Table 4. Effect of temperature on the lipid accumulation and fatty acid composition of *A. niger* AS-101[a]

Temperature (°C)	Biomass (g/l)	Lipid (%)	Relative fatty acid composition[b] (%) 16:0	18:0	18:1	18:2	18:3	UI[c]
20	1.4	12.2	8.6	6.2	26.7	50.0	3.9	1.38
25	1.8	14.5	7.2	4.9	25.5	53.5	4.8	1.47
30	2.0	15.4	10.0	4.5	25.1	54.0	4.2	1.46
35	1.6	11.9	7.5	5.9	24.1	51.4	4.0	1.26

[a] Values are the mean of three replicates. Bagasse (10 g/l) and NH_4Cl (400 mg N/l) were the carbon and nitrogen sources, respectively. [b] Small amounts of other fatty acids, 14:0, 16:1, 17:0 and 20:0 were also detected. [c] Unsaturation index.

from 18:1 to 18:2[19]. The intracellular NH_4^+ concentration has also been reported to affect the regulation of lipid biosynthesis in yeast[19].

Influence of temperature. A. niger AS-101 was grown at 20, 25, 30 and 35 °C in a medium containing bagasse and NH_4Cl as carbon and nitrogen sources, respectively. The mold grown at 25 °C and 30 °C exhibited almost comparable values for lipid content and fatty acid composition (table 4). The yields were decreased when growth was below 25 °C or above 30 °C. This suggested an optimum temperature range of 25–30 °C for culturing this strain, to achieve a maximum lipid yield from cellulose. Earlier observations showed a similar temperature range for the maximum production of cellulase enzyme by this strain[3,4].

Aspergillus niger AS-101 grown on various carbon sources including natural cellulosic residues was able to accumulate lipids to a level of about 15%. An average fatty acid profile showed reasonable agreement with published values from non-cellulolytic strains of this organism. This ability indicates the possibility of using this organism for the conversion of widely-available lignocellulosic materials into single cell oil in addition to single cell protein.

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Age- and sex-related differences in the content of prothymosin α in rat tissues

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Abstract. Differences in the tissue content of prothymosin α during the early postnatal development of male and female rats are reported. Thymus and spleen have been found to contain significantly higher amounts of prothymosin α in the newborn and prepubertal animals, as compared to adults, whereas liver has been found to contain low levels of prothymosin α throughout development. These findings indicate a functional association of prothymosin α with the proliferating lymphoid tissues of the young rat.
Key words. Prothymosin α; thymosin α_1; development.

Prothymosin α (ProTα), a highly acidic polypeptide (pI 3.5) of 109–111 residues[1], mainly produced in the mammalian thymus and spleen[2–5], has been implicated in functions related to the immune system[6–9], and in intracellular regulatory events associated with cell proliferation[10–13].

The primary sequence of ProTα[7,10,11,14–17] includes the immunoactive peptide thymosin α_1 ($T\alpha_1$)[18], which

has 28 amino acid residues, at its N-terminal end. $T\alpha_1$ has been reported to have immuno-restorative properties[18], and has recently been found to interact with specific receptors on T-cell lines[19]. ProTα itself has been reported to protect sensitive mice against opportunistic infections[6,7], and to enhance the proliferation of responder T-cells in the human mixed lymphocyte reaction[8,9]. Recent studies support the view that ProTα is a nuclear polypeptide[13,20] involved in the promotion of cellular proliferation[10–13,21], and that the expression of the ProTα gene is directly controlled by the protooncogene c-myc[22].

Serum levels of $T\alpha_1$ immunoreactive material have been reported to decline significantly with aging[23,24]; this decline has been tentatively ascribed to thymic involution[24–26]. However, there appears to be practically no difference in the tissue concentration of ProTα mRNA and protein between young and aged mice[4], and aging is reported not to affect the levels of ProTα mRNA in rat thymus[27]. The observed developmental changes in ProTα gene expression[27] were interpreted in terms of cellular proliferation and differentiation.

Our results presented here show significant age- and sex-dependent changes in the concentration of ProTα during early stages of rat postnatal development, in rapidly proliferating lymphoid tissues.

Materials and methods

Male and female Wistar rats, ranging in age from newborn to 120 days old, were taken in groups of 4 to 34 animals, according to their age and sex, and sacrificed by decapitation. Tissues of each group were immediately removed, frozen in liquid nitrogen, pooled and stored at − 80 °C. Samples of 0.5 to 1.0 g were taken from each frozen tissue pool and tissue extracts were prepared, as described by Komiyama et al.[28], by pulverization of the tissue under liquid nitrogen, brief boiling in water, homogenization of the cooled suspension, centrifugation (12000 x g, 25 min), acidification to pH 2.8, and centrifugation as above (acid supernatant). Aliquots of 0.1 to 0.2 ml were lyophilized in a Speed Vac concentrator (Savant, Hicksville, IL) and $T\alpha_1$ immunoreacting material was measured by a radioimmunoassay for ProTα, similar to that described by Haritos and Horecker[29].

Our radioimmunoassay utilizes a polyclonal antibody raised in rabbits against synthetic $T\alpha_1$ and a tritium-labeled $T\alpha_1$-derivative as the radioactive tracer. It quantitatively determines both ProTα and $T\alpha_1$ in the range of 1–128 pmol. The antibody used in this study was found to recognize ProTα from rat thymus with one seventh of the sensitivity for $T\alpha_1$.

An aliquot (10 ml) of the acid supernatant from the 50-day-old male rat thymus pool was further analyzed by high pressure liquid chromatography (HPLC). It was passed through two Sep Pak C18 cartridges (Waters Associates, Boston, MA), in series; eluted with 20% (vol/vol) n-propanol in water; concentrated, and chromatographed on an Altex Ultrasphere C18 HPLC column, 5 μm (4.6 × 250 mm, Beckman Instruments, Berkeley, CA). Elution was performed with 10% acetonitrile in water, containing 0.05% trifluoroacetic acid, for 10 min, followed by a linear gradient from 10% to 20% acetonitrile for 10 min, and a second linear gradient from 20% to 45% acetonitrile for 40 min, at a flow rate of 1.5 ml/min. The eluted peptide peaks were detected by their UV-absorbance at 214 nm. This analysis showed the presence of one peptide peak at the elution position of rat thymus ProTα, while no peak was evident at the elution position of synthetic $T\alpha_1$ (not shown).

Synthetic $T\alpha_1$, m.w. 3108, was kindly provided by A. M. Felix, of Hoffmann-La Roche Inc., Nutley, NJ. Rat thymus ProTα, m.w.12311, was a generous gift of B. L. Horecker, of Cornell University Medical College, New York, NY.

Protein concentration in tissue supernatants was determined by the fluorescamine assay[30].

Results and discussion

The procedure of extraction employed prevented proteolytic conversion of ProTα to $T\alpha_1$ or other fragments[1,4,28]. Radioimmunoassay standard curves for aliquots of tissue acid supernatants were superimposable with that of pure ProTα, while the corresponding curve for $T\alpha_1$ had a significantly different slope (not shown).

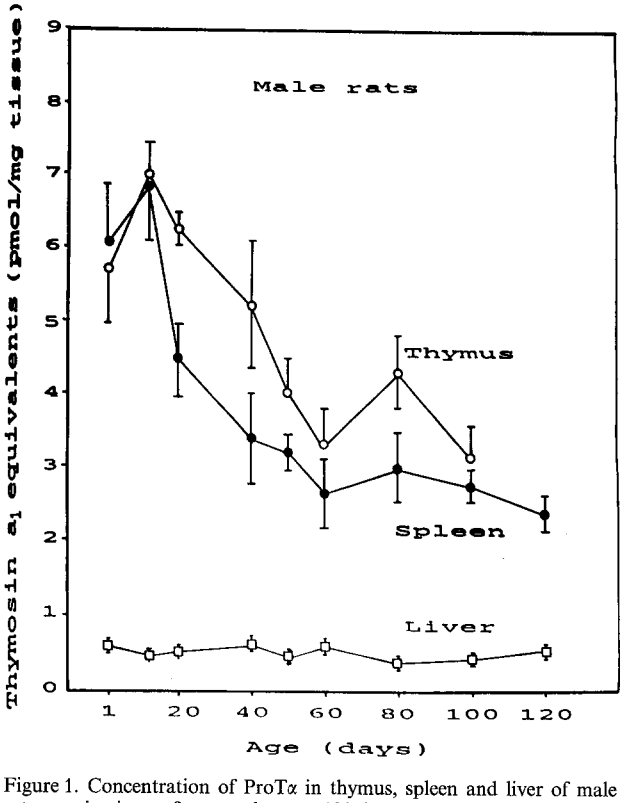

Figure 1. Concentration of ProTα in thymus, spleen and liver of male rats, ranging in age from newborn to 120 days old. Radioimmunoassays were performed on acid supernatants, as described under 'Materials and methods', and results are expressed as pmol $T\alpha_1$ equivalents per g wet tissue. Mean values from three to six determinations are shown, with their standard deviation in vertical bars.

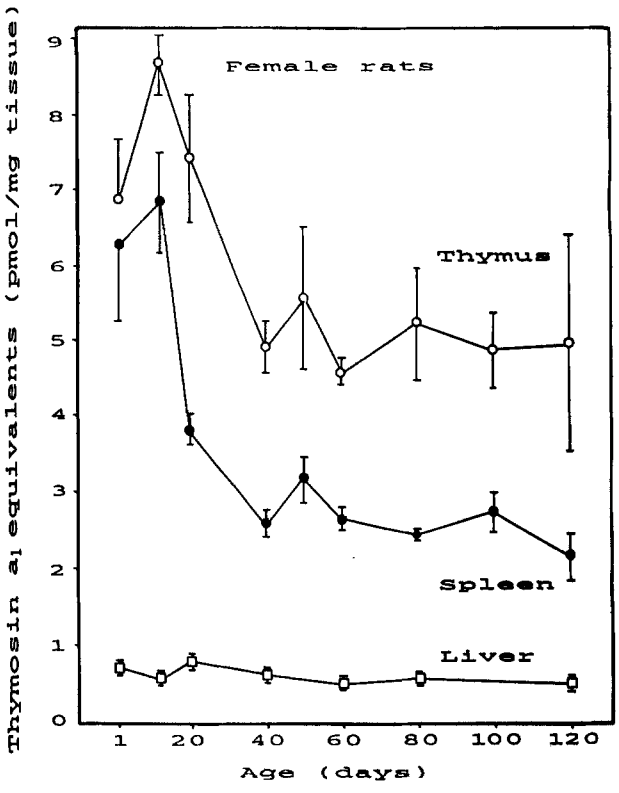

Figure 2. Concentration of ProTα in thymus, spleen and liver of female rats, ranging in age from newborn to 120 days old. Radioimmunoassays were performed as described in the legend to figure 1, and mean values from three to six determinations are shown. Results are expressed as pmol $T\alpha_1$ equivalents per g wet tissue.

These data, in combination with the results of HPLC analysis (see 'Materials and methods'), indicate absence of proteolytically cleaved ProTα from our preparations. In all adult rats examined, ranging from 40 to 120 days of age (figs 1 and 2), the thymus was found to contain 20–50% more ProTα than the spleen, and six- to ten-fold more ProTα than the liver, which agreed with the tissue and age distribution of rat ProTα already reported[2,4]. Newborn and prepubertal male (fig. 1) or female (fig. 2) rats, on the other hand, were found to have significantly higher concentrations of ProTα in thymus or spleen, as compared to adults, while the levels of ProTα in liver remained unaltered throughout postnatal development. Peak concentrations of lymphoid ProTα were observed at 12 days of age, coinciding with a moderate increase in the thymic ProTα mRNA in one and two-week-old rats[27]. The decline in the polypeptide levels of thymus and spleen at puberty can be attributed to hormonal down-regulation and lymphoid tissue involution. Interestingly, we have found that removal of endogenous corticoids of adult male rats by adrenalectomy results in restoration of the levels of lymphoid ProTα to those of pubertal or pre-pubertal animals (manuscript in preparation). It is noteworthy, however, that rat ProTα mRNA[4,27] and protein levels remain high in adult thymus and spleen, possibly in association with self-renewing pools of precursor T-cells, which undergo proliferation and differentiation[26,31].

Age-related differences in the tissue ProTα content of young rodents have not been described by others. In one relevant paper, Clinton et al.[4] did not detect any differences between young (6–8 weeks) and aged (18 months) mice. On the other hand, the differences reported in the present study appear to be consistent with the results of Gómez-Marquez et al.[12], who studied the T-cell lineage of the young rat and showed that ProTα gene expression is linked to lymphoid cell proliferation.

Thymus ProTα content in female rats (fig. 2) was found to be higher compared with that of male rats (fig. 1), throughout development. The decline seen in the levels of thymus and spleen, from 12 to 40 days of age, was more rapid in female than in male animals. These differences may reflect the differential lymphoid effects of gonadal steriods[32].

It has recently been demonstrated that myc protooncogene selectively activates transcription of the gene of ProTα[22]. Age-related differences in the expression of c-myc, in thymus and spleen[33], are consistent with the differences in levels of ProTα reported here. In the liver of newborn rodents, however, high expression of both c-myc[33] and ProTα[27] mRNA has been reported, in contrast to the constantly low levels of liver ProTα from birth onwards, observed in the present work. This finding indicates that the ProTα content of liver, as well as of other non-lymphoid tissues[4], may be regulated primarily at post-transcriptional stages.

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Species-specific differences in the mitogenic activity of heparin-binding growth factors in the sera of various mammals

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Abstract. Sera from different mammalian species displayed great differences in mitogenic activity, as measured by stimulation of DNA synthesis in BALB/c 3T3 cells (3T3 cells). Among the sera examined, fetal bovine serum was least active, and increasing activity was detected in calf serum, human serum, rat serum and mouse serum, in that order. Rat and mouse sera exhibited extremely high mitogenic activity with 3T3 cells, but when TIG-1 human fetal lung fibroblasts were used for the DNA assay instead, the activity levels of all of the sera were lower, and the differences between them were smaller. To determine the reasons for these differences, the heparin-binding growth factors in each serum were separated on a heparin affinity column. Five peaks of DNA-stimulating activity were obtained. Three of these were found in all sera examined, with both 3T3 cells and TIG-1 cells. Two other peaks were found only with 3T3 cells; one was peculiar to rat and mouse sera, with extremely high activity in the rat, and the other was specific to fetal serum. The dependence of the activity of these peaks on the cells used for the test was confirmed using normal rat lung fibroblasts and immortalized rat kidney cells. These findings adequately explain the species-specific differences in mitogenic activity of whole sera, and the variation in activity depending on the cells used for assay of DNA synthesis.
Key words. Heparin-binding growth factors; sera; mammals; species difference; BALB/c 3T3 cell; TIG-1 cell; DNA synthesis.

Many kinds of factors regulating cell growth, both stimulatory and inhibitory, have been discovered recently, and some have been purified and evaluated genetically[1-4]. Some of these factors have been found in serum, although in small quantities[5-7].

The proliferation of animal cells in tissue culture requires the presence of growth factors from serum. To fulfil this requirement fetal bovine serum (FBS) or calf serum (CS) is usually used, since these are commercially available in large volumes. It is not certain, however, whether FBS or CS is the more efficient in terms of stimulating mitogenic activity, because very few reports have been published of studies in which the sera from a variety of mammals have been compared[7-9]. In this paper, we examined the mitogenic activity of sera from different mammalian species by measuring their effect on DNA synthesis in 3T3 cells and TIG-1 cells. Extremely large species differences were discovered. Since there have been no reports explaining why serum mitogenic activity differs among species, we tried to determine the reason by analyzing growth factors