BBA 66351

FACTORS INFLUENCING THE *"IN VITRO"* ENHANCEMENT OF L-ASPARAGINASE ACTIVITY

MAUREEN B. LEE, C. DENISE BOLGER AND J. M. BRIDGES
Department of Clinical Pathology, Royal Victoria Hospital, Belfast (Great Britain)

(Received January 14th, 1971)

SUMMARY

1. The addition of human and animal sera to guinea pig serum or *Escherichia coli* asparaginase (L-asparagine amidohydrolase, EC 3.5.1.1) has been shown to enhance the enzymatic activity *in vitro*.

2. The enhancement by human serum is independent of pH, substrate and product concentration and is not readily destroyed by heat, although all these factors influence the enzyme activity.

3. Enzyme activity was increased after the addition of various plasma fractions to *E. coli* asparaginase, but not by high molecular weight glucose solutions (see also HO AND JONES, *Biochim. Biophys. Acta*, 171 (1969) 172).

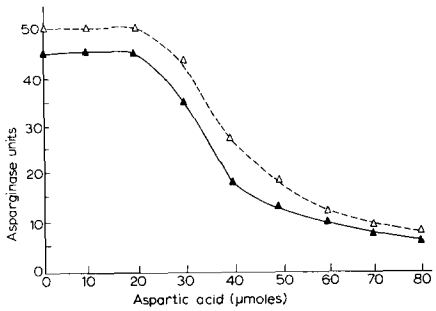
4. It is suggested that the enhancement of asparaginase activity by serum and fractions is due to a non-specific protein stabilisation of the enzyme molecule.

The *in vitro* activity of asparaginase (L-asparagine amidohydrolase, EC 3.5.1.1) derived from guinea pig serum or from *Escherichia coli*[1] is increased by addition of human and various other animal sera[2] but not by high molecular weight glucose solutions[3].

Experiments were carried out to determine whether the enhancement of L-asparaginase by human serum was influenced by changes in pH heat, substrate and product concentrations. Substrate, product concentration and heat had a similar effect on the enhancement of the enzymes from guinea pig serum and *E. coli* extracts (EC_1 and EC_2)[4]. The variation of pH showed a greater increase in the asparaginase activity of *E. coli* extract EC_1 than *E. coli* extract EC_2 and guinea pig serum at acid and alkaline pH values.

Asparaginase activity was assayed as previously described[2]. The enzyme-containing solution (0.5 ml) was added to 1.0 ml of 0.01 M borate/boric acid buffer (pH 8.6) to which 20 μmoles asparagine were added (0.5 ml). The reaction was stopped by adding 0.5 ml ice cold 15% trichloroacetic acid. Liberated ammonia was determined by direct nesslerisation of the protein-free supernatant and the absorbance read at 480 nm. One international unit of enzyme activity will catalyse the formation of 1 μmole of ammonia/min under the conditions of the assay.

To determine the influence of human serum, aliquots of enzyme-containing



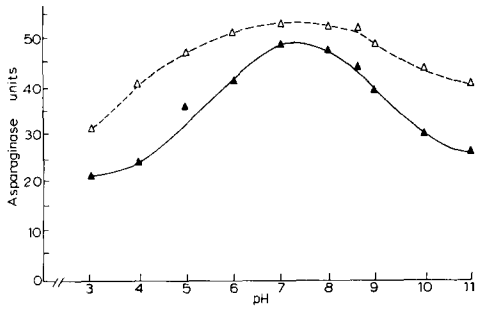


Fig. 1. The influence of product inhibition on the enhancement of EC_2 asparaginase in a system containing 20 μmoles asparagine, 10 μmoles sodium borate buffer (pH 8.5). ▲—▲, asparaginase assayed in aqueous solution; △— — — —△, asparaginase assayed after the addition of human serum.

Fig. 2. The influence of pH on the enhancement of EC_2 asparaginase. ▲—▲, EC_2 asparaginase assayed in aqueous solution; △— — — —△, EC_2 asparaginase assayed after the addition of human serum.

solutions were diluted 1:1 with borate buffer (pH 8.6) or with serum. The rate of liberation of ammonia was determined over a 40-min period; if enzyme or substrate was omitted ammonia was not detected. Enzyme enhancement was demonstrated by an increased rate of ammonia production. If the incubation continued until substrate depletion occurred, the total volume of ammonia generated was unaffected by the presence of serum but this caused substrate depletion to occur sooner. When changes in asparagine and aspartic acid levels were used as indices of asparaginase activity, the addition of serum caused more rapid utilisation of asparagine with a stoichiometric increase in aspartic acid production (measured by the method of PFLEIDERER[5]). Following the heating of the serum to 37°–66° for 10 min, or at 56° for 4 h, there was no decrease in enhancement.

Asparaginase was assayed in a medium containing 20 μmoles of asparagine *plus* 0–80 μmoles aspartic acid in a total volume of 2 ml at pH 8.6. Fig. 1 shows that the enzyme activity was inhibited by 50% and 80% at concentrations of 35 and 60 μmoles of aspartic acid. Product inhibition is dependent on the pH of the assay and may be demonstrable at pH 8.5 but not at pH 5.0 or pH 7.4[6]. Thus the relationship between inhibition and product concentration reported here is valid only at one pH value. Human serum did not alter the pattern of product inhibition measured at pH 8.6 (Fig. 1).

The maximum rate of ammonia liberation remained constant when the concentration of asparagine was increased to 80 μmoles and the enhancement by human serum was not affected by these changes in substrate concentration.

The effect of pH on activity and enhancement was assessed using the following buffers, ranging in pH from 3 to 11: KOLTHOFF's[7] borax/succinic acid buffer, pH range 3–5, KOLTHOFF's[7] borax–monopotassium phosphate buffer, pH range 6–6,8 and CLARKE AND LUBS'[8] borate–hydroxide buffer, pH range 8.6–11.

In buffer, guinea pig serum asparaginase showed the maximum activity between pH 9 and 10, whereas *E. coli* asparaginases EC_1 and EC_2 had maximum activities at pH 7.5; EC_2 being more stable in acid solutions. The addition of serum to the asparaginases increased activity at all values of pH, the degree of enhancement

varying with pH (Fig. 2). At pH 5 the 50% enhancement of EC_2 is comparable with that reported by HO AND JONES[3].

All human plasma fractions (Baxter Hyland Laboratories) reconstituted in borate buffer (pH 8.6) at a concentration of 7 g/100 ml, enhanced enzyme activity to the same extent as whole serum. Various macromolecular solutions produced no significant enhancement (Table I), which is similar to a previous report[3].

There is some evidence that the activity of asparaginase *in vitro* depends on the protein content of the medium. The enzyme from *E. coli* is more stable after the initial precipitation to remove the smaller protein molecules than it is in the crude extract[9]. Pre-incubation with human serum *in vitro* alters the isoelectrofocussing pattern of *E. coli* asparaginase and similar changes occur after the enzyme has circulated in the mouse[10], possibly due to its stabilization by serum proteins. Thus

TABLE I

INFLUENCE OF A VARIETY OF MEDIA ON ASPARAGINASE ACTIVITY

EC_2 asparaginase was diluted 1:1 with the various fractions before assaying as described in the text.

Medium	*Asparaginase activity (I.U./ml enzyme solution)*	*% Enhancement*
Buffer, pH 8.6	0.60	—
Fresh serum	0.80	33
Reconstituted whole plasma	0.72	20
α-Globulin	0.75	25
β-Globulin	0.80	33
γ-Globulin	0.80	33
Albumin	0.80	33
Aminosol*	0.60	0
Rheomacrodex**	0.54	0
Intradex***	0.62	3

* 3% dialysed casein hydrolysate in 5% glucose.
** 10% dextran (mol. wt. 40 000) in normal saline.
*** 6% dextran (mol. wt. 110 000) in normal saline.

the enhancement of asparaginase activity by human serum may result from non-specific protein stabilization of the enzyme molecule. This may occur, together with a decreased denaturation of the enzyme, *via* adsorption onto the walls of the assay vessels, as proposed by other authors[3,11].

ACKNOWLEDGMENTS

We would like to thank the Northern Ireland Leukaemia Research Fund for generous financial support; Dr. J. Teller, Worthington Biochemical Corporation for supplying EC_1 (specific activity 0.10 I.U./mg protein) and Dr. J. Roberts, Wadley Research Institute, Dallas for supplying EC_2 (specific activity 100 I.U./mg protein).

REFERENCES

1 J. D. ROBERTS, M. D. PRAGER AND N. BACHYNSKY, *Cancer Res.*, 26 (1966) 2213.
2 M. B. LEE AND J. M. BRIDGES, *Nature*, 217 (1968) 758.
3 P. P. K. HO AND L. JONES, *Biochim. Biophys. Acta*, 171 (1969) 172.
4 H. A. CAMPBELL, L. T. MASHBURN, E. A. BOYSE AND L. J. OLD, *Biochemistry*, 6 (1967) 721.
5 G. PFLEIDERER, in H. U. BERGMEYER, *Methods of Enzymatic Analysis*, Academic Press, New York, 1965, p. 381.
6 H. A. CAMPBELL AND L. T. MASHBURN, *Biochemistry*, 8 (1969) 3768.
7 I. M. KOLTOFF, *J. Biochem.*, 63 (1925) 135, quoted by K. DIEM, *Documenta Geigy*, Geigy Pharm. Co., Manchester, 6 ed., 1962, p. 314.
8 W. M. CLARK AND H. A. LUBS, *J. Bacteriol.*, 2 (1917) 1, quoted by K. DIEM, *Documenta Geigy*, Geigy Pharm. Co., Manchester, 6 ed., 1962, p. 314.
9 L. T. MASHBURN AND J. C. WRISTON, JR., *Arch. Biochem. Biophys.*, 105 (1964) 450.
10 L. T. MASHBURN AND L. M. LANDIN, in E. GRUNDMANN AND H. OETTGEN, *Recent Results in Cancer Research*, Vol. 33, Springer, Berlin, 1970, p. 48.
11 K. BAUER, R. W. KAUFMANN AND O. WAGNER, in E. GRUNDMANN AND H. OETTGEN, *Recent Results in Cancer Research*, Vol. 33, Springer, Berlin, 1970, p. 31.