Short Communications

On the composition of sheep thyrotropic hormone

Recent communications[1-3] from this laboratory have described the purification and composition of bovine thyrotropic hormone. Starch-gel electrophoresis experiments revealed six biologically active components in purified beef material, two of which were isolated by careful rechromatography on the anion exchanger, diethylaminoethylcellulose. The amino acid compositions of the two isolated thyrotropins were almost identical and a preparation consisting only of active components was shown to contain mannose, fucose, glucosamine and galactosamine. Sheep and whale thyrotropin preparations exhibited chromatographic behavior on diethylaminoethylcellulose similar to that of beef. Several active components were again recognized and approximately the same molecular weight was indicated for the hormones from each species. This paper reports on the composition of hydrolysates of sheep thyrotropin preparations which have been further purified by chromatography on the cation exchanger, carboxymethylcellulose.

The experimental procedures for preparation of the hormone, bioassay, chromatography on the celluloses, starch-gel electrophoresis, and analysis for amino acid and carbohydrate content are as previously described[1-4]. In general, the yields in terms of weight and total units were less than with the beef preparations. Material with a potency of 10–15 U.S.P. units/mg was obtained, a potency similar to that reported by ELLIS[5]. 9 kg of whole sheep pituitaries yielded 2.2 g of 5 unit/mg material which, after chromatography on diethylaminoethylcellulose, yielded two active fractions which emerged from the columns following the bulk of the inactive material (see ref. 3, Fig. 1b). The elution curves from carboxymethylcellulose chromatography and the starch-gel patterns of the sheep material were closely parallel to those reported for beef[2]. The rechromatography on carboxymethylcellulose of the active fraction most strongly retained on diethylaminoethylcellulose yielded the best preparation. From a total of 55 mg (pooled from two runs through the anion exchanger) 21 mg (Sample 1) were obtained from the cation exchanger. A column 1.9 × 17 cm, at pH 6.3 was employed, with a gradient to 0.1 *M* Na^+ concentration applied over 500 ml after emergence of the first peak (inactive). Starch-gel electrophoresis at pH 9.5 showed Sample 1 to consist of essentially only two components, both of which were demonstrated to be biologically active.

Additional material was obtained by rechromatography, under the same conditions, of the active fraction* first emerging from the anion-exchange columns[3]. The rechromatographed material (10 U.S.P./mg), although consisting chiefly of active components, showed some impurities on starch-gel electrophoresis (Sample 2).

* It is of interest to note that an impurity was found in this fraction, which was indistinguishable from the active material by starch-gel electrophoresis at pH 9.5. It was recognized by a different mobility in electrophoresis at pH 5.0 and emerged from the carboxymethylcellulose columns before the active material, shortly after application of the gradient to 0.1 *M* Na^+ concentration.

Because of excessive destruction during hydrolysis prior to amino acid analysis, a third sample was prepared by dissolving a portion of Sample 2 in water and passing it through a column of dextran gel (Sephadex G-25, Pharmacia, Uppsala, Sweden) in order to remove any residual buffer salts[6]. Simultaneously some of the contaminating impurities of Sample 2 were also removed as two protein peaks were found. The second peak to emerge contained the biological activity (Sample 3). Sample 3 showed 2 bands with the expected mobility in starch-gel electrophoresis at pH 9.5. Based on the amount of ammonia present in its hydrolysates, less destruction occurred during hydrolysis of Sample 3.

The carbohydrates of the sheep thyrotropins were found to be the same as for beef, *i.e.*, mannose was the predominating hexose detected by paper chromatography and a weaker but definite spot with the mobility of fucose was also present.

TABLE I

AMINO ACID COMPOSITION OF SHEEP THYROTROPIN PREPARATIONS COMPARED TO BEEF THYROTROPIN *b*

	No. of residues based on a mol. wt. of 28,000				*No. of residues based on a mol. wt. of 28,000**			
	Sheep preparations		*Beef thyrotropin* b		*Sheep preparations*		*Beef thyrotropin* b	
	*Found**	*Arbitrarily corrected***	*(ref. 1)*		*Found*	*Arbitrarily corrected***	*(ref. 1)*	
Aspartic acid	11.4; 11.2	14.4	14.4	Isoleucine	5.6; 5.4	7.0	6.8	
Threonine	12.2; 13.0	16.0	15.6	Leucine	7.2; 7.2	8.5	8.2	
Serine	8.3; 8.2	10.5	10.4	Glucosamine	3.4; 1.9	3.3	4.2	
Proline	13.2; 13.9	17.2	14.7	Galactosamine	0.8; 0.8	1.0	1.6	
Glutamic acid	12.3; 11.1	14.9	14.6	Tyrosine	8.7; 7.6	10.4	10.3	
Glycine	8.3; 9.0	11.0	15.3***	Phenylalanine	5.1; 5.1	6.5	6.3	
Alanine	10.0; 11.5	13.6	12.3	Ammonia	25.0; 17.8	27.2	21.2	
Valine	7.9; 8.8	10.7	11.0	Lysine	10.6; 10.6	13.4	14.8	
Half cystine	10.9; 12.6	14.9	16.6	Histidine	3.6; 3.7	4.6	4.8	
Methionine	4.1; 3.7	4.9	4.9	Arginine	5.5; 6.1	7.3	7.3	

* Values not corrected for moisture or ash. The results were obtained from Sample 1 (left) and Sample 3 (right). A third analysis on another portion of Sample 3 gave similar molar ratios of constituents but recovery could not be calculated because of loss of a small amount of the sample after weighing.

** The results have been arbitrarily corrected to give the same aspartic acid content as in beef thyrotropin in order to directly compare molar ratios of constituents in the two.

*** Free glycine was found in the beef preparations isolated from chromatography in glycine buffers. A value of 11 residues was found for a beef thyrotropin preparation chromatographed in phosphate buffer[2].

Both the anthrone method and an orcinol method[7] indicated the total hexose content of Sample 1 to be 5–8 % and of Sample 2 approx. 5 %. As sufficient material was not available for ash determinations results are not corrected for ash or moisture. In the amino acid analysis two peaks were detected which were ascribed to glucosamine and galactosamine. Material from each peak gave an Elson-Morgan reaction.Table I shows the results of the amino acid analysis together with those for the hexosamines. The values for the latter are low due to destruction during the hydrolysis prior to amino acid analysis[1,2]. The data in Table I show that the ratios of the amino acids found in the sheep thyrotropin are almost identical with those of beef, thus indicating

very similar composition for the hormones from the two species. The recovery of residues of amino acids together with the hexose and hexosamine accounts for 70 % of the weight of sample; with beef material recoveries of 80–85 % were obtained (corrected for moisture and ash[1]). Although the low recoveries may be attributed to losses during hydrolysis the possibility of an unrecognized component in the thyrotropins exists. Analysis of other samples of sheep thyrotropins for sialic acids and tryptophan indicates that the two are not constituents of the molecule.

GRÖSCHEL AND LI[8] have recently reported on the carbohydrates of ovine follicle-stimulating hormone and interstitial-cell-stimulating hormone. In comparing the carbohydrate content of ovine thyrotropin with these hormones, a difference in hexoses is found in that the two gonadotropins contain galactose in addition to mannose; galactose is absent in the best preparations of sheep thyrotropin analysed. The 2.44 % sialic acid found in the follicle-stimulating hormone[8] is consistent with the differences in its electrophoretic behavior as compared to thyrotropin[2].

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Purification of chorionic gonadotropin from the urine of patients with trophoblastic tumors

Human chorionic gonadotropin can be detected in the blood and urine of pregnant women and also, in much larger amounts, in the blood and urine of patients* with such trophoblastic tumors as choriocarcinoma, hydatid mole, chorioadenoma destruens, and syncytial endometritis[1]. The gonadotropin titer of these patients can be used as an index of tumor growth or regression. Whereas the hormone from pregnancy urine has been purified and characterized[2], it has not been concentrated or purified thus far from the urine of patients with malignant tumors. We wish to report here

Abbreviations: Tris, tris(hydroxymethyl)aminomethane; DEAE-, diethylaminoethyl-.
* These patients are referred to collectively in this paper as "tumor patients".