Vol. 88, No. 2, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 28, 1979 Pages 583-588

MULTIPLE FORMS OF RAT LIVER L-ASPARAGINE SYNTHETASE

Rodney S. Markin and Sheldon M Schuster

Department of Chemistry and
School of Life Sciences
The University of Nebraska
Lincoln, NE 68588

Received March 19,1979

Extraction of rat liver asparagine synthetase from rats maintained on normal protein containing diets yields activity in a region corresponding to a molecular weight of 57,000. Decreasing the amount of protein in the diets caused the asparagine synthetase activity to appear in two forms, one being in the 57,000 region and the other in the 113,000 region. If rat were fed a high protein diet, liver asparagine synthetase activity appeared only in the lower molecular weight area. Experiments with synthetic amino acid diets demonstrated that a dietary deficiency in asparagine would cause the appearance of high molecular weight (113,000) hepatic asparagine synthetase.

It has been nearly a quarter of a century since Kidd (1) originally noted that a factor in guinea pig serum had antitumor activity against two strains of murine lymphoma. Several years later, Broome (2) demonstrated that this antitumor activity was due to the presence of L-asparaginase activity, which is particularly high in guinea pig sera. Since that time, L-asparaginase has been used in numerous successful clinical studies (3-5). The basis for the utility of L-asparaginase therapy is the sensitivity of selected tumor cell types to decreased circulatory asparagine.

It was therefore necessary that the enzyme responsible for the production of asparagine from a nitrogen source and aspartic acid be characterized. In this regard, Patterson and Orr (6) described the purification and some properties of the asparagine synthetase of Novikoff hepatoma. These authors found that asparagine was synthesized from aspartic acid and glutamine with the hydrolysis of an ATP to AMP and pyrophosphate. Horowitz and Meister (7) found a similar enzyme in an asparagine-resistant murine leukemia (RADA1).

0006-291X/79/100583-06$01.00/0

A recent report from Hongo et al. (8) describes the partial purification of asparagine synthetase from rat liver. The enzyme from rat liver appears to be very similar to the enzyme from tumors. Such parameters as molecular weight, glutamine requirement and affinity for substrates were similar in normal and tumor derived asparagine synthetases. In all cases the molecular weight of the aparagine synthetase activity was about 110,000. However, it must be noted that these studies were done using either tumors that were asparaginase resistant or normal tissue from rats on low protein diets. Patterson (9) has previously indicated that hepatic asparagine synthetase activity increased in rats fed low asparagine diets. The studies presented here indicate a possible molecular basis for this increased activity, as well as the discovery that asparagine synthetase exists in more than one molecular form.

Materials and Methods

Chemicals - L[4-^{14}C] aspartate and DL-[4-^{14}C] aspartate were obtained from ICN (Riverside, CA). All other reagents and gel filtration materials were obtained from Sigma Chemical Co. in the highest purity available. All scintillation counting supplies were obtained from Research Products, Intl. (Elk Grove Village, Il).

Animals - Long Evans rats were used in these experiments. They ranged in age from 60 to 80 days. After weaning, the rats were maintained on Wayne Rat and Hamster Food containing no less than 24% available protein. Rats used in the dietary experiments were maintained on the following diets: low protein (8%), high protein (64%), Rodger's synthetic amino acid diet with asparagine or Rodger's synthetic amino acid diet without asparagine (U.S. Biochem., Cleveland, Ohio). Each experiment was done with groups of four rats and repeated three times.

Enzyme Preparation - Rats were sacrificed by decapitation and the livers removed. The livers were immediately placed in a buffer consisting of 50 mM Tris-Cl pH 8.0, 1.0 mM DTT and 0.5 mM EDTA at 4°C. Unless otherwise indicated, all procedures were performed at 4°C. The livers were then homogenized in a Waring blender at medium speed for 1 minute. The homogenate was centrifugated at 10,000 rpm in an SS-34 rotor (Sorvall) for 30 minutes, the supernatant recovered and centrifuged again at 20,000 rpm for 2 hours. The supernatant from the second centrifugation was fractionated using ammonium sulfate. A fraction from 30% to 70% saturation was recovered by centrifugation and resuspended in the extraction buffer.

The 30-70% ammonium sulfate fraction was then chromatographed by Sephadex G-100 chromatography on a column (2.6 x 60 cm) that had been equilibrated in the extraction buffer described above. The effluent was collected in 2.5 ml fractions and assayed for asparagine synthetase activity.

Enzyme Assay - Asparagine synthetase was assayed for by the procedure of Horowitz and Meister (7) and also by the method of Luehr and Schuster (10). Both assays yielded the same results.

Determination of molecular weight. - In order to determine the molecular weights of the various asparagine synthetase forms protein markers of known molecular weights were passed through the column and their elution profile determined by absorbance at 280 nm. A graph of eluted volume vs. log molecular weight yields a straight line. Using the calibration curve, the molecular weights of the forms were determined to be 113,000 and 57,000.

Other Methods - 3a70b from RPI was used for all scintillation counting. The samples were counted immediately after preparation. Samples were counted on the Beckman LS 8000 using the Automatic Quench correcting function to correct for the quenching due to ninhydrin.

Results and Discussion

The report of Hongo et al. (8) shows that feeding rats a diet low in protein causes an increase in the overall liver asparagine synthetase activity. In attempting to purify rat liver asparagine synthetase we have observed a similar increase in total enzyme activity. In addition, we have attempted to examine the metabolic basis for this diet-dependent asparagine synthetase activity.

Rats were placed in groups and fed either high (64%), normal (24%) or low (8%) protein diets. At the end of 14 days on these diets the livers were homogenized, treated with ammonium sulfate and chromatographed on Sephadex G-100 as described in "Materials and Methods". When the asparagine synthetase activity was measured the results were as presented in Figure 1. In agreement with the conclusion of Hongo et al. (8) the low protein diet caused an increase in the assayable asparagine synthetase activity (compare Fig. 1B to 1C). However, it appears that there are two peaks of activity from rats fed low protein diets and only one peak in the activity of animals on the control diet. It appears that diet influences not only the quantity of enzymatic activity, but also the molecular form.

Examination of the molecular weights represented by the activity in Figure 1B and 1C show weights of 113,000 and 57,000 for the high and low molecular forms, respectively. Since Hongo et al. (8) found rat liver

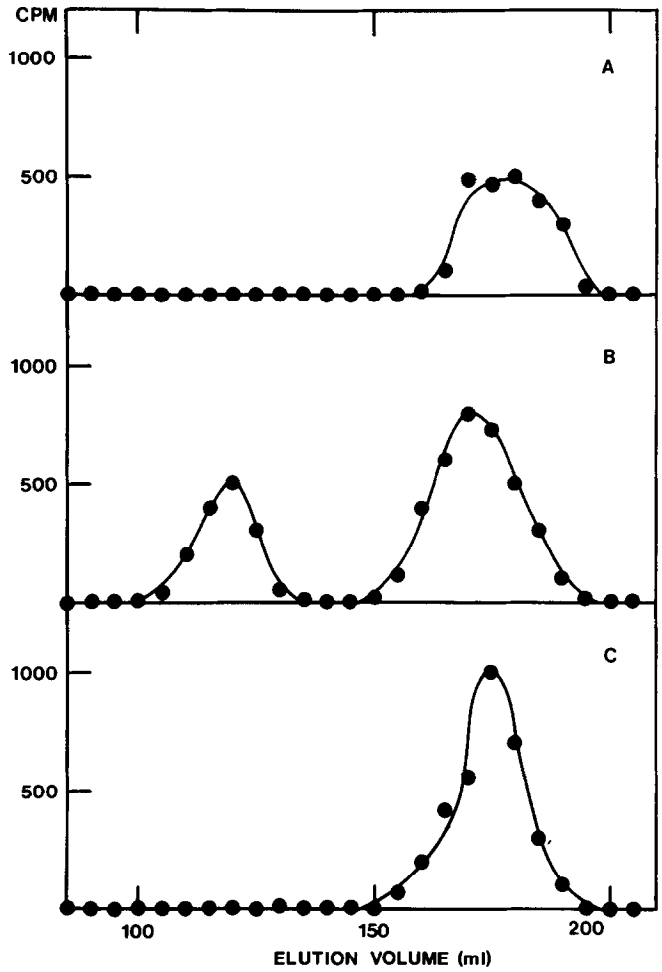

Figure 1. Elution profile of hepatic asparagine synthetase activity from Sephadex G-100; A, from rats fed on a high protein (64%) diet, B, from rats fed a low protein diet (8%); C, from rats fed a normal protein (24%) diet.

asparagine synthetase from rats fed low protein diets to have a molecular weight of 110,000, we are in agreement of their conclusion. In addition we see a distinct form of the enzyme from rats fed normal protein containing diets. This form is approximately half the weight of the larger form.

In support of the two molecular weight forms of asparagine synthetase being diet dependent, the activity from rats fed a high protein diet is shown only in the low molecular weight form (Figure 1A). The overall activity in the livers of rats fed this high protein diet was also decreased.

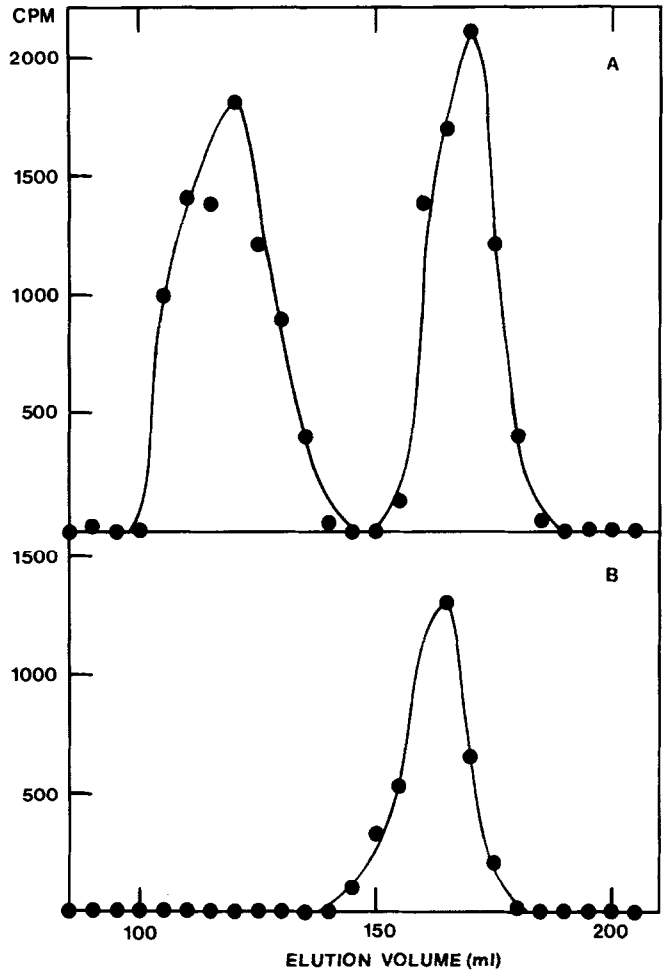

Figure 2. Elution profile of hepatic asparagine synthetase activity from Sephadex G-100: A, from rats maintained on Rodger's synthetic amino acid diet without asparagine and B, from rats fed the identical diet with added asparagine.

In order to determine which component of the various protein diets controls the form of the rat liver asparagine synthetase, rats were fed diets that differed by only one amino acid. When rats were fed a synthetic amino acid containing diet devoid of asparagine, both the high and low molecular weight forms of hepatic asparagine synthetase were observed (Figure 2A). When the asparagine synthetase activity from livers of rats fed the same diet plus asparagine was measured the results were as shown in Figure 2B. It appears that the molecular forms of hepatic asparagine synthetase are res-

ponsive to the presence or absence of dietary asparagine. It is not yet known if other dietary components exert an influence on the molecular forms of hepatic asparagine synthetase. It is however reasonable for one of the asparagine synthetase products (namely asparagine) to control asparagine biosynthesis.

We do not yet fully appreciate the kinetic or stuctural differences of the two forms of asparagine synthetase. However this represents the first report that multiple forms of this enzyme exist. The fact that they are altered in response to dietary factors indicates that they are a central feature in the control of asparagine biosynthesis.

Acknowledgement. This research was supported by a grant from the American Cancer Society #BC-286.

References

1. Kidd, J. G. (1953) J. Exp. Med., 98, 565-583.

2. Broome, J. D. (1963), J. Exp. Med., 118, 99-121.

3. Dolowy, W. C., Henson, D., Cornet, J., and Sellin, H. (1966) Cancer, 19, 1813-1819.

4. Hill, J. M., Roberts, J., Loeb, E., Khan, A., MacLellan, A., and Hill, R. W. (1967) J.A.M.A., 202, 882-888.

5. Oettgen, H. F., Old, L. J., Boyse, E. A., Campbell, H. A., Philips, F. S., Clarkson, B. D., Tallal, L., Leeper, R. D., Schwartz, M. K., and Kim, J. H. (1967) Cancer Res., 27, 2619-2631.

6. Patterson, M. K., and Orr, G. R. (1968) J. Biol. Chem., 243, 376-380.

7. Horowitz, B., and Meister, A. (1972) J. Biol. Chem., 247, 6708-6719.

8. Hongo, S., Matsumoto, T., and Sato, T. (1978) Biochim. Biophys. Acta, 552, 258-266.

9. Patterson, M. K. (1970) Recent Results in Cancer Res., 33, 22-30.

10. Luehr, C. A. and Schuster, S. M., submitted 1979.