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The isolation from wool of a readily extractable protein of low sulphur content

The protein solutions obtained by extracting wool with alkaline solutions of potassium thioglycollate contain several components. One of these, kerateine 2, was obtained in a relatively pure state by fractional extraction from wool[1,2] and was further purified after conversion to the S-carboxymethyl derivative[3,4], SCMK2.

The isolation and purification of a second protein component of reduced wool has now been achieved by the following procedure: solvent-scoured wool top (100 g air–dry weight) was extracted at 50° for 2 h in 3 l 0.1 *M* potassium thioglycollate, pH 10.5, and the proteins precipitated at 20° and pH 5 using acetic acid. The precipitate was redissolved in 1 l 0.1 *M* potassium thioglycollate, pH 8.5, and coupled with iodoacetate at this pH using a two-fold excess. The pH was then adjusted to 6 and the solution dialysed. Electrophoresis in glycine-NaOH buffer at pH 11 and ionic strength 0.1 indicated the presence of at least 3 components (Fig. 1a).

On acidification about two-thirds of the protein precipitated between pH 4.5 and 4.1 and did not redissolve at lower pH values. The remainder of the protein almost completely precipitated at pH 2.9. A sharp separation could thus be effected by precipitation at pH 4.1. The electrophoretic patterns of the precipitate and supernatant obtained at this pH are shown in Figs. 1b and 1c respectively.

Fig. 1 a–d. Ascending electrophoretic patterns of wool protein fractions run in 0.1 ionic strength glycine–NaOH buffer at pH 11.

The supernatant fraction contained at least 4 components which were not further fractionated. It had a high sulphur content, containing about 6.5% compared with 3.5% in whole wool[5], and if present entirely as S-carboxymethyl cysteine (SCMC) residues, this amino acid would constitute at least 30% of the protein fraction.

The fraction precipitating at pH 4.1 also contained at least 4 components. The component corresponding with the main peak (2), which accounted for about 70% of the total area, was separated by precipitation at —5°, pH 7, and ionic strength 0.01 with acetone at a concentration of 50% (v/v). Two re-precipitations under identical conditions gave a protein which moved with a single boundary on electrophoresis at all pH values from 8.5 to 11 at a protein concentration of 1%. Electrophoresis was difficult below pH 10 because of the high turbidity of the protein solution. Fig. 1d shows a typical pattern obtained at pH 11. Ultracentrifuge runs at pH 11 in 0.1 ionic strength glycine–NaOH buffer containing also 0.2 *M* NaCl gave one peak, the s_{20w} being 2.5 at a protein concentration of 0.9%. Ultracentrifuge runs showed only one component but light-scattering measurements indicated the presence of large aggregates which continually accumulated on standing at room temperature. Of the total protein nitrogen, 3.75% was in the form of SCMC and 0.5% as cystine. In addition there was about 1% of combined sulphur in an unknown form.

The S-carboxymethyl protein derivative obtained by the above process and SCMK2 are similar as regards their electrophoretic mobilities ($7.2–7.8 \cdot 10^{-5}$ and $7.0–7.9 \cdot 10^{-5}$) sedimentation coefficients ($2.5 \cdot 10^{-13}$ and $3–4 \cdot 10^{-13}$) and SCMC content (3.75 and 3.53%) though the latter two parameters differ significantly. The new protein also differs from SCMK2 in its greater ease of salting out at pH 6 with $(NH_4)_2SO_4$ (0.17–0.19 *M*, *cf.* 0.3–0.4 *M*), NaCl (1.0–1.2 *M*, *cf.* 1.0–2.0 *M*), and sodium acetate (0.18–0.20 *M*, *cf.* 0.3–0.4 *M*). The pH for 50% precipitation is also lower (4.6–4.7, *cf.* 4.9–5.1). Preliminary analysis by Dr. D. H. SIMMONDS shows them to have a similar but significantly different amino acid composition.

It has been shown[6] that the wool proteins most readily extracted by thioglycollate, including the new protein, originate in the orthocortex while those which are difficult to extract, including kerateine 2, originate in the paracortex. The similarity between these two proteins suggests that they may have a similar function in the architecture of the two segments. They account for 30 to 40% of the fibre and have a relatively low sulphur content. Much of the sulphur is concentrated in the small fraction precipitating at pH 2.9 and this may constitute part of the high-sulphur interfibrillar matrix[7].

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Incorporation of hydropyrimidine derivatives in ribonucleic acid with liver preparations

The biosynthesis of pyrimidine nucleotides, via orotate, has been clarified mainly by KORNBERG and co-workers[1]. Evidence was drawn principally from bacterial preparations, although animal tissues seem capable of using the same pathway[2]. CANELLAKIS[3,4] found that uracil is incorporated into mammalian RNA* but there is some disagreement on the relative importance of pyrimidine precursors of RNA[1,4].

The interconversion of pyrimidines and carbamyl-aminoacids via hydropyrimidines in animal tissues has been presented previously[5,6]. We are evaluating the relative contributions of the orotate and the hydropyrimidine pathways to RNA synthesis in the tissues of several animal species; this note presents evidence that the intermediates related to the hydropyrimidine pathway are incorporated into RNA. The relative activities shown in Tables I and II seem to exclude the mediation of orotate. This is supported by experiments showing that there is no direct interconversion between orotate and the hydropyrimidine derivatives. These enzymic mechanisms are under investigation and several of the isolated enzymic systems are being characterized.

TABLE I

INCORPORATION OF OROTATE AND CARBAMYL-β-ALANINE INTO RNA BY LIVER FRACTIONS OF SEVERAL SPECIES

Preparation	*Precursor*		*Preparation*	*Precursor*	
	Orotate	*C-β-alanine*		*Orotate*	*C-β-alanine*
Dog, Sup. F	19.1	3.9	Chicken, FI	4.5	2.4
Rat, Sup. F	4.8	3.2	Chicken, FII	3.8	0.4
Pigeon, Sup. F	22.0	3.9	Chicken, FIII	33.6	1.9
Chicken, Sup. F	11.5	4.2	Chicken, FIV	4.1	7.7

The incubations contained the following in 3 ml: 1 mg muscle preparation[7], enzyme preparation (about 100 mg protein containing RNA, total RNA made up to 6 mg with added RNA),

* The following abbreviations are used in this paper; RNA, ribose nucleic acid; Sup. F, supernatant fraction, FI, II, III and IV, Fractions I, II, III and IV; C-β-alanine, carbamyl-β-alanine; CβARP, carbamyl-β-alanine ribotide; HUMP, hydrouridine-5'-phosphate; UMP, uridine-5'-monophosphate; CβAR, carbamyl-β-alanine riboside.