[CANCER RESEARCH 41, 1973–1977, May 1981]
0008-5472/81/0041-0000$02.00

Actin Synthesis and Polymerization in the Liver of Fed and Fasted Rats Bearing a Walker 256 Carcinoma[1]

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ABSTRACT

The effect of tumor growth on the amount, state of polymerization, and synthesis of liver actin was investigated in fed and fasted rats bearing a Walker 256 carcinoma. The increase in liver size in the tumor-bearing animal was accompanied by a rise in the total amount of actin and protein, although the amounts present per g liver fell in both cases. Soluble actin increased in both concentration and total amount in the tumor-bearing animals. While the ratio of total actin to total protein in liver was unaltered by tumor growth, the ratio of soluble actin to total actin was increased. The incorporation of [^{3}H]leucine into liver actin relative to that into liver protein, *in vivo*, was not affected by tumor growth, but the radioactivity incorporated into soluble actin relative to total actin in the livers of the tumor-bearing rats was increased. Liver polysome preparations from tumor-bearing rats showed an increased ability to synthesize actin and total protein, whereas polysomes from skeletal muscle of tumor-bearing rats exhibited a decreased synthesis of actin and total protein. These results suggest that, in the liver of the tumor-bearing rat, while there is an increase in actin synthesis in parallel with a net increase in protein synthesis, there is a decrease in the polymerization of actin.

In livers of both control and tumor-bearing rats, the consumption of a meal was accompanied by a decrease in soluble actin relative to total actin and an increase in the synthesis of actin relative to total protein.

INTRODUCTION

The presence of actin in nonmuscle cells including liver is now well established (4, 12, 13, 17, 20, 25). Unlike skeletal muscle where actin exists almost entirely in a filamentous form, in several types of nonmuscle cells, there is thought to be a continuous interchange between filamentous and nonfilamentous forms of actin which occurs close to the plasma membrane and may possibly be controlled by membrane components (17, 29). The nature of the control of this process and its possible function in tissues such as liver remain uncertain, but in cultured cells, filamentous actin has been suggested as playing an important role with regard to cell shape, cell motility, cell proliferation, and cellular contact (25).

As part of a study of the effect of tumor growth on the contractile proteins of the tissues of the host, we have observed alterations in soluble actin in the livers of rats bearing a Walker 256 carcinoma (11). In the present paper, we have extended our earlier studies and have investigated the effect of the tumor on both the polymerized and unpolymerized forms of liver actin and their interrelationship.

[1] This work was supported by a grant from the Cancer Research Campaign.
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Received August 25, 1980; accepted February 2, 1981.

MATERIALS AND METHODS

Reagents and Isotopes. L-[4,5-^{3}H]Leucine (55 Ci/mmol) and L-[*U*-^{14}C]leucine (348 mCi/mmol) were from the Radiochemical Centre, Amersham, United Kingdom. Purified bovine pancreatic DNase I (EC 3.1.4.5.), calf thymus DNA, and disodium *p*-nitrophenyl phosphate were from Sigma Chemical Co., Ltd., London. Cyanogen bromide-activated Sepharose 4B was from Pharmacia Fine Chemicals, Ltd., London.

Treatment of Animals. Female Wistar rats of fasting body weight, 180 to 200 g, were used. Rats were given injections s.c. in the dorsal region with a suspension of Walker carcinoma cells as described previously (8). Groups of 2 tumor-bearing and 2 control rats of similar initial body weight were housed individually and fed daily at 6 p.m. 14 g ground rat cake (B. P. Nutrition, Stepfield, United Kingdom) made into a paste with water, all of which was consumed by 9:30 p.m. by rats in both groups. The animals were maintained on a 12-hr light-dark cycle (lights on at 6 a.m.). After 10 to 12 days (when the tumor weighed 20 to 30 g), one tumor-bearing and one control rat in each group were killed at 9:30 p.m., 3.5 hr after the last meal was offered. The other 2 rats were killed at 9:30 a.m. One hr before killing, rats were given an i.p. injection of 10 μCi L-[4,5-^{3}H]leucine per 100 g body weight.

Estimation of Liver Actin Content and Radioactivity. Livers were minced and homogenized at 4° in 5 volumes of a medium containing 150 mM NaCl, 2 mM $MgCl_2$, 0.2 mM dithiothreitol, 0.5% (w/v) Triton X-100, 0.2 mM ATP, 0.01 mM phenylmethylsulphonyl fluoride, 5 mM Tris-HCl, pH 7.6, with an Ultra-Turrax homogenizer (Janke and Kunkel, Kg., Staufen i Br., W. Germany) running at 80 V for 3 periods of 3 sec. The total actin content of the homogenate was measured by the DNase I inhibitor assay in the presence of an actin-depolymerizing buffer as described by Blikstad *et al.* (2). An aliquot of the homogenate was centrifuged at 120,000 × *g* at 4° for 2 hr, and the actin content of the supernatant, measured by the DNase I inhibitor assay, was taken as an estimate of soluble or unpolymerized actin. In order to standardize the assay with that for total actin, actin-depolymerizing buffer was added to the 120,000 × *g* supernatant. That amount of sample which produced 50% inhibition of DNase I activity was taken to contain 1.5 μg actin.

Samples of soluble and total actin were obtained by subjecting either the 120,000 × *g* supernatant or the liver homogenate treated in both cases with actin-depolymerizing buffer to affinity chromatography on DNase I-Sepharose (15). The final actin fractions, eluted from the column in 3 M guanidine-HCl, were exhaustively dialyzed against distilled water at 4° to remove the guanidine-HCl. The dialysates were lyophylized and taken up in a small volume of 0.1 M NaOH, and suitable aliquots were counted on glass fiber discs (8). The protein content of the alkaline solution was estimated by the method of Lowry *et al.*

(18), and the specific activity (dpm per mg actin) of the isolated actin was calculated. To obtain the total radioactivity incorporated into soluble or total actin, the specific activity was multiplied by the respective amounts of soluble or total actin present per g liver as estimated by the DNase I inhibitor assay. The specific activity and the amount of trichloroacetic acid-precipitable protein per g liver were measured in an aliquot of the whole homogenate (7).

In Vitro Synthesis of Actin by Polysome Preparations from Liver and Skeletal Muscle. Liver polysomes were prepared from fasted control and tumor-bearing rats by the method of Staehelin and Falvey (28). Polysome preparations from tumor-bearing rats were incubated with [^{3}H]leucine, and equivalent amounts (with respect to RNA content) of polysomes from control rats were incubated with [^{3}H]leucine or [^{14}C]leucine. Conditions of incubation were as described previously (8) except that a Sephadex G-50 filtrate of rat liver cytosol (3) was used as a source of soluble factors. The reaction was stopped after 30 min by the addition of puromycin to a final concentration of 5 mM. Equal aliquots of incubates of liver polysomes from control or tumor-bearing animals labeled with [^{3}H]leucine were mixed with an equal aliquot of incubate from control polysomes incubated with [^{14}C]leucine.

In a separate series of experiments, polysomes prepared from mixed hind-leg muscles of fasted control and tumor-bearing rats by the method of Chen and Young (5) were incubated under the same experimental conditions described for liver polysomes.

Additional Sephadex G-50 filtrate was added to the pooled mixtures of polysomes as a source of carrier actin and samples of actin isolated by affinity chromatography on DNase I-Sepharose. The guanidine-HCl used to elute actin from the column was removed by exhaustive dialysis, the samples were lyopholized, and the residue was taken up in a small volume of 0.1 M NaOH. Suitable aliquots were counted in a dioxan-based scintillation fluid (BDH Chemicals, Ltd., Poole, United Kingdom), and the actin ^{3}H to ^{14}C incorporation ratio was measured. The incorporation of [^{3}H]leucine into protein by polysome preparations from control and tumor-bearing rats was measured as described previously (8).

Actin Content of Plasma Membrane. Liver plasma membranes were isolated according to the method of Ray (26). Final membrane preparations were assayed for actin content by the DNase I inhibitor method in the presence of 0.75 M guanidine-HCl (2). The recovery of the marker enzyme alkaline *p*-nitrophenylphosphatase (26) was used to calculate the concentration of membrane-associated actin initially present per g liver.

RESULTS

In the present series of experiments, we have examined the amount, synthesis, and state of polymerization of liver actin at times corresponding to those reported for maximal and minimal synthesis of protein in rat liver. A diurnal variation in liver protein synthesis has been demonstrated in rats subjected to a 12-hr light-dark cycle. A rise in protein synthesis was apparent shortly after the onset of the dark period and the commencement of food consumption (16, 23). Minimal levels of protein synthesis were observed 12 hr later. In order to ensure the same pattern of food intake by both groups of rats, a fixed amount of food was offered only at the beginning of the dark cycle.

The effects of tumor growth and nutritional status on liver weight and on the amounts of liver protein and actin are shown in Table 1. Soluble actin refers to the actin present in the 120,000 × *g* supernatant of liver homogenate, and total actin is the amount estimated in the whole homogenate in the pres-

Table 1

The effect of tumor growth on the amount of protein and actin in livers of fed and fasted rats

Animals in the fed and fasted groups were killed 3.5 and 15.5 hr after the last meal was offered, and total protein and soluble and total actin were estimated as described in "Materials and Methods."

			Fed	Fasted	p^a	
Liver wt[b]	g	Control	3.80 ± 0.14[c]	3.13 ± 0.09	Control *versus* tumor	<0.001
		Tumor	4.98 ± 0.26	4.21 ± 0.22	Fed *versus* fasted	<0.005
Total protein	mg/g liver	Control	138 ± 2.5	159 ± 4.0	Control *versus* tumor	<0.025
		Tumor	130 ± 3.5	150 ± 3.2	Fed *versus* fasted	<0.001
	mg/liver[b]	Control	524 ± 21	496 ± 15	Control *versus* tumor	<0.001
		Tumor	645 ± 27	630 ± 33	Fed *versus* fasted	NS
Total actin	mg/g liver	Control	4.54 ± 0.10	4.31 ± 0.18	Control *versus* tumor	<0.025
		Tumor	3.76 ± 0.28	4.11 ± 0.20	Fed *versus* fasted	NS
	mg/liver[b]	Control	17.22 ± 0.66	13.44 ± 0.48	Control *versus* tumor	<0.025
		Tumor	18.55 ± 1.31	17.36 ± 1.36	Fed *versus* fasted	<0.025
Soluble actin	mg/g liver	Control	1.25 ± 0.08	1.44 ± 0.11	Control *versus* tumor	<0.005
		Tumor	1.55 ± 0.05	1.99 ± 0.17	Fed *versus* fasted	<0.025
	mg/liver[b]	Control	4.71 ± 0.26	4.37 ± 0.31	Control *versus* tumor	<0.001
		Tumor	7.69 ± 0.50	8.44 ± 0.91	Fed *versus* fasted	NS
$\frac{\text{Total actin}}{\text{Total protein}} \times 100$		Control	3.4 ± 0.1	2.6 ± 0.1	Control *versus* tumor	NS
		Tumor	3.0 ± 0.2	2.7 ± 0.2	Fed *versus* fasted	<0.005
$\frac{\text{Soluble actin}}{\text{Total actin}}$		Control	0.28 ± 0.02	0.33 ± 0.02	Control *versus* tumor	<0.001
		Tumor	0.42 ± 0.03	0.50 ± 0.05	Fed *versus* fasted	<0.05

[a] Statistical analysis was carried out by analysis of variance; there were no significant interactions between tumor growth and nutritional status.
[b] Expressed per 100 g initial body weight.
[c] Mean ± S.E. of 6 experiments.

ence of depolymerizing buffer (2). Because of the increase in size of the livers of the tumor-bearing rats, the results have been expressed as the amount per g liver and per liver per 100 g initial body weight. The concentrations of protein and total actin per g liver both showed a slight decrease in the tumor-bearing rat while, accompanying the hepatomegaly observed in these animals, there was an absolute increase in the amount of protein and total actin present. Liver-soluble actin was increased both in concentration and total amount in tumor-bearing rats, and the ratio of soluble to total actin was markedly increased. Thus, it would appear that the proportion of actin existing in a polymerized form is reduced in the tumor-bearing rat. These effects of the tumor on liver actin were apparent whether the animal was in a fed or fasted state. In the fed animal compared to the fasted animal, while the total amount of actin relative to protein was increased, there was a decrease in the amount of soluble actin relative to total actin.

The incorporation of radioactive leucine into total liver protein and into actin isolated by affinity chromatography was studied both with cell-free systems from livers of control and tumor-bearing rats and also in whole-animal experiments where rats were given injections i.p. in the fed or fasted state with [^{3}H]-leucine and killed 1 hr later. Polysome preparations from livers of tumor-bearing rats exhibited an enhanced capacity to incorporate labeled leucine into total protein (Table 2). Lundholm *et al.* (19) have shown in livers of sarcoma-bearing mice that, while there was a net increase in protein synthesis, the synthesis of all liver proteins was not affected to the same extent and in some cases appeared to be diminished. Inspection of the actin ^{3}H to ^{14}C ratios from mixed liver polysome preparations (Table 2) would suggest that there is an increase in the synthesis of this protein in rats with a Walker tumor. In contrast to these findings, polysomes from skeletal muscles of rats with a Walker tumor showed a decreased synthesis of total protein and also of actin (Table 3). The polysome data showing diminished actin synthesis by muscle polysomes from tumor-bearing rats are in keeping with previous work which showed that the synthesis of contractile proteins in skeletal muscles of these animals is impaired (7).

In experiments with whole animals, the radioactivity incorporated into the soluble or total actin present per g liver was determined from the product of the specific activity (dpm per mg actin) of isolated samples of soluble or total actin and the amounts of these estimated to be present per g liver. The incorporation of radioactivity into total protein per g liver was also determined. In order to overcome fluctuations which can occur in the specific activity of the precursor amino acid pool for protein synthesis caused by changes in the influx of amino acids from the diet and from protein degradation, the incorporation of [^{3}H]leucine into actin has been expressed relative to that into total liver protein. In this way, we can relate the effect of tumor growth on the synthesis of actin to its action on liver protein synthesis in general. The results in Table 4 show that the ratio of radioactivity incorporated into total actin to that into total protein was not significantly altered from control levels in rats bearing a tumor, indicating that there was no preferential alteration in liver actin synthesis due to tumor growth. However, in both the control and tumor-bearing groups, the ratio of the incorporation of leucine into total actin relative to that into total protein was increased in those animals in the fed state, indicating a preferential increase in actin synthesis. The ratio of leucine incorporated into soluble actin relative to that incorporated into total actin is also shown in Table 4. Relatively more radioactivity would appear to be present in soluble actin than in polymerized actin in livers of tumor-bearing rats compared to the distribution in control animals. In contrast, the ratio of radioactivity in soluble actin relative to that in total actin was decreased in the fed animals of both groups (Table 4). The plasma membrane is believed to play a role in the polymerization of actin, and the plasma membrane of liver has been shown to contain actin (13). We have examined the amount of actin remaining in association with purified liver plasma membranes and found it to be 0.61 ± 0.07 mg and 0.59 ± 0.13 mg per membrane fraction per g liver (mean of 5 experiments) in control and tumor-bearing rats, respectively.

Table 2

The in vitro incorporation of radioactive leucine into total protein and actin by polysomes from livers of control and tumor-bearing rats

Conditions of incubation are as described in "Materials and Methods."

	Control	Tumor
Incorporation of [^{3}H]leucine into total protein (dpm/100 μg RNA)	1319 ± 258[a]	2166 ± 166[b]
Incorporation into actin (^{3}H to ^{14}C)	0.94 ± 0.10[c]	1.64 ± 0.19[b, d]

[a] Polysome preparations from livers of 4 rats were pooled for each experiment. Results are expressed as the mean ± S.E. of 3 experiments.
[b] Significantly different from control level, $p < 0.01$ (Student's t test).
[c] Radioactivity in actin synthesized by control polysomes incubated with [^{3}H]leucine to radioactivity in actin synthesized by control polysomes incubated with [^{14}C]leucine.
[d] Radioactivity in actin synthesized by polysomes from tumor-bearing rats incubated with [^{3}H]leucine to radioactivity in actin synthesized by polysomes from control rats incubated with [^{14}C]leucine.

Table 3

The in vitro incorporation of radioactive leucine into total protein and actin by polysomes from skeletal muscles of control and tumor-bearing rats

Conditions of incubation are as described in "Materials and Methods."

	Control	Tumor
Incorporation of [^{3}H]leucine into total protein (dpm/100 μg RNA)	1865 ± 253[a]	1028 ± 123[b]
Incorporation into actin (^{3}H to ^{14}C)	2.86 ± 0.33[c]	1.71 ± 0.28[b, d]

[a] Polysome preparations from muscles of 4 rats were pooled for each experiment. Results are expressed as the mean ± S.E. of 3 experiments.
[b] Significantly different from control level, $p < 0.01$ (Student's t test).
[c] See Table 2, Footnote *c*.
[d] See Table 2, Footnote *d*.

DISCUSSION

In the absence of information on precursor pool radioactivity in the present work, it is not possible to estimate the absolute rate of actin synthesis *in vivo*. However, the data in Table 4 show that, compared to control values, actin synthesis relative to that of total protein synthesis in livers of tumor-bearing rats was unaltered at either time of study. Polysome preparations isolated from livers of tumor-bearing rats exhibited an increased ability to incorporate leucine into actin and also into total protein (Table 2). This rise in liver protein synthesis at the polysome level has also been observed by other workers (9, 19). These observations would suggest that actin synthesis is

Table 4

In vivo incorporation of L-[4,5-^{3}H]leucine into actin and total liver protein of control and tumor-bearing rats

Control and tumor-bearing rats were treated as described in the legend to Table 1 except that, 1 hr prior to sacrifice, fed and fasted animals in both groups were given injections i.p. with 10 μCi L-[4,5-^{3}H]leucine per 100 g body weight. Radioactivity incorporated into total actin, soluble actin, and total protein per g liver was estimated as described in "Materials and Methods."

[^{3}H]Leucine incorporation		Fed	Fasted	p^a	
Total actin / Total protein	Control	0.11 ± 0.01[b]	0.05 ± 0.01	Control *versus* tumor	NS[c]
	Tumor	0.10 ± 0.02	0.06 ± 0.01	Fed *versus* fasted	<0.005
Soluble actin / Total actin	Control	0.26 ± 0.02	0.43 ± 0.03	Control *versus* tumor	<0.005
	Tumor	0.44 ± 0.02	0.57 ± 0.03	Fed *versus* fasted	<0.005

[a] Statistical analysis was carried out by analysis of variance; there were no significant interactions between tumor growth and nutritional status.
[b] Mean ± S.E. of 5 experiments.
[c] NS, not significant.

increasing at the same rate as total protein synthesis in the liver of the tumor-bearing rat. Although the concentrations of both protein and total actin were decreased in the liver of the tumor-bearing rat, the amount of actin and protein per liver were both increased. The fall in concentration may be explained by the increase in water content of liver shown to occur in rats bearing a Walker 256 carcinoma (22). The difference in response to tumor growth of actin synthesis by polysome preparations from liver and muscle (Tables 2 and 3) may be a reflection of the fact that actin in adult skeletal muscle is present in a single form (α-actin) which differs from the 2 forms (β- and γ-actin) present in liver (12), and therefore, different gene products are involved. There was no evidence of the dietary state of the animal having an effect on the concentration of total liver actin in either the control or tumor-bearing animals (Table 1). However, the total actin per liver and the ratio of total actin to total protein were significantly increased in both groups of fed animals. This appeared to be due to a preferential increase in actin synthesis relative to that of total protein (Table 4).

Actin in many nonmuscle cells appears to be present in both polymerized and unpolymerized forms (17), and it would appear from the present work that this is true for rat liver. Significant variations in the relative amounts of these 2 forms were observed in response to tumor growth and dietary status. In studies with whole tissues such as those described here, consideration has to be given to the possibility that the homogenization and extraction techniques might cause some alteration in the state of aggregation of actin. Care was therefore taken to ensure that conditions of homogenization and volumes of solutions relative to tissue were constant. The fact that a similar change in the pattern of distribution of actin between soluble and polymerized forms was observed following the consumption of a single meal in both control and tumor-bearing animals (Tables 1 and 4) does suggest that the procedures used can be used to indicate alterations in the relative amounts of the 2 forms of actin.

The concentration of actin existing in a soluble form was increased in the liver of the tumor-bearing rat while the concentration of total actin was decreased. The radioactivity data in Table 4 suggest that newly synthesized actin is rapidly polymerized in the liver of the normal rat, but in the tumor-bearing rat, the amount of this actin undergoing polymerization is decreased. These findings support the view that actin polymerization in the liver of the tumor-bearing rat is diminished. On the other hand, in both the control and tumor-bearing rats, the polymerization of liver actin is increased in the absorptive phase of digestion. This is particularly apparent with the radioactivity data (Table 4), suggesting that, at this time, a rapid increase in the polymerization of newly synthesized actin is occurring.

The role of the plasma membrane in the alteration in the polymerization of actin due to tumor growth is not clear from the results obtained in the present work. The actin found firmly associated with purified plasma membrane fractions amounted to only 14% of the total actin present and was not altered in the tumor-bearing animal. Moreover, if this actin represents that firmly bound to nucleation sites on the membrane, these would appear to be unaffected by tumor growth. Recently, several workers (14, 24, 27) have reported the existence of factors in serum and plasma which can bring about the depolymerization of filamentous actin. Whether such factors are involved in the state of polymerization of actin observed in the present work remains to be investigated.

Actin filaments occur in various parts of the cell where they fulfill different functional roles. They are found associated with cell junctions and in many cell types, including the hepatocyte and constitute a cytoskeletal network which plays a role in maintaining cell shape (10, 29). In livers of patients with cholestasis, alterations in microfilament structure have been observed. In this case, there was an increase in hepatocyte microfilaments and in the density of the microfilament network (1). The liver of the rat with a Walker carcinoma has been shown to have an increase in cell number (6), and thus the changes arising in actin polymerization may be related in some part to this, although one might have expected to see some change in the membrane-bound actin if this were so. It has also been suggested that actin filaments in nonmuscle cells play a role in the control of metabolism by the specific adsorption of certain enzymes (21). The changes observed in liver actin might therefore be involved in the metabolic alterations known to occur in liver, both in response to the demands of the tumor and also to deal with the products of digestion.

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