The Activities and Kinetic Properties of Purine Phosphoribosyltransferases in Developing Mouse Liver

By A. W. MURRAY*

*Department of Biochemistry and Nutrition, University of New England,
Armidale, New South Wales, Australia*

(Received 12 December 1966)

1. The total activity of adenine phosphoribosyltransferase/liver of mice remained constant from 1 to 16 days after birth despite a fourfold increase in liver weight. The total activity of this enzyme increased fivefold from 16 to 36 days and then remained relatively constant at least until 96 days after birth. Total hypoxanthine-phosphoribosyltransferase activity/liver steadily increased between 1 and 57 days after birth. 2. The mean K_m of 5-phosphoribosyl pyrophosphate with adenine phosphoribosyltransferase was $10.1 \mu\text{M}$ between 3 and 11 days, at 64 days and at 96 days after birth. Between 17 and 51 days the mean K_m value was $3.0 \mu\text{M}$. The K_m of 5-phosphoribosyl pyrophosphate with hypoxanthine phosphoribosyltransferase remained constant at $28.2 \mu\text{M}$ between 2 and 64 days. 3. Adenine-phosphoribosyltransferase activity was stimulated between 15 and 83% by $60 \mu\text{M}$ -ATP when extracts were made between 3 and 11 days, at 64 days or at 96 days after birth. Between 17 and 51 days ATP had little stimulatory effect on the activity of this enzyme. 4. AMP competed with 5-phosphoribosyl pyrophosphate in the reaction catalysed by adenine phosphoribosyltransferase. Liver extracts containing enzyme with a low value of K_m for 5-phosphoribosyl pyrophosphate ($3 \mu\text{M}$) had a K_m/K_i ratio approximately half that of extracts with a high value of K_m ($10 \mu\text{M}$). 5. The results indicate that two different forms of adenine phosphoribosyltransferase can exist in mouse liver at different stages of development. The physiological significance of these findings is discussed.

Recent work has implicated the purine phosphoribosyltransferases in the metabolism of rapidly growing tissues (Atkinson & Murray, 1965a,b; Murray, 1966a). The activity of both adenine phosphoribosyltransferase and hypoxanthine phosphoribosyltransferase has been found to increase markedly during the rapid growth phase of Ehrlich ascites-tumour cells and of regenerating rat liver (Murray, 1966a). In the present paper the activities of adenine phosphoribosyltransferase and of hypoxanthine phosphoribosyltransferase in extracts from postnatal mouse liver are reported. The results suggest the importance of both enzymes during the rapid growth phase of mouse liver. Evidence is also presented for the existence of form(s) of adenine phosphoribosyltransferase with different kinetic properties during rapid liver growth.

EXPERIMENTAL

Substrates. Samples of radioactive and non-radioactive purines were similar in purity to those described before

(Murray, 1966b). PRPP* was obtained as the magnesium salt from the Sigma Chemical Co. (St Louis, Mo., U.S.A.) and was not purified further before use.

Assay of purine-phosphoribosyltransferase activity. Extracts were prepared from postnatal mouse liver as described before (Murray, 1966a). Conditions of assay with radioactive adenine and hypoxanthine were also as described before (Murray, 1966a).

RESULTS

Activities of adenine phosphoribosyltransferase and of hypoxanthine phosphoribosyltransferase in developing mouse liver. The activity of both adenine phosphoribosyltransferase and hypoxanthine phosphoribosyltransferase in extracts of mouse liver at various times after birth is shown in Fig. 1. Time of birth was established by examining the pregnant mice every 12 hr. as they approached parturition; this time therefore represents the maximum error in the estimations of the ages of the mice. The mice were weaned at 21 days, but observation indicated that voluntary weaning may have occurred several days before this. The adenine-phosphoribosyltransferase activity/g. wet wt. of liver decreased

* Present address: School of Biological Sciences, the Flinders University of South Australia, Adelaide, South Australia.

* Abbreviation: PRPP, 5-phosphoribosyl pyrophosphate.

Fig. 1. Activities of adenine phosphoribosyltransferase (○) and of hypoxanthine phosphoribosyltransferase (●)/g. wet wt. of tissue in extracts of mouse liver at different stages of development. Assays were carried out at pH 7.8 as described in the Experimental section. Values represent the means $\pm$ S.E.M. of separate determinations on at least four mice taken from separate litters.

after birth until about 16 days after birth and then varied between about 15 and 25 mμmoles of AMP formed/min./g. of liver until at least 96 days after birth. Hypoxanthine-phosphoribosyltransferase activity increased from a value of 47 mμmoles of IMP formed/min./g. of liver on the day of birth to a value of 62 mμmoles of IMP formed/min./g. of liver 2 days after birth. After this time the activity decreased until a plateau value of 30–40 mμmoles of IMP formed/min./g. of liver was reached about 25 days after birth. In a previous study (Murray, 1966a) adenine- and hypoxanthine-phosphoribosyltransferase activities were found to be respectively 26 and 43 mμmoles of nucleotide formed/min./g. of liver; liver extracts were made when the mice were between 49 and 56 days old.

In Fig. 2 the total activities of adenine phosphoribosyltransferase and of hypoxanthine phosphoribosyltransferase/liver for postnatal mice are plotted; the liver weights of mice over the experimental period are also plotted in Fig. 2. The total adenine-phosphoribosyltransferase activity/liver remained constant at a value of about 5 mμmoles of AMP formed/min. until 16 days after birth, although the total liver weight increased fourfold over this period. This is reflected in the decrease in adenine-phosphoribosyltransferase activity/g. of liver that occurred during the same period (see Fig. 1). After 16 days the total activity of adenine phosphoribosyltransferase increased, reaching a constant value of about 25 mμmoles of AMP formed/min./liver at 36 days. In contrast, the total activity of

Fig. 2. Total activities of adenine phosphoribosyltransferase (○) and of hypoxanthine phosphoribosyltransferase (●)/liver in extracts of mouse liver at different stages of development. The mean wet weights of livers at intervals during the experimental period are also plotted (Δ). These results were derived from the same series of experiments shown in Fig. 1.

hypoxanthine phosphoribosyltransferase increased steadily until about 57 days, when a constant value of 50–55 mμmoles of IMP formed/min./liver was reached. Between about 29 and 50 days after birth there appeared to be a slight increase in the rate at which the total activity of this enzyme increased. Total liver weight had reached an approximately constant value of 1.6 g. after 57 days.

Kinetic properties of adenine phosphoribosyltransferase in extracts from mouse liver at different stages of development. Fig. 3 shows the values of the Michaelis constant, K_m , of PRPP with adenine phosphoribosyltransferase in extracts from mouse liver at various times after birth. The percentage stimulation of adenine phosphoribosyltransferase by 60 μM-ATP in the presence of 12.5 μM-PRPP is also shown in Fig. 3 (see Murray, 1967a,b). Between 3 and 11 days after birth the K_m value was in the range 8.4–11.2 μM (mean 10.0 μM), and between 17 and 51 days after birth in the range 2.6–3.8 μM (mean 3.0 μM). At both 64 and 96 days after birth the K_m value was 10.3 μM. Between 17 and 51 days ATP had little stimulatory effect on adenine-phosphoribosyltransferase activity. However, the activity of this enzyme was considerably stimulated by ATP when extracts were made between 3 and 11 days, at 64 days or at 96 days after birth. The

Fig. 3. Values of K_m for PRPP with adenine phosphoribosyltransferase in extracts of mouse liver at different stages of development ($\circ$). In these determinations the adenine concentration was kept constant at $15\mu\text{M}$ and the PRPP concentration was varied between 2.8 and $37\mu\text{M}$. All assays were carried out at pH 7.8. The percentage stimulation of adenine phosphoribosyltransferase by $60\mu\text{M}$ -ATP (PRPP concentration $12.5\mu\text{M}$) is also shown.

K_m of PRPP with hypoxanthine phosphoribosyltransferase was in the range 25 – $32\mu\text{M}$ (mean $28.2\mu\text{M}$; seven determinations) with extracts of mouse liver made between 2 and 64 days after birth. With this enzyme there was no trend in the values of K_m found at any stage during the experimental period.

AMP was found to compete with PRPP in the reaction catalysed by adenine phosphoribosyltransferase (see also Murray, 1966b, 1967a). However, the ratio of K_m to the inhibitor constant, K_i , varied in liver extracts made at different times after birth. At 5 days after birth the K_m/K_i ratio was 2.4, at 11 days it was 2.1, at 29 days it was 0.9, at 51 days it was 1.2, at 64 days it was 2.1 and at 96 days it was 2.4. Thus the ratio was higher in extracts with a high K_m for PRPP (see Fig. 3), suggesting that AMP is not as effective an inhibitor of the adenine phosphoribosyltransferase in these extracts as of the enzyme with a lower K_m for PRPP.

The maximum velocity of the reaction catalysed by adenine phosphoribosyltransferase was determined at eight pH values between 5.3 and 8.8 with liver extracts made 5 and 51 days after birth. A composite buffer of tris and histidine was used in this experiment (see Murray, 1967b). Plots of activity against pH were very similar for the two extracts,

each showing a maximum velocity between pH 7.4 and 7.8.

DISCUSSION

Up to 16 days after birth there was no change in the total activity of adenine phosphoribosyltransferase/liver, although the total liver weight increased fourfold over this period. This could be explained by assuming little or no turnover of the enzyme protein present at or near birth, so that the addition of new tissue, which itself does not synthesize adenine phosphoribosyltransferase, merely 'dilutes' the enzyme already present. This would give the observed effect of decreasing adenine phosphoribosyltransferase activity/g. of liver for the first 16 days after birth. Alternatively, the constant adenine-phosphoribosyltransferase activity would result if the breakdown of enzymically active protein was equal to its synthesis. Either explanation suggests a direct control of enzyme synthesis or activity or both for 16 days after birth. From 16 to about 36 days there was an increase in the total adenine-phosphoribosyltransferase activity/liver. There is no way of deciding from the results of this study if the increase was due to increased net protein synthesis or to increased activity of enzyme already present.

The kinetic properties of adenine phosphoribosyltransferase in mouse-liver extracts were found to differ during development. The altered enzymic activity could have arisen by synthesis of a new enzyme with different kinetic characteristics or by modification of the enzyme already present. The small stimulatory effect of ATP on the adenine phosphoribosyltransferase with a low K_m for PRPP suggests that there may be a mixture of the two forms of the enzyme in extracts made between 16 and 51 days after birth. It was not possible to confirm this with kinetic experiments as described by Ballard & Oliver (1964), as the K_m values of the two forms were not sufficiently different. The existence of a number of different forms of adenine phosphoribosyltransferase in Ehrlich ascites-tumour cells has been implied by previous workers (Atkinson & Murray, 1965a; Hori & Henderson, 1966). Thus it appears that two forms of adenine phosphoribosyltransferase can exist in mouse liver, one with allosteric properties (i.e. activated by ATP, which is bound at a site separate from the substrate-binding site; see Murray, 1967a,b) and the other without these properties. It is clear that the increased affinity of adenine phosphoribosyltransferase for PRPP (as reflected by a lower K_m) could be an advantage during the rapid growth phase of mouse liver if concentrations of PRPP were not high enough to enable the enzyme with a higher K_m to function at its maximum rate. However,

desensitization of an allosteric enzyme to an effector can result in increased affinity of the substrate for the enzyme (Gerhart & Pardee, 1964; Murray, 1967a). Thus the decrease in K_m may only be a secondary effect resulting from the lack of ATP stimulation. From these considerations, the results could be explained by postulating a reversible dissociation of catalytic sub-units (binding PRPP and adenine) and regulatory sub-units (binding ATP) during rapid liver growth. However, although substrates and ATP are bound at different sites on adenine phosphoribosyltransferase (Murray, 1967a,b), there is no evidence for the existence of regulatory and catalytic sub-units. The physiological meaning of the loss of allosteric properties between 17 and 51 days after birth is not apparent. It is perhaps significant, however, that AMP is less effective as an inhibitor of the adenine phosphoribosyltransferase that has a lower K_m for PRPP than of the enzyme that has a higher K_m for PRPP. The activity of this

enzyme may be less susceptible to metabolic control during rapid tissue growth.

The skilful technical assistance of Mr F. Ball is gratefully acknowledged. This work was supported by grants from the Australian Research Grants Committee and from the New South Wales State Cancer Council.

REFERENCES

- Atkinson, M. R. & Murray, A. W. (1965a). *Biochem. J.* **94**, 64.
Atkinson, M. R. & Murray, A. W. (1965b). *Biochem. J.* **94**, 71.
Ballard, F. J. & Oliver, I. T. (1964). *Biochem. J.* **90**, 261.
Gerhart, J. C. & Pardee, A. B. (1964). *Fed. Proc.* **23**, 727.
Hori, M. & Henderson, J. F. (1966). *J. biol. Chem.* **241**, 3404.
Murray, A. W. (1966a). *Biochem. J.* **100**, 664.
Murray, A. W. (1966b). *Biochem. J.* **100**, 671.
Murray, A. W. (1967a). *Biochem. J.* **103**, 271.
Murray, A. W. (1967b). *Biochem. J.* **104**, 669.