TRANSLATION OF TYPE I AND TYPE II PROCOLLAGEN MESSENGERS IN A CELL-FREE SYSTEM DERIVED FROM WHEAT GERM

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1. Introduction

Analyses of collagens from various tissues have demonstrated the existence of four genetically distinct collagen molecules which have been designated Types I–IV (for review see ref. [1]). Best characterised of these types are the Type I collagens from tissues such as skin, bone and tendon having the chain composition $(\alpha 1\ I)_2\alpha 2$ and the Type II collagens from cartilagenous structures having the chain composition $(\alpha 1\ II)_3$. Biosynthetic data have established that both collagen Types I and II are synthesised in a precursor form (procollagen) in which the pro-α chains are approximately 25% larger than their respective α chains because they have additional extensions at their amino termini (for reviews see refs. [2,3]). Recent studies from this laboratory have been concerned with the assembly of these procollagens in matrix-free cells released from embryonic chick tendon and sternal cartilage [4–7]. We recently reported that in tendon cells the major mRNA species is of a size appropriate to presumptive procollagen mRNA [8] and we now present evidence that mRNAs extracted from tendon and cartilage cells can direct in a cell-free system the synthesis of polypeptides having the characteristics of procollagen Type I and procollagen Type II, respectively.

2. Experimental

Phenol chloroform and isoamyl alcohol were used to extract RNA [9] from cells isolated from leg tendons [10] and sternal cartilage [11] of 17-day-old chick embryos. The RNA was precipitated with cold ethanol and mRNAs therein were examined for their ability to direct the synthesis of procollagen in a cell-free system derived from wheat germ. The wheat germ extract was prepared according to the method of Roberts and Paterson [12] except that the preincubation step was omitted.

Standard protein synthesis assays contained in a final volume of 50 μl, 20 μl of a 30 000 $\times$ *g* supernatant of wheat germ, 20 mM HEPES (pH 7.6), 2 mM dithiothreitol, 1 mM ATP, 20 μM GTP, 8 mM creatine phosphate, 40 μg/ml creatine phosphokinase, 25 μM unlabelled amino acids excluding proline, KCl, magnesium acetate and [^{14}C] proline as indicated. Reactions were incubated for up to 90 min at 25 °C and the syntheis of [^{14}C] proline-labelled protein was determined using the trichloroacetic acid precipitation technique [12]. That the polypeptides synthesised were in part collagenous was demonstrated by digestion of an aliquot of the reaction mixture with a highly purified bacterial collagenase having negligible proteolytic activity towards a [^{3}H] tryptophan-labelled substrate [13]. Determination of molecular size of the synthesised [^{14}C] polypeptides was carried out by SDS-polyacrylamide gel electrophoresis [14].

3. Results

The ability of cytoplasmic RNA extracted from 20×10^9 tendon cells to act as template for the synthesis of labelled protein in a heterologous cell-free system was examined. Initial experiments were designed to establish optimal concentrations of Mg^{2+} and K^+ for the incorporation of [^{14}C] proline into TCA-precipitable polypeptides. The results in

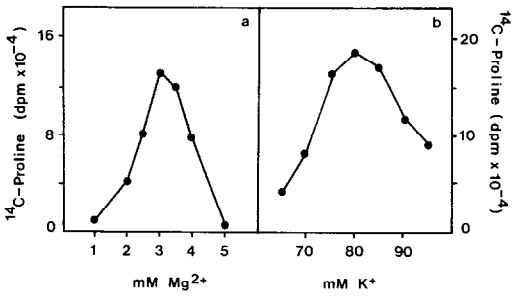

Fig.1. Determination of optimal ionic conditions for the translation of tendon RNA. Protein synthesis assays containing 15 μg tendon RNA and 5 μCi [^{14}C]proline were conducted for 60 min as described in the text. (a) Influence of potassium concentration at 3 mM Mg^{2+}; (b) influence of magnesium concentration at 80 mM K^+.

fig.1 indicate K^+ and Mg^{2+} optima of 80 mM and 3 mM, respectively, when using the tendon RNA extract. Similar results were obtained when protein synthesis was directed by 5 μg of globin mRNA (Searle Res. Labs., High Wycombe, Bucks., U.K.). Under these optimal conditions the extent of incorporation of [^{14}C]proline was directly proportional to the amount of tendon RNA added to the cell-free system (fig.2a). A time course of [^{14}C]proline incorporation demonstrated a linear relationship for up to 75 min at which point the incorporation was approx. 120 times greater than control (Fig.2b).

In order to assess whether the tendon RNA was directing the synthesis of collagenous polypeptides

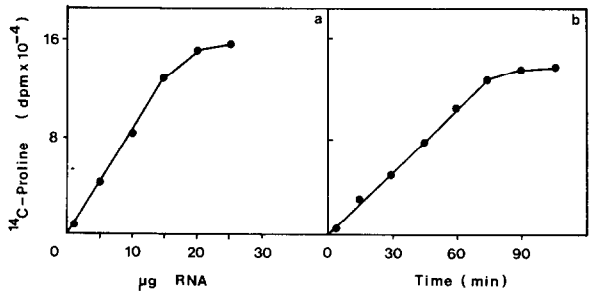

Fig.2. Characteristics of protein synthesis directed by tendon RNA. Protein synthesis assays contained 3 mM Mg^{2+}, 80 mM K^+ and 5 μCi [^{14}C]proline. (a) Influence of added RNA on [^{14}C]proline incorporated in 60 min into TCA-precipitable protein, (b) time course of protein synthesis directed by 15 μg RNA. Incorporation due to endogenous activity has already been subtracted.

the [^{14}C]proline-labelled products of the cell-free system which included 3 mM Mg^{2+} and 80 mM K^+ were digested with 3 μg highly purified bacterial collagenase in 1 ml 0.02 M Tris–HCl buffer (pH 7.6) containing 5 mM $CaCl_2$ for 2 hr at 37 °C. Fifty-five per cent of the radioactivity incorporated became dialysable following collagenase treatment suggesting that the tendon RNA extract contained collagen mRNA. However, analysis of the translational products by SDS-polyacrylamide gel electrophoresis revealed the absence of ^{14}C-polypeptides greater than 60 000 daltons indicating that although procollagen translation was being initiated, completion of the polypeptide chains was not achieved under these conditions.

Previous studies have indicated that procollagen is the major protein synthesised by tendon cells [10] and that the major mRNA species in these cells appears to have some of the anticipated characteristics of procollagen mRNA [8]. Therefore, further experiments were carried out to determine the conditions which would give levels of collagen synthesis nearer to those observed with intact cells and also yield complete pro-α chains. A major increase in the synthesis of collagenase-susceptible polypeptides was observed when the concentration of K^+ was increased to 150 mM (Fig.3).

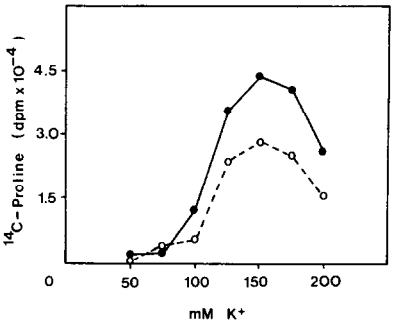

Fig.3. Influence of K^+ concentration on the synthesis of collagenase-susceptible [^{14}C]proline-labelled polypeptides. Protein synthesis assays containing 3 mM Mg^{2+}, 15 μg tendon or cartilage RNA, 5 μCi [^{14}C]proline and increasing K^+ concentrations were incubated for 60 min at 37 °C. The sample was dialysed exhaustively against water prior to collagenase digestion. Collagenous peptides were recovered after dialysis against two changes of 25 ml H_2O and the diffusate was concentrated and counted as described previously [6]. ●——●, tendon RNA; ○- - -○, cartilage RNA.

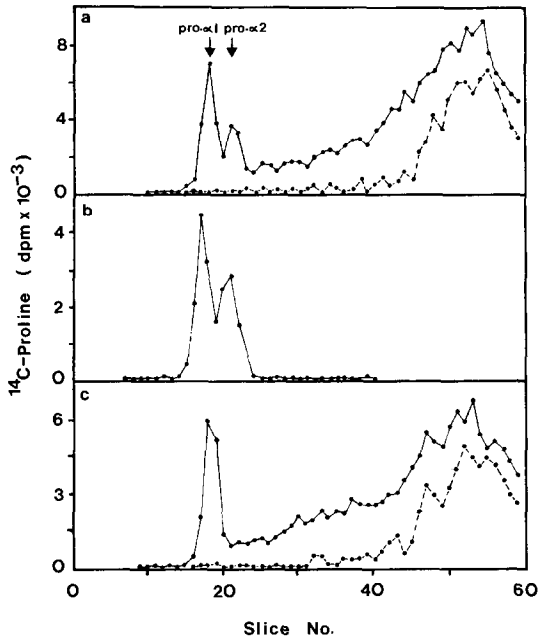

Fig.4. SDS-polyacrylamide gel electrophoresis of the products of cell-free synthesis. Protein synthesis assays containing 3 mM Mg^{2+}, 150 mM K^+, 15 μg RNA and 10 μCi [^{14}C]proline were incubated for 60 min. Samples were treated with SDS and mercaptoethanol [14] and applied to 5% polyacrylamide gels. Electrophoresis was for 4 hr at 8 mA/tube after which gels were sliced and counted. (a) Products of tendon RNA; (b) standard of tendon procollagen secreted by chick tendon cells [10]; (c) products of cartilage RNA. ——, translational product, - - -, translational product collagenase-treated.

When the tendon RNA translation products obtained with the higher K^+ concentration were analysed by SDS-polyacrylamide gel electrophoresis two labelled peaks of high molecular weight were observed (Fig.4a). These two peaks which were collagenase-susceptible contained [^{14}C]proline in the ratio of approx. 2:1 (Fig.4a) and were identified as pro-α1 and pro-α2 chains on the basis of their identical mobility to standards of chick tendon pro-α chains (Fig.4b).

In parallel experiments in which cytoplasmic RNA extracted from cartilage cells was used to direct protein synthesis in the wheat germ system a K^+ concentration of 150 mM was again found to be optimal for collagen synthesis (Fig.3). Only one major high molecular weight peak of protein was observed on SDS-polyacrylamide gel electrophoresis when the system was programmed with cartilage RNA (Fig.4c). This peak had the same mobility as cartilage pro-α1(II) chains (not shown) and its collagenous nature was demonstrated by its susceptibility to bacterial collagenase

4. Discussion

The data presented here provide the first demonstration of the synthesis of intact tendon and cartilage pro-α chains in a heterologous cell-free system. Although [^{14}C]proline incorporation was maximal in the presence of 80 mM K^+, the synthesis of pro-α chains required the presence of K^+ at a higher concentration (150 mM). This result suggests that the ribosomal stability necessary for the successful translation of long mRNAs such as procollagen mRNA is enhanced by the elevated K^+ concentration. Support for this conclusion comes from recent studies with encephalomyocarditis viral RNA (45S) in a Krebs II ascites cell-free system which also demonstrated the requirement for an increased K^+ concentration for complete translation [15]. This observation may explain the results of a previous report [16] in which the translation of collagen mRNA from chick embryo calvaria yielded incomplete procollagen polypeptides in a cell-free system derived from Krebs II ascites cells.

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