Activation and stabilization of asparaginase by anti-asparaginase IgG and its Fab

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Modified asparaginase, in which 4 tryptophan residues were modified with 2-hydroxy-5-nitrobenzyl bromide, had little enzymic activity and retained immunoreactivity [(1976) FEBS Lett. 65, 11–15]. Addition of IgG or its Fab towards asparaginase to the modified asparaginase gave rise to marked enhancement of the enzymic activity. Native asparaginase (4 subunits) lost the enzymic activity due to dissociation into subunits by dilution of the enzyme solution. However, in the presence of Fab, asparaginase did not lose enzymic activity on dilution, probably due to no dissociation into subunits occurring.

Asparaginase *2-Hydroxy-5-nitrobenzyl bromide* *Anit-asparaginase IgG*

1. INTRODUCTION

Celada et al. [1–3] demonstrated the activation of mutant β-galactosidase by its antibodies, and Melchers and Messer [4] reported the protection from thermal denaturation of β-galactosidase with its antibodies. These phenomena have been explained by the alternation or protection of protein conformation due to interaction of enzyme with specific antibodies [5].

In a previous study [6], it was found that 4 tryptophan residues in the asparaginase molecule from *E. coli* (M_r 136000, 4 identical subunits) were specifically modified with 2-hydroxy-5-nitrobenzyl bromide (HNB). This study deals with activation of HNB-modified asparaginase together with stabilization of native asparaginase by IgG or its Fab towards native asparaginase.

2. MATERIALS AND METHODS

A crystallized asparaginase (L-asparagine amidohydrolase, EC 3.5.1.1) from *E. coli* was kindly donated by Kyowa Hakko Kogyo Co. (Tokyo). The molar extinction coefficient at 278 nm is 8.53×10^4 $M^{-1} \cdot cm^{-1}$. HBN was purchased from Dojin Laboratory (Kumamoto, Japan). Other reagents were of analytical grade.

Asparaginase was modified with HNB by the method described previously [6–8]. The degree of modification of tryptophan residues in the asparaginase molecule with the reagent was spectrophotometrically determined, and it was found that all 4 tryptophan residues in the molecule were modified. The modified asparaginase dissociated into subunits and lost almost completely the enzymic activity [6].

Anti-asparaginase serum was obtained from rabbit immunized with native asparaginase [9]. The IgG fraction was isolated from the serum by ammonium sulfate precipitation (35% saturation) followed by ion-exchange chromatography with DEAE-Sephadex [10]. The Fab fragment was obtained by digestion of the IgG with papain [11]. The enzymic activity (hydrolysis of L-asparagine) was determined from the amount of released ammonia using Nessler reagent.

3. RESULTS AND DISCUSSION

Fig.1 represents the enhancement of the enzymic activity of the HNB-modified asparaginase with

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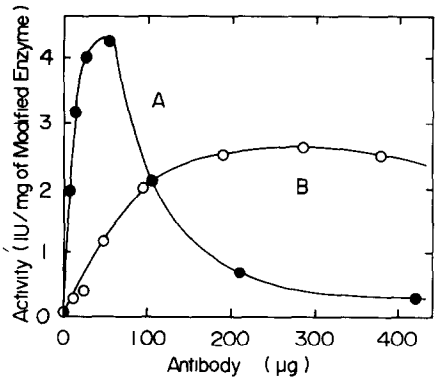

Fig.1. Activation of the enzymic activity of HNB-modified asparaginase with IgG (A) and its Fab (B). The amount of modified asparaginase, 5 μg.

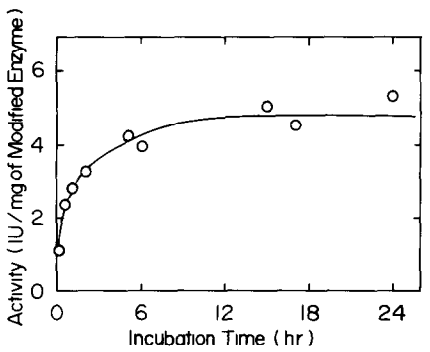

Fig.2. Effect of incubation time of the modified asparaginase (5 μg) with IgG (53 μg) at 25°C on the enzymic activity.

IgG (curve A) and its fragment, Fab (curve B). Addition of IgG to the modified asparaginase gave rise to a marked increment of the enzymic activity, as shown by curve A. The activity reached to the maximum level and then gradually decreased by increasing the concentration of IgG. The maximum activity obtained at 53 μg IgG was approx. 60-times greater than the activity of the modified asparaginase in the absence of IgG (0.07 IU/mg protein). Enhancement of the enzymic activity of the modified asparaginase was also observed in the presence of Fab, which is shown by curve B. The activity was enhanced with increasing concentration of Fab but kept a constant level (2.6 IU/mg protein) at higher than 200 μg Fab. The decrease of once-enhanced activity with IgG shown by curve A can be explained by insolubilization due to the formation of enzyme-IgG complexes.

Fig.2 shows the effect of incubation time of the modified asparaginase with IgG on the enzymic activity. The activity was enhanced by increasing the incubation time. This result was obtained by the incubation of 5 μg modified asparaginase with 53 μg IgG at 25°C, which is an optimal condition with the maximum activity (fig.1, curve A). In the absence of IgG, the activity was not enhanced by incubation at all. Note that enhancement of the enzymic activity of modified asparaginase takes place not only with IgG but also with Fab, which was obtained by immunization of native asparaginase. The same phenomenon as above was never observed with IgG obtained from other antigens such as uricase instead of asparaginase.

Since the modified enzyme would interact with IgG or Fab to form an antigen-antibody complex, a conformational change of the modified enzyme takes place, and the active site of the enzyme may be partially rearranged to exert the enzymic activity.

Kirschbaum et al. [12] reported that the asparaginase molecule (8.6 S) is dissociated into subunits (5.6 S and 4.0 S) by the dilution of an asparaginase solution. Here, we studied the dilution effect on the enzymic activity of asparaginase in the presence and absence of Fab. The result is shown in table 1. The solution containing 1 mg/ml native asparaginase was diluted approx. 10^4 times

Table 1

Retaining the enzymic activity of asparaginase by Fab fragment

Incubation time (min)	Enzymic activity of asparaginase (IU/mg)	
	In the absence of Fab	In the presence of Fab
0	156	149
40	83	140
80	12	141
240	2	144

One mg/ml of native asparaginase was diluted to approx. 10^4 times (0.09 μg/ml) with phosphate-buffered saline (pH 7.0) in the presence and absence of Fab (3.6 μg/ml)

(0.09 μg/ml) with phosphate-buffered saline (pH 7.0) with and without Fab. The diluted solution was incubated at 25°C. At a given time of incubation, the enzymic activity was determined. In the absence of Fab, the enzymic activity was markedly decreased by increasing the incubation time, probably due to dissociation of the asparaginase molecule into subunits. On the other hand, the activity was not reduced at all in the presence of Fab.

From these results, together with the data by Kirschbaum et al. [12], it can be said that the asparaginase molecule dissociates into subunits, accompanied by loss of the enzymic activity. The stabilization of asparaginase may be due to the protection from dissociation of the molecule into subunits with Fab. The activation of the HNB-modified asparaginase (monomeric form) by IgG or Fab may be attributed to a partial association of subunits to form a tetrameric structure.

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REFERENCES

[1] Rotman, M.B. and Celada, F. (1968) Proc. Natl. Acad. Sci. USA 60, 660–667.
[2] Accolla, R.S. and Celada, F. (1976) FEBS Lett. 67, 299–302.
[3] Accolla, R.S., Cina, R., Montesoro, E. and Celada, F. (1981) Proc. Natl. Acad. Sci. USA 78, 2478–2482.
[4] Melchers, F. and Messer, W. (1970) Biochem. Biophys. Res. Commun. 40, 570–575.
[5] Celada, F. and Strom, R. (1983) Biopolymers 22, 465–473.
[6] Saito, T., Obata, M., Todokoro, K., Okubo, M., Minowa, M., Yamazaki, S., Tamaura, Y. and Inada, Y. (1976) FEBS Lett. 65, 11–15.
[7] Barman, T.E. and Koshland, D.E. jr (1967) J. Biol. Chem. 242, 5771–5776.
[8] Barman, T.E. (1972) Biochim. Biophys. Acta 258, 297–313.
[9] Henney, C.S. and Isizaka, K. (1967) J. Immunol. 99, 695–702.
[10] Yagi, Y., Maier, P. and Pressman, D. (1962) J. Immunol. 89, 442–451.
[11] Porter, R.R. (1959) Biochem. J. 73, 119–126.
[12] Kirschbaum, J., Wriston, J.C. jr and Ratch, O.T. (1969) Biochim. Biophys. Acta 194, 161–169.