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POLYMERS WITH COMPLEXING PROPERTIES. SIMPLE POLY(AMINO ACIDS)

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16. Abstract

Some properties of simple thermal poly(amino acids) are described. The free amino (0.3 equiv/residue) and carboxyl (0.5 equiv/residue) groups of thermal polylysine increase dramatically on treatment with distilled water. The total hydrolysis of such a polymer is "abnormal" in that only about 50% of the expected amino acids are recovered. Poly(lysine-co-alanine-co-glycine) under usual conditions hydrolyzes completely in 8 hours, whereas when it is pretreated with diazomethane, a "normal" period of 24 hours is required to give (nearly) the same amounts of each free amino acid as compared with those obtained from the untreated polymer. The amino groups of the basic thermal poly(amino acids) are sterically hindered. The existence of nitrogen atoms linking two or three chains and reactive groups (anhydride, imine) are proposed. The latter can account for the observed decrease in total yield of free amino acids by leading, on hydrolysis, to aldehydes and α-keto acids.

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POLYMERS WITH COMPLEXING PROPERTIES. SIMPLY POLY(AMINO ACIDS)

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ABSTRACT. Some properties of simple thermal poly(amino acids) are described. The free amino (0.3 equiv/residue) and carboxyl (0.5 equiv/residue) groups of thermal polylysine increase dramatically on treatment with distilled water. The total hydrolysis of such a polymer is "abnormal" in that only about 50% of the expected amino acids are recovered. Poly(lysine-co-alanine-co-glycine) under usual conditions hydrolyzes completely in 8 hours, whereas when it is pretreated with diazomethane, a "normal" period of 24 hours is required to give (nearly) the same amounts of each free amino acid as compared with those obtained from the untreated polymer. The amino groups of the basic thermal poly(amino acid) are sterically hindered. The existence of nitrogen atoms linking two or three chains and reactive groups (anhydride, imine) are proposed. The latter can account for the observed decrease in total yield of free amino acids by leading, on hydrolysis, to aldehytes and α-keto acids, whose ability to react with free amino groups is known.

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One approach to the solution of the problem of separating different metal ions, or their derivatives, from a mixture in solution consists in the preparation of a substance having suitable complexing properties. Among the substances with complexing properties there are several polymers which are suitable for application with chromatographic techniques. Among these, the proteinoids [1] are known for their complexing and catalytic properties [2]. The chemical nature of these polymers has been only partially studied, and a more extensive investigation is necessary in order to be able to rationalize these properties and to establish their applications. /667*

In this communication, we shall describe the determination of some of the properties of polymers having a basic character, which are obtained as indicated below. In particular, reference will be made to polylysine (PK) and to poly(lysine-co-alanine-co-glycine) (PKAG) [3].

It is known [4] that, when basic proteinoids are prepared, they form cross links owing to the reaction of the ε-amino group of the lysine. It has now been proven by determination [5] of PK that, when it is formed, the major proportion of the amino groups of the lysine react. Indeed, it is found that the number of equivalents of amino groups per residue is of the order of 0.3, while in polylysine prepared by the Leuchs [6] method, this value would be 1.0. On the other hand, the number of equivalents of carboxyl group per lysine residue is 0.5, and is thus very high as compared with a polylysine which contains only peptide linkages. In a structure which takes these results into account, nitrogen atoms (α-N or ε-N) should connect different lysine residues. /668

The behavior due to hydrolysis is anomalous as compared with typical proteinoids [2] or with proteins [3]. By treatment of PK

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*Numbers in the margin indicate pagination in the original foreign text.

dissolved in water for 24 hours at 110° C in a sealed tube, after evacuation, a small quantity of free lysine is formed which represents 0.5% by weight of the original polymer, and at the same time one observes in the remaining polymer, by automated analysis, an increase in the number of free amino and carboxyl groups. This increase amounts to 50% relative to the original polymer (expressed as meq/residue). This result implies the existence of reactive groups such as anhydride and imine groups. This second possibility has not been found to be supported in PK by the formation of ammonia during such treatment.

It is known [3] that the treatment of a protein with 2N HCl for 24 hours at 110° C is sufficient for complete hydrolysis. An analogous treatment applied to PK gave 50% of the theoretical amount of free lysine. The unhydrolyzable components were separated by a combination of filtration with BioGel P-2 and chromatography on carboxymethylcellulose. Their molecular weight is high relative to that of lysine, but their nature has not been studied in more detail.

A sample of PKAG was hydrolyzed with 6N HCl for increasing lengths of time, and the results shown in Table 1 were obtained.

TABLE 1. YIELD OF AMINO ACIDS (mg/g POLYMER) IN THE HYDROLYSIS OF PKAG FOR VARYING LENGTHS OF TIME, AND OF "METHYLATED" PKAG*

	Time (hours)	Lysine	Alanine	Glycine	Total
PKAG	0,5	22,136	26,396	46,860	95,392
	3	395,340	87,536	126,536	609,412
	8	664,628	116,708	179,256	940,592
	10	523,476	88,592	142,156	754,224
	24	509,664	94,070	131,051	734,785
	32	512,962	83,732	122,936	719,630
	96	419,587	57,748	99,444	576,779
PKAG-Me	24	660,128	121,248	182,964	964,340

*Translator's note. Commas in numbers represent decimal points.

Total hydrolysis occurs in 8 hours, and prolonging the treatment leads to lower yields. This fact may be due in part to the formation of aldehydes and/or α-keto acids, particularly glyoxilic acid, during the hydrolysis of PKAG, owing to the reaction of reactive groups such as imines. PKAG treated with diazomethane gives a polymer which is completely hydrolyzed by treatment with 6N HCl for 24 hours at 110° C. This fact does not appear to have any immediate explanation.

The method of Sanger [3] for the determination of N-terminal amino acids, when applied to PK, PKAG, and to other basic polymers, has given negative results. This may be due to the fact that the small number of free amino groups (see above) are in position poorly accessible to 1-fluoro-2,4-dinitrobenzene.

The results presented here indicate the existence of a particular structure of these polymers which clearly differentiates them from the proteins, with which it has been attempted to relate them [2]. The possible formation of thermal polymers on the primitive Earth, and their subsequent evolution into proteins has been debated [7, 8]. These results do not permit any definitive answer to be made to this problem, but they do shed some light on their profound structural differences.

The practical application of the existence of reactive groups with complexing and catalytic properties is now under active study.

Experimental Section

Preparation of thermal polylysine

Lysine (14.6 g) was heated in a nitrogen atmosphere at 176 - 180° C for 5 hours. The resulting mixture (9.6 g) was allowed to cool, water (80 ml) was added, and the insoluble portion (5.2 g) was removed by filtration. The aqueous solution was dialyzed at 0° C for 3 days. The solution was lyophilized, and a slightly colored product (1.6 g) was obtained (thermal polylysine, PK). The product proved to be homogeneous by filtration with BioGel P-6 in a sodium chloride gradient (position of greatest concentration, 23.4 ml). An electrophoresis with pH 1.85 buffer gave R_F 0.75 - 0.85, taking lysine as the reference (R_F 1.00).

Preparation of poly(lysine-co-alanine-co-glycine)

Equimolar quantities of L-lysine (14.6 g), DL-alanine (8.9 g), and glycine (7.5 g) were mixed, and the mixture was heated in a nitrogen atmosphere at 176 - 180° C for 4 hours. During the course of the reaction, water was evolved along with volatile compounds which gave an alkaline reaction to pH paper. The resulting mixture (24.6 g) was allowed to cool and water (180 ml) was added. The insoluble portion (1.4 g) was filtered. The aqueous solution was treated as in the preceding preparation, and finally poly(lys-ala-gly) (PKAG) was obtained. Analysis by electrophoresis under the same conditions as above gave a broad band, R_F 0.00 - 0.68, and indicated the absence of free amino acids.

Treatment of PKAG with diazomethane

A cold ethereal solution of diazomethane, prepared with N,N-nitrosomethylurea (50 mg) and 40% KOH (0.2 ml) (theoretical quantity of CH_2N_2 1/3 mmol) was added to a cold aqueous solution (8 ml) of PKAG (0.5 g), and the mixture was allowed to stand overnight at 0° C. Acetic acid was added to destroy the excess diazomethane. The product could not be extracted with ether. The aqueous solution was concentrated and lyophilized. The residue was soluble in methanol. An aqueous solution of this residue (42 mg) was dialyzed against water (800 ml) for 6 hours. The resulting solution was lyophilized and the residue (PKAG-Me) was hydrolyzed for /669 analysis of its amino acids, which was then carried out by electrophoresis:

	Buffer, pH 6.5	Buffer, pH 1.85
Lysine	1.00	1.00
PKAG	0.20	0.68 - 0.55
PKAG-Me	0.45	0.67 - 0.57

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