Note

The reaction of dextran carbonate with amino acids and polypeptides

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Dextran carbonate has been prepared previously[1], and the same workers reported the reaction of sugar carbonates with glycine and ammonia[2]. The preparation of cellulose carbonate[3] and its reaction with β-D-glucosidase[4] have also been reported. The present investigations were pursued in order to determine whether a soluble, synthetic glycopeptide might be prepared by the reaction of proteins with dextran carbonate.

Preparation of several dextran carbonates indicated that derivatives of linear dextran were less soluble than those of branched dextrans. It was also noted that the

TABLE I

COMPETITIVE COUPLING OF AMINO ACIDS TO DEXTRAN CARBONATE IN VARIOUS MEDIA

Amino acid (μmole/100 mg) coupled	*Reaction time, 30 min*					*1 h*
Conditions	*Phosphate 0.5M, pH 5.7*	*Phosphate 0.1M, pH 5.7*	*Phosphate 0.1M, pH 7.8*	*Phosphate 0.1M, pH 9.0*	*Methyl sulphoxide*	*Phosphate 0.1M, pH 7.8*
Aspartic acid	0.210	0.287	0.112	0.300		0.115
Threonine	0.108	0.113	0.100	0.106		0.073
Serine	0.302	0.275	0.340	0.266		0.281
Glutamic acid	0.419	0.608	0.183	0.472		0.220
Proline	0.151	0.086	0.084	0.085		0.059
Glycine	0.349	0.251	0.287	0.229	0.472	0.254
Alanine	0.208	0.179	0.166	0.154	0.280	0.137
Valine	0.141	0.148	0.081	0.136	0.115	0.060
Cysteine	n.d.[a]	0.046	n.d.	0.060	0.015	0.025
Methionine	0.020	0.066	n.d.	0.054	0.023	n.d.
Isoleucine	0.098	0.135	0.046	0.129	0.078	0.040
Leucine	0.178	0.199	0.079	0.169	0.119	0.065
Tyrosine	0.062	0.106	0.042	0.111	0.055	0.034
Phenylalanine	0.095	0.125	0.040	0.121	0.061	0.036
Lysine	0.112	n.d.	0.071	0.086	0.243	0.062
Histidine	0.125	0.104	0.091	0.119	0.070	0.107
Arginine	0.094	0.097	0.036	0.087	0.082	n.d.
Total % (w/w)	0.283	0.314	0.174	0.283	0.156	0.157

[a]n.d. = not detectable.

higher the degree of substitution the less soluble was the carbonate. A mixture of amino acids in equimolar proportions was used to study the relative reactivity of the amino acids under various conditions. Previous work[5], using methyl 4,6-*O*-benzylidene-α-D-glucopyranoside 2,3-carbonate as the model compound, had suggested that triethylamine, or a similar tertiary base, was necessary for the ring opening of *trans* cyclic carbonates. Table I shows, however, that the 17 amino acids tested reacted in aqueous phosphate buffer or aqueous methyl sulphoxide. The ring opening occurs under weakly acidic and basic conditions. The lower degree of coupling at pH 7.8 can be explained by a slower rate of reaction under the almost neutral conditions, which is typical of a general acid/base-catalysed reaction. Increasing the reaction time to 4 h failed to increase the total amount of amino acid coupled under these conditions (amino acids 3mM; pH 7.8). With serine at high concentration (0.383M), coupling was incomplete after 30 min, but no further coupling occurred after 4 h (Table IIa).

TABLE II

REACTION OF DEXTRAN CARBONATE WITH SERINE

(*a*) Variation of molar ratio; 30-min reaction time.

Molar ratio of carbonate groups–serine	*Serine* (%, *w/w*)
5:1	0.20
1:1	0.54
1:5	1.17

(*b*) Variation of reaction time; molar ratio of carbonate–serine, 1:5.

Reaction time	*Serine* (%, *w/w*)	
	Carbonate I[a]	*Carbonate VI*[b]
30 min	1.18	0.77
1 h		0.84
2 h	1.25	1.2
4 h	1.35	1.07

[a]Water-soluble carbonate. [b]Water-insoluble carbonate.

In general, the total amount of amino acid coupled in aqueous methyl sulphoxide was intermediate between that attached in aqueous buffer at pH 7.8 and 9.0. However, the relative proportions of glycine, alanine, and lysine coupled were increased. The relative reactivity of the amino acids does not relate to their basicity. Cysteine and methionine are partially destroyed during the subsequent acid hydrolysis

used for analysis, particularly in the presence of such a large excess of carbohydrate, and the analysis figures probably do not reflect the true reactivity of these amino acids. Cysteic acid was not determined in these samples.

Cellulose carbonate, when reacted with a similar amino acid mixture[6] at pH 7.8 in phosphate buffer, coupled less amino acid than the dextran carbonate. With cellulose, the basic amino acids were coupled to the greatest extent. Serine was coupled to water-insoluble dextran carbonate to a lesser extent than to the water-soluble carbonate (Table IIb). The experiments with serine show that only 5–6% of the available carbonate groups react with the amino acid, although i.r. analysis of the product indicated the disappearance of all the cyclic carbonate groups. These results are possibly due to competition between the amino acids and the solvent. Reactions of dextran carbonate with human serum albumin, insulin, and insulin peptide A emphasise the importance of steric considerations. The larger the polypeptide the less efficient is the coupling reaction (Table III). The amount of human serum albumin coupled to dextran carbonate was below the limit of detection by amino-acid analysis, but human serum albumin (I-125) was coupled[6] to the extent of 0.06% to cellulose carbonate (d.s. 40%).

TABLE III

COMPOSITION OF DEXTRAN CARBONATE DERIVATIVES

Polypeptide	*Amount coupled*	
	μmole/100 mg	% (*w/w*)
Insulin peptide A	0.537	1.90
Insulin	0.102	0.60
Human serum albumin	<0.004	<0.1
Amino acid derivative		
N-Acetylasparagine	7.5	1.3
Cystine	1.3	0.3
Cystine + sodium hydroxide (trace)	2.1	0.5
S-Benzylcysteine	n.d	n.d
Cysteine	19.8	2.4
N-Acetylcysteine	9.1	1.12

The experiments with amino acid derivatives indicate that amide and thiol groups are reactive towards *trans* cyclic-carbonate groups (Table III). The lack of coupling by cystine and *S*-benzylcysteine can be explained by the low solubility of these compounds under the conditions used.

Thus, although amino acids and their derivatives react with cyclic carbonate groups, the efficiency with which proteins are coupled to dextran carbonate is low. Sufficient enzyme can be attached to cellulose to form useful insolubilised-enzymes, but the protein content of the derivatives is generally less than 2%.

EXPERIMENTAL

An industrial grade dextran (mol. wt., 110,000) and dextran T70 (Pharmacia) were used to prepare[1] dextran carbonates (d.s. 40%).

Reactions with amino acids. — Dextran carbonate (60 mg) was dissolved in phosphate buffer (4 ml; 0.5M, pH 5.7; 0.1M, pH 5.7; 0.1M, pH 7.8; 0.1M, pH 9.0), and a standard amino-acid mixture (0.4 ml; 2.4 μmoles of each of 17 amino acids/ml) was added. The mixture was stirred at room temperature for 30 min or 1 h, and the product was recovered after dialysis and lyophilisation. In one case, methyl sulphoxide (4 ml) and water (2 ml) were used in place of phosphate buffer. Products were analysed, using a Technicon Autoanalysis system, after acid hydrolysis (6M HCl, 105°, 22 h).

Reaction with serine. — Dextran carbonate (60 mg) was dissolved in methyl sulphoxide (4 ml) and serine (76, 15, or 3 mg) in phosphate buffer (2 ml; 0.1M, pH 7.8) was added. The ratio of cyclic carbonate groups to serine was 5:1, 1:1, and 1:5, respectively. The reaction mixture was stirred at room temperature for 30 min. Using a 5-fold excess of serine (76 mg) to available carbonate groups (dextran carbonate, 60 mg), the reaction mixture was stirred for periods of 1, 2, and 4 h. All the dextran derivatives were recovered by dialysis and lyophilisation. They were analysed, after standard, acid hydrolysis (6M HCl, 105°, 22 h), using ion-exchange chromatography on Dowex AG-50W x8 resin.

Reaction with amino acid derivatives. — Dextran carbonate (30 mg) dissolved in methyl sulphoxide (3 ml) was added to a solution of *N*-acetylasparagine (64 mg; carbonate–*N*-acetylasparagine, 1:5) in phosphate buffer (1 ml; 0.1M, pH 7.8), and the mixture was stirred at room temperature for 4 h. In the same way, cystine (88 mg), *S*-benzylcysteine[7] (77.5 mg), cysteine (44.5 mg), and *N*-acetylcysteine (60 mg) were reacted with dextran carbonate (30 mg). The amount of material coupled was determined by acid hydrolysis, followed by ion-exchange chromatography. Cysteine derivatives were oxidised to cysteic acid with performic acid, prior to acid hydrolysis.

Reaction with polypeptides. — Dextran carbonate (60 mg) was added to human serum albumin (60 mg), insulin or insulin peptide A (60 mg) dissolved in phosphate buffer (6 ml, 0.1M, pH 7.8), and the mixture was stirred for 4 h at room temperature. Uncoupled insulin or insulin peptide A was removed by gel filtration using Sephadex G-100. Unreacted, human serum albumin was removed by ion-exchange chromatography on DEAE cellulose. The carbohydrate fractions were combined, dialysed, and lyophilised. The polypeptide content of the products was estimated by hydrolysis (6M HCl, 105°, 22 h) and analysis, using a Technicon Autoanalysis system[2].

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