acceptance of polysaccharides as supports for enzyme immobilization and particularly in affinity chromatography, it was of interest to compare the relative stability of the linkage between low molecular weight ligands and a polysaccharide matrix in conjugates prepared via isonitrile and via CNBr-activated derivatives of the polysaccharide. Recent reports drawing attention on the leakage of ligands from polysaccharide conjugates prepared by the CNBr method enhance the relevance of such comparison [35—39].

Table IV summarizes experiments carried out with Gly-LeuNH$_2$ conjugates of cellulose prepared by a four-component reaction with the appropriate isonitrile derivative and via CNBr activation [32,34]. The two peptide cellulose conjugates were incubated at 30°C for 48 h with 0.1 M carbonate (pH 9) and with a nucleophile, 0.2 M propylamine, pH 9.

A 30—35% decrease in peptide conent was found for the cellulose-Gly-LeuNH$_2$ conjugate prepared b; Br activation. No loss of bound ligand was observed for cellulose-Gly-Le , prepared via a four-component reaction with isonitrile-cellulose.

Concluding remarks

Isonitrile derivatives of polysaccharides offer several advantages as supports for the fixation of high and low molecular weight ligands via stable covalent bonds.

The isonitrile functional groups are attached to the polysaccharide backbone by O-alkyl side chains. The -NC groups generate peptide bonds in four-component reactions, with the ligand moiety. The ligand polysaccharide conjugates, hence carry no residual charged groups deriving from either the modification or coupling reactions.

The use of four-component reactions for coupling to isonitrile containing polymers allows the covalent fixation of ligands carrying -COOH as well as -NH$_2$ functional groups to the same polymeric support [1—5]. In the case of

enzymes sensitive to aldehyde, protection against the harmful effects of the reagent can be obtained through the lowering of the aldehyde concentration in the aqueous buffer without impairing the efficiency of binding. The -NC moiety on a polysaccharide matrix can, according to the envisaged application, be also transformed into many other types of reactive groups by four-component and other simple one-step reactions [1—7].

Coupling procedures based on isonitrile derivatives of polysaccharides, could thus provide alternatives to coupling by cyanogen bromide activation.

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References

1 Goldstein, L., Freeman, A. and Sokolovsky, M. (1974) Biochem. J. 143, 497—509
2 Freeman, A., Sokolovsky, M. and Goldstein, L. (1976) J. Solid Phase Biochem. 1, 261—274
3 Freeman, A., Granot, R., Sokolovsky, M. and Goldstein, L. (1976) J. Solid Phase Biochem. 1, 275—286
4 Goldstein, L., Freeman, A., Blassberger, D., Granot, R. and Sokolovsky, M. (1977) in Biotechnological Applications of Proteins and Enzymes (Bohak, Z. and Sharon, N., eds.), pp. 153—167, Academic Press, New York
5 Blassberger, D., Freeman, A. and Goldstein, L. (1978) Biotechnol. Bioeng. 20, 309—315
6 Ugi, I. (1962) Angew. Chem. Int. Edn. Engl. 1, 8—21
7 Ugi, I. (ed.) (1971) Isonitrile Chemistry, Academic Press, New York
8 Axén, R., Vretblad, P. and Porath, J. (1971) Acta Chem. Scand. 25, 1129—1132
9 Vretblad, P. and Axén, R. (1971) FEBS Lett. 18, 254—256
10 Vretblad, P. and Axén, R. (1973) Acta Chem. Scand. 27, 2769—2780
11 Matteson, D.S. and Bailey, R.A. (1968) J. Am. Chem. Soc. 90, 3761—3765
12 Hjerten, S., Rosengren, J. and Pahlman, S. (1974) J. Chromatogr. 101, 281—288
13 Lowry, D.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
14 Layne, E. (1957) Methods Enzymol. 3, 447—454
15 Dische, F. (1962) in Methods in Carbohydrate Chemistry (Whistler, R.L. and Wolform, M.L., eds.), Vol. I, p. 488, Academic Press, New York
16 Arora, A.S., Hinrichs, E. von and Ugi, I. (1974) Z. Anal. Chem. 269, 124
17 Jacobsen, C.F., Leonis, J., Lindestrom-Lang, K. and Ottesen, M. (1957) Methods Biochem. Anal. 4, 171—210
18 Walsh, K.A. and Wilcox, P.E. (1970) Methods Enzymol. 19, 31—41
19 Chaney, A.L. and Marbach, E.P. (1962) Clin. Chem. 8, 130—132
20 Weatherborn, M.W. (1967) Anal. Chem. 39, 971—974
21 Martin, D., Weise, A. and Niclas, J.J. (1967) Angew. Chem. Int. Edn. Engl. 6, 318—344
22 Ledwith, A. and McFarlane, N. (1964) Proc. Chem. Soc. 1964, 108
23 Steiner, E.C. and Gilbert, J.M. (1963) J. Am. Chem. Soc. 85, 3054—3055
24 Steiner, E.C. and Gilbert, J.M. (1965) J. Am. Chem. Soc. 87, 383—384
25 Arnett, E.M. and Small, L.E. (1977) J. Am. Chem. Soc. 99, 808—816
26 Snyder, C.H. and Soto, A.A. (1965) J. Org. Chem. 30, 673—676
27 Kennedy, J.F. (1974) Adv. Carbohydr. Chem. Biochem. 29, 305—405
28 Goldstein, L. and Manecke, G. (1976) in Applied Biochemistry and Bioengineering, Vol. I: Immobilized Enzymes, Principles (Wingard, L.B., Katchalski-Katzir, E. and Goldstein, L., eds.), pp. 23—126, Academic Press, New York
29 Axén, R., Myrin, P. and Janson, J.C. (1970) Biopolymers 9, 401—413
30 Goldstein, L. (1976) Methods Enzymol. 44, 397—443
31 Engasser, J.M. and Horvath, C. (1976) in Applied Biochemistry and Bioengineering, Vol. I: Immobilized Enzymes, Principles (Wingard, L.B., Katchalski-Katzir, E. and Goldstein, L., eds.), pp. 127—220, Academic Press, New York
32 Porath, J., Aspberg, K., Drevin, H. and Axén, R. (1973) J. Chromatogr. 86, 53—56
33 Freeman, A. (1978) Ph.D. Thesis, Tel-Aviv University
34 Porath, J. and Axen, R. (1976) Methods Enzymol. 44, 19—45
35 Wilchek, M. and Hexter, C.S. (1976) Methods Biochem. Anal. 23, 348—385
36 Wilchek, M., Oka, T. and Topper, Y.J. (1975) Proc. Natl. Acad. Sci. U.S. 72, 1055—1058
37 Jost, R., Miron, T. and Wilchek, M. (1974) Biochim. Biophys. Acta 362, 75—82
38 Wilchek, M. and Miron, T. (1976) Biochem. Biophys. Res. Commun. 72, 108—113
39 Lasch, J. amd Koelsch, R. (1978) Eur. J. Biochem. 82, 181—186