INFLUENCE OF SERUM ALBUMIN ON THE ACTIVITY OF A MICROSOMAL AMINOPEPTIDASE *

P. DEHM and A. NORDWIG
Max-Planck-Institut für Eiweiss- und Lederforschung, D8 München 15/Germany

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1. Introduction

One of two exopeptidases recently detected in the microsomal fraction of swine kidney homogenates [1] is an aminopeptidase. It is probably a typical "proline peptidase", specific for cleaving the *N*-terminal amino acid residue, X, from peptides of the sequence H_2N-X-Pro... [2]. This action appears to be independent of the substrate chain length and the tentative designation "X-prolyl-aminopeptidase" is suggested. Details of the assay procedure, of the isolation and partial characterization of the enzyme [2] are in preparation.

During our investigations it was found that the activity of the enzyme was dependent on the presence of manganese ions and that this effect was much more pronounced when the assay solution also contained serum albumin. A further important effect of albumin was its ability at high concentration to protect both microsomal and purified preparations of X-prolyl-aminopeptidase against decrease of specific activity caused by dilution. Since such types of activation are seldom well understood, (e.g., [3]) experiments were designed to investigate the influence of albumin on the activity of the peptidase in more detail. They revealed that membranes and comparable cell particles could also prevent the decrease of specific activity of X-prolyl-aminopeptidase obtained by lowering its concentration. The observed influence of albumin *in vitro* is, therefore, interpreted in terms of a substitution of membranes by this protein as some sort of "cofactor" for enzymatic catalysis.

2. Materials and methods

The X-prolyl-aminopeptidase used was, if not otherwise stated, a microsomal preparation obtained by stepwise centrifugation of swine kidney homogenate at 12,000 *g* and 100,000 *g* [4] or a preparation purified 250-fold by treatment of the microsomes with *n*-butanol and subsequent DEAE-cellulose chromatography and Sephadex G-200 gel filtration (see legends of table and figures). The quantitative assay for enzymic activity was based on the incubation of enzyme with Gly-Pro-Hyp (Cyclo Chem. Corp., Los Angeles, USA) at 40° in barbiturate buffer, pH 7.75, under conditions giving a linear time course for the reaction (zero order kinetics). The amount of glycine released was measured by a variation [5] of the chromotropic acid procedure of Alexander et al. [6].

Swine erythrocyte membrane was prepared according to Burger et al. [7], brain microsomes as mentioned above for kidney microsomes. Bovine serum albumin (dry, "reinst"; purity as checked by electrophoresis 100%) was purchased from Behringwerke (Marburg, Germany). The remaining compounds of table 1 were obtained from different commercial sources.

3. Results

Fig. 1 shows that enzymic activity in the presence of manganese ions is increased by X-prolyl-aminopeptidase and other kidney peptidases, e.g. imidodipepti-

* Part 1 of this series: see ref. [1].

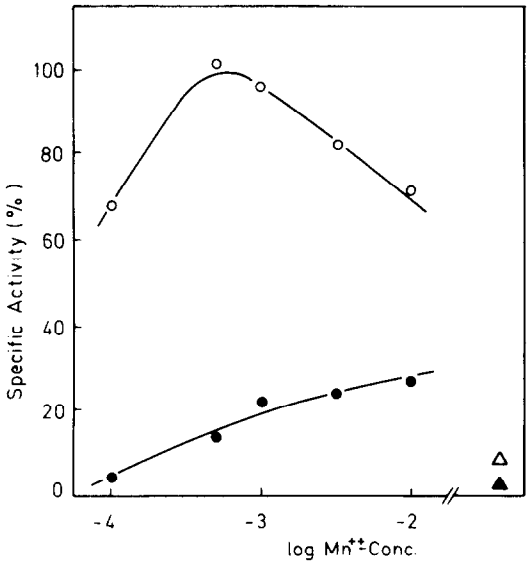

Fig. 1. Dependence of activity of X-prolyl-aminopeptidase on the concentration of manganese ions with and without albumin added. The enzyme used was an approximately 250-fold purified preparation. •—•, incubation without albumin. ○—○, albumin added (10 mg per ml of test solution). (Controls: ▲, incubation without Mn^{2+} and without albumin. △, without Mn^{2+}, but in the presence of albumin.)

dase (EC 3.4.3.7., [8]) or prolineiminopeptidase (EC 3.4.1.a, [9]). In the presence of albumin this effect is enhanced, showing a maximum of specific activity at a $MnCl_2$ concentration of 5×10^{-4} to 1×10^{-3} M (fig. 1).

No influence of albumin on the activity of the enzyme was found up to concentrations of approximately 10^{-2} mg per ml (fig. 2). At higher concentrations of albumin a dramatic increase of enzyme activity was observed; the slope of the semilogarithmic plot was identical for different preparations and for different concentrations of the peptidase. For technical reasons, still higher concentrations of albumin added could not be used.

The decrease of specific activity of X-prolyl-aminopeptidase by diluting microsomal preparations of the enzyme and the ability of added albumin to keep the specific activity constant under otherwise identical conditions is shown in fig. 3. The same effect was obtained with partially purified enzyme fractions (not shown in the figure).

The following explanations for these findings

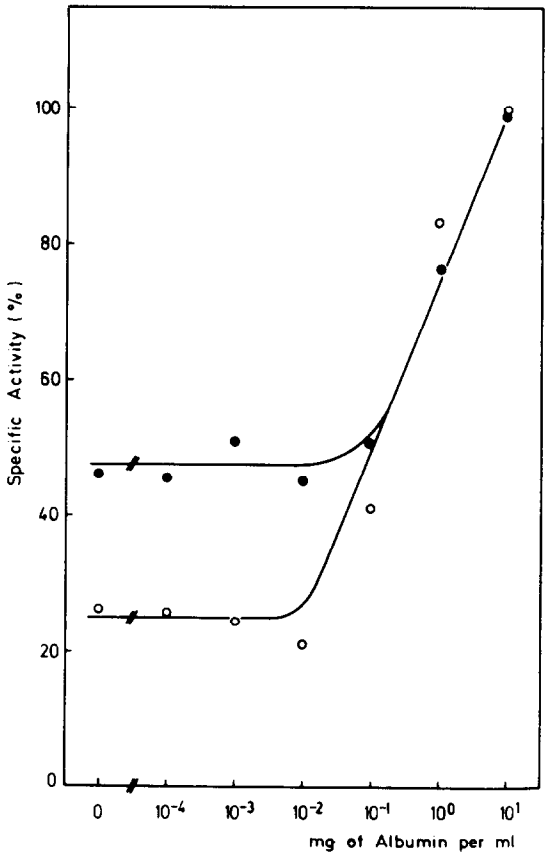

Fig. 2. Dependence of activity of X-prolyl-aminopeptidase on the concentration of albumin in the test solution. Enzyme used: preparation obtained after DEAE-cellulose chromatography, purification factor about 120. •—•, 110 μg of enzyme per ml of test solution. ○—○, 17 μg of enzyme per ml. All incubations were carried out in the presence of 10^{-3} M $MnCl_2$.

could be excluded experimentally: denaturation of the diluted peptidase solutions under the incubation conditions used; presence of inhibitory impurities i.e., heavy metal ions in one of the solvents or reagents used; albumin as an activator of SH-groups necessary for full enzyme activity; dissociation of the peptidase molecule into inactive subunits by dilution.

We were, however, able to show that a significant increase of X-prolyl-aminopeptidase activity was brought about not only by addition of serum albumin or ovalbumin, but also by fractions of membrane structures in their native state, such as erythrocyte

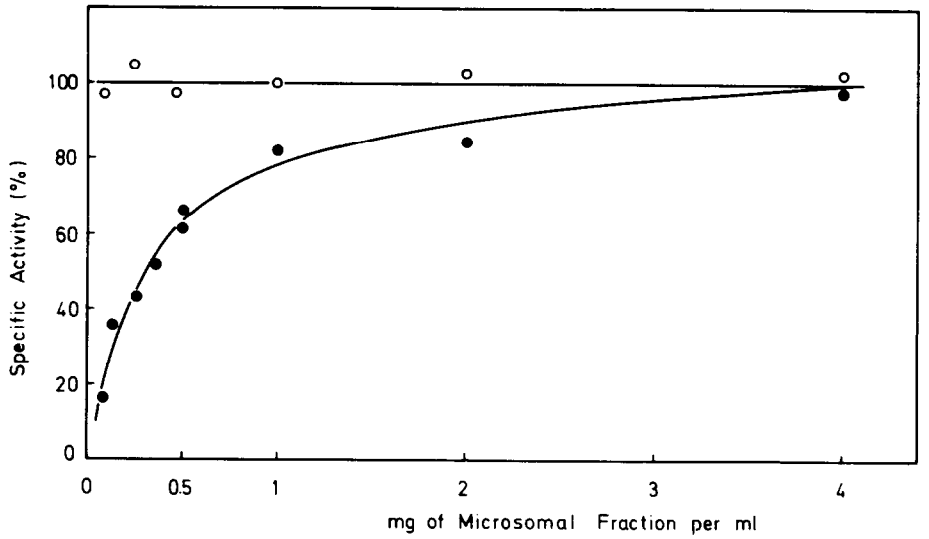

Fig. 3. Influence of enzyme concentration of the specific activity of X-prolyl-aminopeptidase. Enzyme used: microsomal fraction of swine kidney homogenate. •—•, incubation without albumin. o—o, albumin added (10 mg per ml of test solution). All incubations were carried out in the presence of 10^{-3} M $MnCl_2$.

Table 1
Effect of different additives on the enzymatic activity of X-prolyl-aminopeptidase

Addition	mg per ml of test solution	Activity (%)
None	–	29
Bovine serum albumin	10	100
Ovalbumin	10	100
Swine erythrocyte membrane	5	83
Swine brain microsomes	3	75
Swine kidney microsomes (inactivated by 5% TCA)	12	77
Swine kidney microsomes (inactivated by heating to 100° for 1 min)	12	58
Fibrinogen	10	30
Cytochrome *c*	10	26
Casein	10	11
Protamine sulfate	10	29
Dextran plasma expander	20	25

Enzyme purified approximately 250-fold was used for these experiments. All incubations were carried out in the presence of 10^{-3} M $MnCl_2$. Activities are given as percentage of the activity measured under conditions of optimal albumin concentration.

membrane or brain microsomes (table 1). Kidney microsome preparations also enhanced the reaction at least two-fold, even when they had been treated with trichloroacetic acid or by heating. It should be noted that the albumin content of preparations of microsomes was reported to be less than 0.01 mg per mg protein [10]. Other proteins (fibrinogen, cytochrome *c*, protamine sulfate) and a plasma substitute had no effect, whereas casein in preliminary experiments was slightly inhibitory (table 1).

4. Discussion

On the basis of these results, we suggest that the mechanism of action of albumin (or membrane material) is best understood as promoting formation of the native enzyme structure *in vitro*. Membranes appear to be important for the maintenance *in vivo* of a configuration of the peptidase which is optimum for its activity; albumin at high concentrations can apparently substitute *in vitro* for the particles normally present *in vivo*. This influence of albumin could be due to its property to aggregate [11, 12] and to the ability of such aggregates to mimic cell structures. This would be consistent with the findings of fig. 2.

It is of interest to note that two other proteolytic enzymes isolated from kidney microsomes, aminopeptidase "M" [13] and carboxypeptidase "P" [2], are not affected by albumin or dilution, as shown during our investigations. Those enzymes were studied as pure proteins whereas X-prolyl-aminopeptidase was obtained as a lipoprotein complex [2]. Further, a dipeptidase detected in the same preparation and probably attached to the membrane in the vicinity of X-prolyl-aminopeptidase [2], is also clearly activated by albumin and even more strongly by albumin plus manganese ions. Therefore, we assume that the *in vitro* effects described above are characteristic for enzymes isolated as lipoprotein complexes. The ability of albumin to combine easily with lipids might be another explanation for the above observations.

Our interpretation, i.e. participation of lipid components in enzymically catalysed reactions in a cofactor-like manner, is supported by several reports. Recently, Hawiger et al. [14] showed activation of lysosomal enzymes from isolated leucocytic granules by phospholipid spherules.

It is at present difficult to understand how an enzyme in its particle-bound state is activated by addition of membranes or related compounds (fig. 3). Structural changes not only of the enzyme protein itself but also of its supporting membranes on dilution *in vitro* must be considered, both effects resulting in a decrease of enzyme activity. This will remain an open question until more is known of the native structure of the organelles of the cell.

Manganese ions seem to play a role not only in the catalytic mechanism. This is indicated by the increase of activity in the presence of both the metal ions and albumin (fig. 1), an effect which is far greater than additive. Also, it should be stressed that lower concentrations of manganese ions are needed in the presence of albumin.

The findings described here could be of greater significance for the active state of particle-bound enzymes *in vivo*. Further work is needed to clarify this question.

Acknowledgements

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