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Immunochemical Studies on Blood Groups. XXXIII. The Effects of Alkaline Borohydride and of Alkali on Blood Group A, B, and H Substances*

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ABSTRACT: Degradation of blood group A, B, and H substances by alkali with or without sodium borohydride causes destruction of almost all of the serine and threonine and of the internal *N*-acetylgalactosamine of the blood group substances. Some destruction of galactose also occurs. Most of the other amino acid constituents remained unchanged but some destruction of arginine and histidine was also found. In the presence of reducing agent, α-aminobutyric acid and additional alanine were formed from threonine and serine and an additional peak was found on the chromatograms after valine. The findings suggest that oligosaccharide side chains bearing the A, B, and H determinants are linked to the serine and threonine probably by an *N*-acetylgalactosamine residue, but the possibility of linkage by a galactose cannot be excluded.

Earlier studies from this laboratory (Schiffman *et al.*, 1964a,b; Lloyd and Kabat, 1964) have shown that treatment of blood group A, B, and H substances with alkali in the presence of sodium borohydride degrades these substances with the appearance of low molecular weight dialyzable reduced oligosaccharides, some of which showed highly potent specific blood group activity as measured by inhibition of precipitation or hemagglutination with their corresponding specific antibodies. In their study of the effects of alkali on mucopolysaccharides, Anderson *et al.* (1964a,b, cf. 1963) reported that a sample of hog mucin A + H blood group substance upon treatment with alkali showed destruction of 8% of its threonine and 28% of its serine, showing that the sugars were released by a β-carbonyl elimination (cf. Ballou, 1954) of *O*-substituted serine and threonine. The reduced oligosaccharide fragments identified (Lloyd and Kabat, 1964) indicated that the oligosaccharide chains liberated by this reaction were further degraded by a peeling reaction (Whistler and BeMiller, 1958; Ballou, 1954) from the reducing end. In connection with our studies on the structure of the blood group substances an effort was made to study the effects of the alkaline elimination reaction with and without borohydride (or borodeuteride) on the amino acid and sugar compositions of the dialyzable and nondialyzable portions of blood group A, B, and H substances. The data confirm and extend the findings of Anderson *et al.* (1964a,b) that selective destruction of serine and threonine occur, and also establish that the *N*-acetylgalactosamine in the interior of the oligosaccharide chain is selectively destroyed in the A, B, and H substances. More recently in studies of a polysaccharide from a human colloid breast carcinoma, alkaline liberation of *N*-acetylgalactosamine was accompanied by destruction of threonine (Adams, 1965). Glycosidic linkages of sugars or amino sugars to serine and threonine have been shown to exist in other polysaccharides (Lindahl and

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Rodén, 1964; Harbon *et al.*, 1964; Gregory *et al.*, 1964; Tanaka *et al.*, 1964).

Experimental

Three samples of blood group substance were used, an A substance from human ovarian cyst (MSS, 10%), hog mucin A + H substance, and a human ovarian cyst B substance (Beach phenol-insol.) (see Schiffman *et al.*, 1964a; Lloyd and Kabat, 1964). The procedure followed was essentially that of Schiffman *et al.* (1964a) in which the substances at a concentration of 10% were exposed to 0.2 M alkali in the presence of 1% sodium borohydride. However the sample of MSS (3 g) and a 1-g sample of Beach were treated with NaOD and $NaBD_4$. An additional sample of Beach was exposed only to NaOD. In the studies with deuterium, exchangeable hydrogens were removed by two lyophilizations from D_2O.[1] A sample of 35 g of hog mucin A + H substances was treated with NaOH and $NaBH_4$ and a sample of 3.2 g was treated with NaOH only. After standing at room temperature for 1 week the samples were neutralized and the dialyzable and nondialyzable fractions were obtained as described previously. The weights recovered were obtained, and solutions of the original substances and the nondialyzable and dialyzable fractions were analyzed for nitrogen by the ninhydrin method (Schiffman *et al.*, 1964a); for hexosamine, *N*-acetylhexosamine, and methylpentose by the methods previously used (see Kabat, 1961); and for galactose by the method of Nolan and Smith (1963) and correcting for the color due to fucose (see Lloyd and Kabat, 1964). Samples (1–3 mg) were hydrolyzed with 10 ml 6 N HCl at 110° (see Carsten and Kabat, 1956) for 22 hours; the solutions were evaporated to dryness overnight over NaOH, taken up in 0.5 ml starting buffer, *p*H 2.83, and analyzed in an amino acid analyzer (Spackman *et al.*, 1958; Piez and Morris, 1960). Glucosamine and galactosamine as well as the amino acids were determined.

Results in Table I are presented for the total recoveries and for the composition of the dialyzable and nondialyzable fractions expressed as percentages of the original amount of each constituent used. No correction factors have been applied. To calculate the amount theoretically present in the dialyzable fraction the amount found in the nondialyzable fraction was subtracted from the total amount of each constituent used. In this way recoveries of any sugar or amino acid in the dialyzable and nondialyzable fractions may be compared with the weight recovered. Values for glucosamine and galactosamine are only relative because of the destruction occurring on hydrolysis with 6 N HCl and are considerably lower than the true values (see Clamp and Putnam, 1964). Thus the sum of the glucosamine and galactosamines found in the amino acid analyzer after 6 N HCl hydrolysis as compared with the hexosamine values after 2 N HCl for 2 hours at 100° indicated 32, 56, and 41% destruction for the human A, hog A + H, and human B substances, respectively. These values are comparable to the destruction of up to 50% reported by Clamp and Putnam (1964) for hydrolysis under similar conditions.

[1] Analysis of oligosaccharides for deuterium will be the subject of a future communication.

Results

The data in Table I show that the weights of material recovered were quite good. In the case of the hog mucin and the Beach B substance, in which direct comparisons of alkali with and without borohydride or borodeuteride were made, the extent of the degradations, as evidenced by the amount of dialyzable fragments, was greater with the reducing agent than without it and total recoveries were also somewhat lower. Except in the case of Beach, treated with NaOD, there appeared to be some loss of nitrogen, probably as a result of deamination during alkaline degradation. Recovery of methylpentose was generally equal or slightly higher than recovery of weight, and recovery of galactose was always somewhat lower than the weight recovered.

The most striking findings are in the hexosamine, *N*-acetylhexosamine, glucosamine, and galactosamine values and in the serine and threonine; these two amino acids make up about one-third or more of the total amino acids in the blood group substances (Carsten and Kabat, 1956; Pusztai and Morgan, 1963, 1964). Considering total recoveries, it is evident that for the different preparations only between 48 and 74% of the hexosamine in the original sample was recovered; the *N*-acetylhexosamine recoveries were always higher, ranging from 62 to 86%. Since the *N*-acetylhexosamine color of *N*-acetylgalactosamine is only 31% that of glucosamine, but they give equal colors in the Elson-Morgan hexosamine reaction (see Kabat, 1961), these findings indicate selective destruction of *N*-acetylgalactosamine. This is definitely confirmed by the glucosamine and galactosamine analyses in which recoveries of glucosamine ranged from 75 to 94%, while those of galactosamine ranged from 16 to 59%. Examination of these values for the nondialyzable and dialyzable fractions shows that the destruction was confined largely to the dialyzable fraction as indicated by the greater recoveries of *N*-acetylhexosamine than of hexosamine and of glucosamine than of galactosamine in the dialyzable fractions, only relatively slight differences being found in the nondialyzable fractions. It is also significant that the smallest amount of destruction of *N*-acetylgalactosamine was found for the A substance (MSS) and that more destruction was found with hog mucin which is a mixture of A and H substances, since A activity is associated with terminal *N*-acetylgalactosamine residues which are lacking in H substance. The B substance, which has no terminal nonreducing *N*-acetylgalactosamine, showed the greatest destruction of *N*-acetylgalactosamine, amounting to 92 and 80% for the dialyzable fraction. In the samples treated with reducing agent the values for galactosamine found with the amino acid analyzer might be high to the

TABLE I: Effect of Alkali with and without Sodium Borohydride or Sodium Borodeuteride on the Cleavage and Destruction of Constituents of Blood Groups A, A + H, and B Substances.

	Total Recovery (%)					Nondialyzable (%)					Dialyzable[a] (%)				
Constituent	A MSS A NaOD $NaBD_4$	A + H Hog Mucin NaOH $NaBH_4$	A + H Hog Mucin NaOH Only	B Beach NaOD $NaBD_4$	B Beach NaOD Only	A MSS A NaOD $NaBD_4$	A + H Hog Mucin NaOH $NaBH_4$	A + H Hog Mucin NaOH Only	B Beach NaOD $NaBD_4$	B Beach NaOD Only	A MSS A NaOD $NaBD_4$	A + H Hog Mucin NaOH $NaBH_4$	A + H Hog Mucin NaOH Only	B Beach NaOD $NaBD_4$	B Beach NaOD Only
Weight	93	82	89	80	98	21	12	45	22	38	91	79	78	74	98
N	83	78	74	76	103	30	15	41	37	64	76	73	56	62	108
Methylpentose	94	83	102	81	106	18	6.5	46	15	28	96	82	104	78	107
Galactose	90	73	85	72	87	19	8	44	14	25	87	70	73	68	83
Hexosamine	65	60	74	48	65	15	7.5	41	12	24	58	57	57	40	54
N-Acetylhexosamine	74	73	86	62	70	17	9	46	16	27	70	70	74	55	60
Glucosamine	87	84	94	75	82	20	9	50	16	30	83	83	88	70	75
Galactosamine	53	35	59	16	36	14	9	47	8	20	46	28	24	8	20
Thr	19	26	67	20	39	12	7	60	15	33	8	20	18	7	10
Ser	23	23	39	25	42	13	5	32	17	35	12	19	11	10	12
Asp	88	91	84	61	113	52	20	52	37	89	75	88	66	38	[b]
Glu	89	84	104	83	115	56	21	71	59	91	75	83	108	57	[b]
Pro	88	86	108	75	107	49	22	92	48	91	77	83	210	51	[b]
Gly	88	89	99	82	114	46	18	67	52	90	77	87	98	63	[b]
Ala	140	143	107	134	111	77	34	79	85	89	273	166	132	330	[b]
Val	88	105	97	83	112	52	34	75	55	88	76	107	90	65	[b]
Ileu	80	93	102	88	108	48	22	72	58	88	62	91	100	71	[b]
Leu	87	93	133	88	108	52	27	103	60	88	74	91	≫100	70	[b]
Tyr[c]	102	290	20	53	47	45	40	6	48	47	103	425	15	10	[b]
Phe	87	88	51	87	93	54	27	34	63	93	72	84	25	64	[b]
Lys	85	72	72	91	112	54	21	48	65	91	66	64	46	73	[b]
His	59	62	86	58	80	30	16	58	40	68	42	55	65	31	40
Arg	73	72	31	92	13	45	19	11	38	10	52	65	20	87	5
[cys A]	37	36	31	47	94	21	12	26	32	82	18	29	7	26	67
OH lys		161	85				57	44							

[a] Amount of dialyzable constituent found/(total constituent − constituent in nondialyzable fraction) × 100. [b] The small amounts of amino acids which were dialyzable rendered calculation of per cent recovery in the dialyzable fraction subject to very large errors. [c] The tyrosine contents are very low and may represent impurity.

TABLE II: Destruction of Hexosamine, Galactose, Serine and Threonine, Arginine, and Histidine by Alkaline Degradation and Formation of Other Constituents.

Constituent	A MSS A NaOD $NaBD_4$	A + H Hog Mucin NaOH $NaBH_4$	A + H Hog Mucin NaOH Only	B Beach NaOD $NaBD_4$	B Beach NaOD Only
		Mmoles Destroyed			
Hexosamine	2.4	23.9	1.5	0.68	0.45
Galactose	0.38	11.1	0.74	0.52	0.23
Serine + Threonine	1.58	12.9	0.63	0.51	0.39
Serine	0.60	5.0	0.43	0.19	0.15
Threonine	0.99	7.9	0.20	0.32	0.24
Arginine	0.032	0.37	0.086	0.005	0.045
Histidine	0.04	0.53	0.007	0.014	0.007
		Mmoles Formed			
α-Aminobutyric acid found	0.01	1.8	None	0.04	None
Additional alanine	0.16	1.55	0.014	0.04	0.014
Unknown after valine	0.18	1.78	None	0.065	None
		Ratio Destroyed			
Hexosamine/ (Serine + Threonine)	1.50	1.85	2.40	1.32	1.15
Galactose/ (Serine + Threonine)	0.24	0.86	1.17	1.02	0.59
		Per Cent by Weight			
Nondialyzable	21	12	45	22	38

extent that glucosaminitol or galactosaminitol was formed, since both of those traveled with galactosamine and gave about one-half of its color value per mole. Thus even more galactosamine could have been destroyed than the values indicate.

The data show destruction of up to 80% of the total serine and threonine in the MSS, in hog mucin, and in the Beach materials treated with alkali and borohydride or borodeuteride, and less destruction by alkali alone. Examination of the values for dialyzable and nondialyzable fractions shows that with alkali alone the residual serine and threonine were mostly in the nondialyzable fraction, indicating a lesser degree of degradation of the blood group substance by alkali.

In contrast to the findings with serine and threonine, recoveries of most of the other amino acids were approximately the same as recoveries of weight. In the presence of the borohydride or borodeuteride the alanine recoveries were greater than 100% as a consequence of the reduction of dehydroalanine (Tanaka *et al.*, 1964); a peak for α-aminobutyric acid also was found with the reduced samples (Table II) which represented only 12–22% of the threonine destroyed with the hog A + H and the human B substance; with the human A only 1% of the threonine was α-aminobutyric acid. The additional alanine with reducing agent amounted to one-fifth to one-third of the serine destroyed. Some evidence of destruction of histidine in the presence of reducing agent and of arginine especially in the absence of reducing agent was found; small amounts of ornithine were found in all of the nondialyzable fractions and occasionally in a dialyzable fraction. Histidine and arginine occur in relatively small amounts in blood group substances (Carsten and Kabat, 1956; Pusztai and Morgan, 1963, 1964); the moles destroyed constitute a small fraction of the other constituents destroyed. Values for tyrosine, which is present in extremely small amounts and is probably an impurity, are erratic. The peak which moved in the region of cysteic acid was probably not cysteic acid. An additional peak which traveled behind valine was observed only in samples treated with reducing agent; this was not glucosaminitol or galactosaminitol, both of which traveled in the same position on the chromatogram as galactosamine.

Another important finding with respect to the distribution of amino acids is seen from the data on the nondialyzable fractions. It is evident that those amino acids which are not destroyed are selectively concentrated in the nondialyzable fractions as compared

to the weight distribution or the distribution of the sugars. Thus in MSS, the nondialyzable fraction made up 21% of the weight but contained 52% of the aspartic acid, 56% of the glutamic acid, 49% of the proline, and so on. Similar findings are evident for the other amino acids. The same results are seen for all preparations studied.

However, if the percentages of each of these amino acids in the nondialyzable fractions are compared with the total recoveries of the amino acids in Table I, it is seen that the alkaline borohydride treatment rendered a considerable portion of each amino acid dialyzable. Thus in the case of the B substance treated with sodium borohydride, 59% of the glutamic acid remained in the nondialyzable fraction while the total recovered represented 83% of the amount initially present in the sample. Values for proline were 48 and 75%, those for glycine were 52 and 82%, and so on. Thus from one-fourth to one-half of these amino acids has become associated with dialyzable fragments. On the other hand, the B substance treated with alkali alone showed substantially less fragmentation of amino acid–containing materials into dialyzable fragments. The nondialyzable amino acids ranged from 88 to 91% of the amounts initially used although total recoveries were from 107 to 115%. Similar findings are seen for the other samples.

Discussion

The findings presented here contribute significantly to attempts at formulation of an overall structure of the blood group substances. They confirm the findings of Anderson *et al.* (1964a,b) of β elimination from serine and threonine of sugar residues, indicating that the attachment of the oligosaccharide chains, some or many of which contain the determinants of blood group A, B, or H specificity, to the amino acids is through serine and threonine. Moreover the selective concentration of amino acids relative to weight or of sugar in the nondialyzable fraction indicates that much of the amino acid is in the form of polypeptides of sufficiently large size to be nondialyzable. In all probability however the alkali and the borohydride are also causing some splitting of peptide bonds with the formation of dialyzable peptides (see Crawhall and Elliott, 1955).

Another important finding is the selective destruction of the internal *N*-acetylgalactosamine residues in the blood group substances. The A substance, which contains terminal nonreducing *N*-acetylgalactosamine residues associated with blood group A activity (see Kabat, 1956; Morgan, 1960; Watkins, 1964) and that have been shown to be present in oligosaccharide fragments isolated after alkaline borohydride (Schiffman *et al.*, 1964a; Lloyd and Kabat, 1964), shows less destruction of *N*-acetylgalactosamine than does the A + H mixture, which in turn shows less destruction of *N*-acetylgalactosamine than does the B substance. Neither H nor B substances have terminal nonreducing *N*-acetylgalactosamine as part of their antigenic determinants (see Kabat, 1956; Morgan, 1960; Watkins, 1964), and H activity has been obtained from A substances enzymatically (Iseki and Masaki, 1953) by removal of *N*-acetylgalactosamine (Watkins, 1960, 1962, 1964; see Marcus *et al.*, 1964) or by enzymatic de-*N*-acetylation of terminal *N*-acetylgalactosamine (Marcus *et al.*, 1964).

Whether the *N*-acetylgalactosamine residue which is destroyed by alkali is attached directly to the serine and threonine residues is not clear. While this is a distinct possibility, it is also possible that the serine and threonine are attached to some other sugar residue and that the peeling degradation of the liberated oligosaccharide from the reducing end may occur (see Whistler and BeMiller, 1958) and proceed sufficiently to include the internal *N*-acetylgalactosamine residue. None of the oligosaccharide fragments thus far isolated from alkaline borohydride–treated blood group substances contains an internal *N*-acetylgalactosamine. The isolation in small amounts of free *N*-acetylgalactosaminitol from hog mucin substance and galactitol from human and hog A substances treated with alkaline borohydride (Lloyd and Kabat, 1964) is additional evidence for peeling degradation; if this takes place in the presence of borohydride by the same mechanism as with alkali alone, these substances must have originated from terminal nonreducing ends. Alkaline borohydride or borodeuteride has yielded reduced *N*-acetylamino sugars (acetylated hexosaminitols) which were different from *N*-acetylglucosaminitol or *N*-acetylgalactosaminitol as established by paper chromatography (K. O. Lloyd and E. A. Kabat, unpublished data), and alkali alone has yielded two chromogens (see Morgan, 1956). Formation of these chromogens (Kuhn and Kruger, 1956, 1957) is probably associated with the disappearance of the internal galactosamine on treatment with alkali alone. In the dialyzable portion, for the hog A and H substance, destruction of galactosamine appeared to be only slightly greater with reducing agent, but with the B substance it was considerably greater, amounting to 92% as compared with 80%.

Table II summarizes data on the numbers of millimoles of hexosamine, galactose, and of serine plus threonine destroyed for the various substances. The values for hexosamine are used rather than those for galactosamine, since the latter were obtained after hydrolysis with 6 N HCl for 22 hours while the former were obtained after hydrolysis with 2 N HCl for 2 hours. The hexosamine values therefore are closer to representing the actual values for destruction while the galactosamine values are only relative. The values for hexosamine destroyed, however, would be high to the extent that any glucosamine was lost by alkaline degradation. Similarly the galactose values would also tend to be high insofar as alkaline peeling degradation continued from the reducing end and reached additional galactose units. Considering these limitations, the data in Table II indicate that the molar destruction of hexosamine (galactosamine) ranges from 1.15 to 2.4 times the moles serine + threonine destroyed, while the molar destruction of galactose varied from 0.24

to 1.17 times the moles of serine + threonine destroyed. Although a definite conclusion cannot be drawn as to which of these two sugars is attached directly to the serine and threonine, the finding of three values for galactose substantially less than required from the serine + threonine destroyed, while all of the values for hexosamine destroyed are greater, suggests that the site of attachment is probably an *N*-acetylgalactosamine. Destruction of galactose would then result by the peeling reaction after alkaline degradation and cleavage of the *N*-acetylgalactosamine. The rate of this alkaline degradation relative to the rate of action of borohydride under the conditions used would then determine how much galactose and additional amino sugar was destroyed for each blood group substance. All of the fragments isolated thus far (Lloyd and Kabat, 1964; K. O. Lloyd and E. A. Kabat, unpublished data) contain either a galactitol or the degraded reduced unsaturated residue R, both of which are formed from galactose, thereby accounting for much of the galactose destroyed. Evidence already exists from the reduced oligosaccharide fragments isolated that the two chains involved in the A, B, and H antigenic determinants, based on the isolation from each of two trisaccharides (Cheese and Morgan, 1961; Painter *et al.*, 1963; Schiffman *et al.*, 1962; Rege *et al.*, 1963, 1964), are degraded by alkali to different extents (K. O. Lloyd and E. A. Kabat, unpublished data). It is perhaps also of some interest that the compound β-GNAc-(1→3)-β-Gal-(1→3)-GalNAc has been isolated from A, B, H, and Le^a substances on partial acid hydrolysis (Rege *et al.*,1963). This compound with *N*-acetylgalactosamine at the reducing end could be linked to the serine and threonine. No oligosaccharide with an *N*-acetylgalactosamine between two sugar residues has yet been isolated.

The data in Table II also indicate differences in the susceptibility of the various substances to alkaline degradation. Thus the human A substance showed much more degradation of hexosamine relative to galactose than did the hog A + H or the human B substance. Comparing the effects of the borohydride with those of alkali alone, the hog A + H substances showed relatively little difference in the proportion of hexosamine and galactose to serine + threonine destroyed despite the more extensive total degradation of the molecule with the borohydride as evidenced by the lesser amount of nondialyzable material remaining. With the B substance, however, although the total amount of dialyzable material was considerably greater, the ratio of hexosamine to galactose destroyed was lower when sodium borodeuteride was used.

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Immunogenicity of a Series of α,*N*-DNP-L-Lysines*

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ABSTRACT: A homologous series of α,*N*-DNP-oligo-L-lysines, ranging in size from the tetramer to the nonamer, were prepared from poly-ϵ,*N*-carbobenzoxy-L-lysine ($\bar{n}$ = 5.7) by reaction with dinitrofluorobenzene to substitute the single α-NH_2 position of each chain. The ϵ,*N*-carbobenzoxy groups were removed, the mixture of α,*N*-DNP-oligo-L-lysines was resolved according to peptide chain length by CM-cellulose chromatography, and the separated materials were used for immunization in Hartley, strain 2, and strain 13 guinea pigs.

This homologous series of compounds, because of the chemical definition in both positions of the hapten and peptide chain length, provide obvious advantages over previously used materials in studies attempting to elucidate the chemical basis of immunogenicity. Immunogenicity was observed in Hartley and strain 2 guinea pigs with α,*N*-DNP-hepta-, octa-, and nona-L-lysine, whereas smaller α,*N*-DNP-oligo-L-lysines were not immunogenic. The L configuration and the presence of a hapten were also required for immunogenicity in this system. The same antigen, e.g., α,*N*-DNP-octa-L-lysine, induced the formation of both delayed and immediate sensitivity.

Synthetic polypeptide antigens have provided a powerful tool for studying the chemical basis of the antigenicity of proteins (Stahmann *et al.*, 1955; Sela, 1962, 1965; Maurer, 1964). For the most part, these studies have indicated that homopolymers of α-amino acids are not antigenic, whereas both linear and branched random copolymers or DNP- or other hapten-substituted polymers of amino acids may be antigenic. While the above-mentioned studies have provided important information as to the chemical basis of antigenicity, most studies were performed with materials which, though less complex than proteins, were in themselves still heterogeneous with respect to sequence and chain length.

Advances in the chromatography of proteins have recently been applied to the purification of lysine oligopeptides (Stewart and Stahmann, 1962; Sober, 1962) and now enable one to prepare oligolysines of known chain length (Yaron *et al.*, 1964). These peptides, when conjugated with haptens in a defined position, provide simple, chemically defined molecules whose immunogenicity can be studied.

The studies to be reported in this paper describe the preparation and immunogenic properties of a homologous series of α,*N*-DNP-substituted L-lysine oligopeptides. These materials were prepared so as to preclude antigen heterogeneity, either in peptide chain length or in hapten position, since each member of this series contains a DNP group in the single α-amino position of the oligopeptide; the ϵ-amino groups remain unsubstituted.

Materials

Poly-ϵ,*N*-benzyloxycarbonyl-L-lysine (LY-50) was prepared for us by Yeda, Rehovoth, Israel. The *N*-carboxy-α-lysine anhydride was polymerized in dioxane using butylamine as the initiator. This preparation had an average degree of polymerization ($\bar{n}$) of 5.7 as determined by anhydrous titration with perchloric acid. Poly-ϵ,*N*-benzyloxycarbonyl-L-lysine ($\bar{n}$ = 60) was also prepared for us by Yeda. Poly-ϵ,*N*-benzyloxycarbonyl-D-lysine ($\bar{n}$ = 80) was provided by Dr. M. Sela, Weizmann Institute, Rehovoth, Israel.

1-Fluoro-2,4-dinitrobenzene and triethylamine were obtained from Eastman Organic Chemicals and dimeth-

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