

Role of Nucleolar Ribonucleic Acid in Incorporation of Ribosomal Amino-acid

It is known that incorporation of amino-acids into protein by ribosomes is dependent on RNA, and thus sensitive to RNase¹. It is believed that this incorporation requires a 'messenger RNA', which may have a nucleolar origin².

To test this hypothesis we measured the effect of nucleolar RNA, prepared according to Allfrey *et al.*³, on ribosomes treated with RNase (they lost 75 per cent of their RNA this way).

Table 1 gives the results of 12 experiments, which show that incorporation of either ¹⁴C-glycine or ¹⁴C-valine is enhanced by the addition of nucleolar fraction. Pre-treatment of this fraction with RNase destroyed its activity.

Table 1. EFFECT OF NUCLEOLAR RNA ON AMINO-ACID INCORPORATION

No.	Ribosome	Nucleolar fraction	Ribosome + nucleolar fraction	Ribosomes + RNase-treated nucleolar fraction
1	72	3	844	
2	21	100	320	
3	158	157	398	
4	20	41	474	
5	190	229	1,308	
6	223	246	679	
7	480	507	2,322	
8	383	448	935	383
9	269	333	821	369
10	321	234	652	
11	280	756	512	242
12	26	55	121	102

To 0.5 ml. of an incubating mixture containing 2 μ mol. of ATP, 0.25 μ mol. of GTP, 0.04 μ mol. (90,000 c.p.m.) of ¹⁴C-glycine or ¹⁴C-valine (exps. 10, 11, 12), 10 μ mol. of phosphoenolpyruvate, 12 μ mol. of *tris* buffer pH 7.5 and 50 μ g of crystalline pyruvate kinase, we added 6.5 mg of ribosomal protein (ref. 1) (in 0.5 ml. of *tris* buffer pH 7.5, 0.045 M), 1.5 mg of pH 5 enzyme (in 0.25 ml. of sucrose 0.44 M) and 0.4 mg of nucleolar RNA fraction (ref. 3) (in 0.5 ml. of 0.44 sucrose). The mixture was incubated for 2 h at 30° C and the reaction stopped by adding 1 ml. of cold 10 per cent TCA. Precipitated proteins were washed by the procedure of Siekevitz (ref. 8), modified by washing for 1 h at room temperature in N sodium hydroxide.

These results agree with Sibatani, de Kloet, Allfrey and Mirsky's hypothesis². As a matter of fact Perry⁴ demonstrated that some nucleolar RNA migrates to ribosomes. Recent papers⁵⁻⁷ have shown that synthetic polyribonucleotides can stimulate amino-acid incorporation into pre-incubated microsomes, and it is reasonable to assume that the latter are depleted of active RNA.

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Prekeratin

FIBROUS keratin is formed in the germinal or differentiating cells of keratinizing tissues and appears in the cytoplasm as birefringent fibrils. The fibrils are rich in sulphhydryl groups, and electron microscopy shows that they consist of long filaments with a diameter of about 60 Å (refs. 1-3). The molecular structure of prekeratin as revealed by X-ray diffraction appears to be similar to that of keratin^{4,5}. Since the α -pattern of prekeratin is readily

disoriented by concentrated urea solution⁶, it seems that the molecular framework is primarily held together by hydrogen bonds; disulphide linkages are absent.

Only a few investigators have examined the properties of prekeratin *in vitro*. Rudall found that the buffered aqueous solvents commonly used for the extraction of proteins are unsatisfactory for the extraction of prekeratin. He noted, however, that copious amounts of protein can be removed from the epidermis with concentrated urea solution. In view of this, Rudall⁴ selected 6 M urea solution for the extraction of prekeratin (epidermin) from the epidermis of cow's nose, and Rogers⁵ used 8 M urea solution for the isolation of proteins from the wool root. Analyses of the extracts thus obtained showed that they contain globular-type proteins in addition to the precursor of fibrous keratin. Complete separation of these globular proteins from prekeratin has proved very difficult^{6,7}. The partially purified prekeratin preparations were commonly found to have a relatively low sulphur content; furthermore, the bulk of the sulphur present was in the —SH form. The average molecular weight of the preparations was found to be in the range of 60,000-100,000, thus indicating that urea splits the macromolecules of prekeratin into relatively small units. So far as the shape and molecular structure of the isolated prekeratin are concerned, it appears significant that dry films of the preparations prepared from the urea extracts were anisotropic in polarized light and showed an α pattern in X-ray diffraction investigations^{4,6}.

The purpose of this communication is to report a new isolation technique by which pure prekeratin can be obtained from the epidermis of the cow's nose. This prekeratin has a high molecular weight and can be readily spun into fibres. For the isolation 0.1 M citric acid-sodium citrate buffer (CASC) of pH 2.6 and ionic strength of 0.6 was used.

After thorough washing of the cow's noses in running tap water, the epidermis is removed in sheets 0.5 mm thick with a keratome (obtained from the Storz Instrument Co., St. Louis, 10, Missouri). The epidermal sheets are collected in a beaker, and, after addition of a few millilitres of CASC buffer, are cut into small pieces with scissors. The minced epidermis is afterwards suspended in CASC buffer in the ratio of 1 : 5 and homogenized in the 'VIR-TIS' apparatus for 4 min. The homogenate is then filtered through gauze and centrifuged for 10 min at 3,500 r.p.m. The resulting supernatant is centrifuged for 30 min at 20,000 r.p.m. The supernatant of this centrifugation is then diluted 1 : 1 with CASC buffer and the pH adjusted to 7 by the addition of 1 N sodium hydroxide drop by drop and constant stirring with a magnetic bar. The first precipitates of prekeratin appear at about pH 3.6. The large, sticky fibrous precipitates formed at pH 7 have a tendency to fuse and form an insoluble clot. It is advisable, therefore, to decant the supernatant fluid as soon as the precipitates settle and immediately to add fresh CASC buffer to the precipitate. After adjustment of the pH to 2.6 with 0.1 N hydrochloric acid, the solution is stirred until all the precipitates are dissolved and the solution becomes clear. For further purification, prekeratin is serially reprecipitated at pH 6, pH 5 and finally at pH 4.5. The precipitate thus obtained is less coherent than that previously obtained at pH 7 and may, therefore, be collected by brief centrifugation at 600 r.p.m. The final preparation is dialysed against CASC buffer and cleared by high-speed centrifugation for 1 h. By this procedure approximately 1 g prekeratin may be extracted from the epidermis taken from the noses of 20 cows.

The purity of prekeratin preparations was determined by moving-boundary electrophoresis and by sedimentation in the Beckman-Spinco analytical ultracentrifuge. The preparations appear monodisperse on electrophoresis in CASC buffer (Fig. 1) and form a single component on ultracentrifugation (Fig. 2). The sedimentation coefficient of prekeratin, corrected to water and extrapolated to