the active principle is lecithin (or one of the lecithins), and not lysolecithin or one of the minor contaminants of the preparation.

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Separation of the Aminoethylated A and B Chains of Insulin

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An investigation of the structure of a protein frequently involves the cleavage of one or more disulfide bridges. When tryptophan is absent, as in insulin [1] and ribonuclease,[2] oxidation with performic acid has been used as a convenient tool for cleaving S—S-bonds. Proteins containing tryptophan may, however, after oxidation give undesirable side products. Since tryptophan is normally stable to reduction several investigators have studied the reduction of —S—S— to —SH followed by protection of the sulfhydryl groups. Reduction with mercaptoethanol or thioglycolic acid seems to be the method of choice in this connection.[3] The SH-groups can then be stabilized by alkylation.

The present report describes a convenient method for the separation of the two chains of insulin obtained after reduction and aminoethylation of the SH-groups.

Procedure. The general method of Cavallini *et al.*[4] and Hofman [5] was used.

a) Reduction. The insulin (250 mg = 40 μmoles) was dissolved in 25 ml of 0.05 M tris-HCl-buffer pH 8 which was 8 M with respect to urea in a tube fitted with a three-way stopcock. Air was removed with an oilpump and substituted by argon. Mercaptoethanol (2.5 ml = 40 mmoles) was added and the reaction mixture was again carefully deaerated and left for 24 h at + 2°C.

b) Coupling. β-Aminoethylbromide·HBr (40 g = 200 mmoles) was completely dissolved in the reduction mixture. After 1 h at room temperature the solution was transferred to a water bath and incubated at 37°C for 4.5 h. It was placed in a coldroom over night. During the first 5.5 h the pH was kept at 8 (indicator paper) with 10 N sodium hydroxide. The final volume was 49 ml. The part of the solution which was not chromatographed immediately was stored at —20°C.

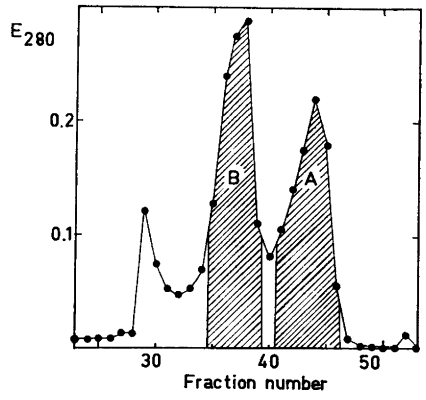

Fig. 1. A chromatogram of the aminoethylated chains from insulin. The shadowed areas were pooled and correspond to the A- and B-chains, respectively. Column: Sephadex G-50, 4 × 100 cm, eluted with 50 % acetic acid; flow rate 25 ml/h, fraction volume 12 ml.

Table 1.

	A			B		
	nitrogen	residues		nitrogen	residues	
	μg	found	theoretical	μg	found	theoretical
α-Aminoethylcystein	59.4	3.30	4	25.5	1.48	2
Lysine			0	17.9	1.04	1
Histidine			0	45.0	1.75	2
Arginine			0	34.6	1.00	1
Aspartic acid	18.4	2.04	2	9.2	1.06	1
Threonine	3.0	0.39 *	0	8.1	0.92	1
Serine	16.4	1.82	2	8.4	0.97	1
Glutamic acid	35.5	3.97	4	26.4	3.06	3
Proline				8.4	0.98	1
Glycine	9.3	1.02	1	25.7	2.98	3
Alanine	6.9	0.77 *	1	18.0	2.10	2
Valine	13.2	1.47	2	25.8	3.00	3
Isoleucine	9.4	1.04	1	0.9	0.10	0
Leucine	18.8	2.08	2	34.2	3.97	4
Tyrosine	17.4	1.94	2	15.6	1.81	2
Phenylalanine				23.7	2.76	3
Ammonia	55.4	6.48	4	30.0	3.50	2
Sum	263.1			356.9		
Kjeldahl analyses	300			404		

* The insulin used in this investigation was a commercial mixture from ox and pig.

c) *Separation.* 10 ml of the reaction mixture were chromatographed on a column of Sephadex G-50 (4 × 100 cm) with 50 % acetic acid. A typical chromatogram is given in Fig. 1. The peaks were pooled according to the figure and lyophilized. In three such experiments the recovery from 10 ml of reaction mixture was between 11.4 and 12.9 mg of the A-chain derivative and between 16.6 and 18.4 mg of the B-chain derivative. Efforts to separate the aminoethylated chains from insulin on Sephadex G-50 in 0.2 N acetic acid and on Sephadex G-25, Biogel P-4, or P-6 in 0.2 N or 50 % acetic acid gave no or only partial resolution.
d) *Characterization of the purified chains.* 1.99 mg of the aminoethylated A-chain and 2.43 mg of the aminoethylated B-chain, respectively, were hydrolyzed with constant boiling hydrochloric acid at 110°C for 20 h and chromatographed on a Beckman Amino Acid Analyser 120 B.[6] Table 1 shows that the amino acid analyses for the separated A- and B-chain are in good agreement with the expected values.

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