Haptoglobin acting as a Natural Inhibitor of Cathepsin B Activity

OBSERVATIONS in this laboratory on various homogenates and on tumour ascites fluid[1,2] have suggested that a component in blood plasma acts as a powerful cathepsin inhibitor. In order to study this interesting finding, cathepsins B and D were first separated by gel filtration from the lysosomal pellet of calf liver homogenates and subsequently studied in their partially purified form. For the colorimetric assays edestin denatured with urea was used as a substrate in 0·1 molar acetate buffer at *p*H 5·0 according to Ottoson and Sylvén[1]. Both 0·004 molar cysteine and 0·05 EDTA must be added for maximum cathepsin B activity. Without their addition, or with added cysteine alone, extinction increments which were too small and erratic were obtained. By means of this system the cathepsin B and D activity changes could then be studied separately after the addition of small amounts of all the different available purified plasma protein fractions from normal human blood. One fraction only prepared from Cohn's fraction IV–b containing the haptoglobins exhibited a marked inhibitory effect on the cathepsin B activity. This inhibition was noted even at weak haptoglobin concentrations amounting to a quarter of the amount of enzyme. No inhibition was found on addition of other highly purified serum proteins such as transferrin, ceruloplasmin or α_1-acid glycoprotein, nor by addition of pure sialic acid, which is a component of haptoglobin. The cathepsin D activity, however, remained totally uninfluenced by all of the plasma fractions.

Through the courtesy of the KABI Co., a sample of higher purity (human haptoglobin *RFE* 76) was supplied containing more than 90 per cent haptoglobin. This preparation showed a much stronger inhibition at the same concentrations as the first fraction (Fig. 1). The inhibition was reversible. When a certain amount of enzyme and haptoglobin were mixed and a dilution curve of the activity of this sample was determined, a non-linear curve was obtained showing a downward curvature suggesting the presence of a dissociable inhibitor–enzyme complex. Preincubation of haptoglobin and cathepsin B for different lengths of time did not abolish the effect. Further analyses were made using various substrate and haptoglobin concentrations; evaluation in a Lineweaver–Burk diagram[3] showed that the kinetics of the enzyme reaction behaved as expected for a non-competitive type of inhibition. A study of the *p*H-dependence showed further that the inhibition occurred over the whole *p*H range of enzyme activity. In addition to the protein substrate edestin, haemoglobin denatured with urea was also investigated and the same result was obtained. When the artificial substrate BANA (*N*-benzoyl-DL-arginine-β-naphthylamide) was used, however, according to Goldbarg and Rutenburg[4], no enzyme inhibition was found. This may be because of the hydrophobic character of BANA.

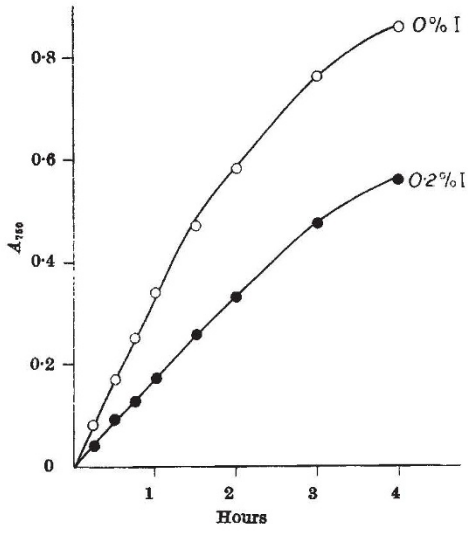

Fig. 1. Hydrolysis of urea-denaturated edestin by purified cathepsin B at *p*H 5·0. Each sample of 50 μl. contained 600 μg edestin, 20 μg cathepsin B. I = 0, no haptoglobin added; I = 0·2, 20 μg haptoglobin added; cysteine + EDTA present.

Experiments were further designed to separate a mixture of haptoglobin and cathepsin B on a 'Sephadex *G*-200' column. The two components could be separated and the original activity of cathepsin B was regained. In the separation diagram, however, the curve of cathepsin B seemed distorted when compared with a diagram where cathepsin B alone had been filtrated. This is to be expected when the small molecule of cathepsin B is dragged along with the much larger haptoglobin molecule before they dissociate.

Trypsin was investigated in the same way, but no inhibition was found when haptoglobin was added. There is thus a considerable difference in behaviour between cathepsin B and trypsin toward haptoglobin. Both enzymes split peptide chains at the same amino-acids. Trypsin splits the haptoglobin molecule easily[5], but cathepsin B cannot hydrolyse haptoglobin. It seems that the inhibitor is reversibly bound to cathepsin B in a manner which alters the enzyme structure so that its active group cannot work.

It seems, from the literature, to be very difficult to obtain a haptoglobin free from contaminations of other proteins. The haptoglobin we used carries less than 10 per cent of contaminating substances. Results so far obtained strongly suggest that haptoglobin or one of its major components (?) is the cathepsin inhibitor. Because part of the cathepsin B activity extends above *p*H 7 the presence of a physiological plasma inhibitor would seem of importance for the protection against active proteolysis. It is also noticeable that conditions such as inflammatory processes, tumour diseases and major tissue injury where cathepsin B will be liberated are associated with increased amounts of haptoglobin in the blood plasma[6].

We thank Dr R. Ottoson and Dr B. Sandberg of the Research Department of the KABI Group, Inc., Stockholm, for the supply of purified serum protein fractions.

This work was partially supported by institutional grants from the Jubilee Fund and Cancer Society of Stockholm.

O. SNELLMAN
B. SYLVÉN

The Cancer Research Division,
Radiumhemmet,
Karolinska Institute,
Stockholm 60.

Received September 18, 1967.

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Improved Multi-enzyme Analyser

BY a relatively simple adaptation of the Technicon auto-analyser system, it was possible to develop a technique for the automatic assay of groups of enzymes[1]. The reaction mixture—for example, a tissue homogenate, buffer and any co-factors common to all the enzymes in the group—flows continuously through the analyser. Different substrates are then introduced through the automatic sampler, so that the various enzymes in the homogenate which react with these substrates are assayed in sequence.