ANTAGONISTIC ACTION OF CHONDROITIN SULPHATE AND CETYLPYRIDINIUM CHLORIDE ON HUMAN LIVER β-GALACTOSIDASE

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1. Introduction

The reduced activity of β-galactosidase found in the liver of patients with mucopolysaccharidosis [1–3] can be explained by assuming the formation of a complex between the β-galactosidase molecules and different kinds of mucopolysaccharides [4]. Chondroitin sulphate binds with several hydrolases in different ways. As a result of this binding a new isoenzyme profile is seen, when glycosidases are mixed with acid mucopolysaccharides, whereby some isoenzymes are unaffected while others show a more acid isoelectric point. Total hydrolase activity, however, as measured with synthetic methylumbelliferyl substrates, is only slightly inhibited, except in the case of β-galactosidase which is strongly inhibited [4]. I now present further data on the occurrence of such complexes, the resulting inhibition of the β-galactosidase activity and the possible dissociation of the complex.

2. Methods

All experiments were done with post-mortem adult human liver which had been removed 6 hr after death and stored at −20°C. Liver homogenate (10% w/v) in distilled water was frozen and thawed 5 times, sonicated for two periods of 30 sec and centrifuged at 15 000 rpm.

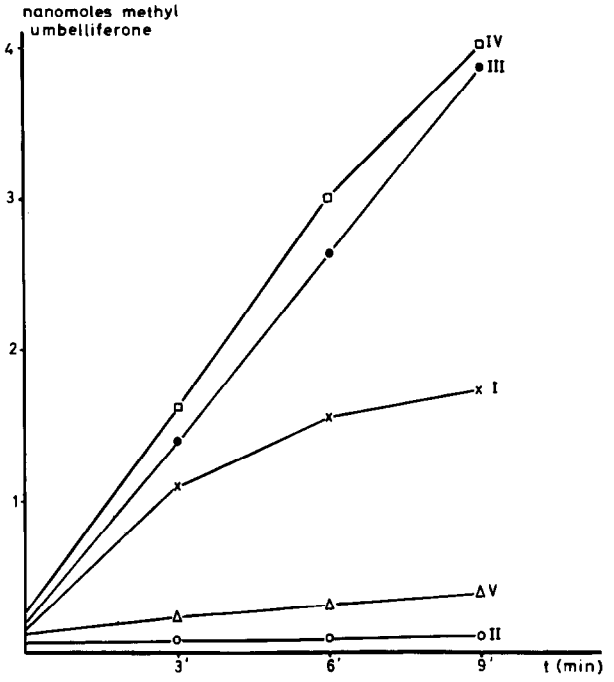

Fig. 1. Effect of chondroitin sulphate and CPC on liver β-galactosidase. The final concentration of the supernatant was made 3.3% with: I) 1.60 mg/ml chondroitin sulphate; II) 1.60 mg/ml CPC; III) 1.60 mg/ml chondroitin sulphate and 1.60 mg/ml CPC; IV) distilled water; V) 1.60 mg/ml of chondroitin sulphate. After adding the chondroitin sulphate to the liver homogenate the mixture was left for 10 min at room temperature. Then CPC was added as indicated. In V) CPC was added first and chondroitin sulphate 10 min later.

Different amounts of chondroitin sulphate from shark cartilage (Koch-Light, Colnbrook, England)

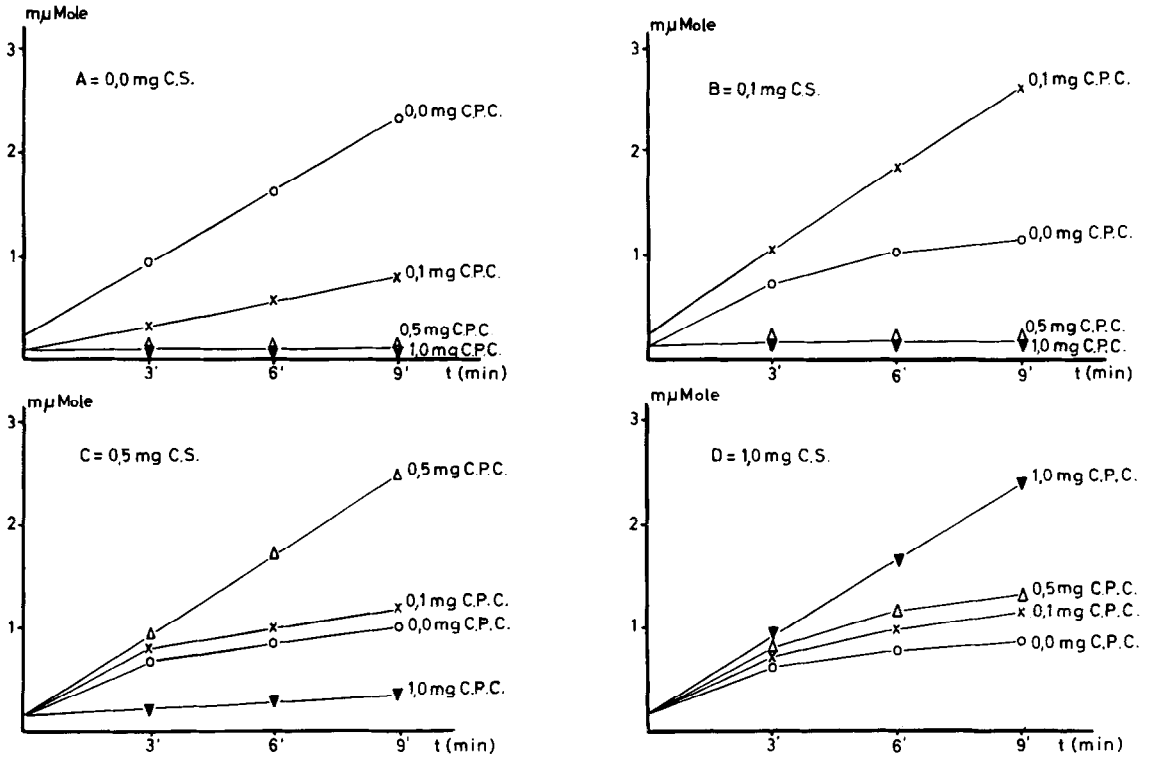

Fig. 2. Antagonistic effect of chondroitin sulphate and CPC. Liver homogenate (3%) was treated without (A) and with 0.1 mg/ml (B), 0.5 mg/ml (C) and 1.0 mg/ml (D) of chondroitin sulphate. After 10 min water or CPC 0.1 mg/ml, 0.5 mg/ml or 1.0 mg/ml was added. Enzyme activity was then measured as indicated in fig. 1.

were mixed with this liver homogenate. Cetylpyridinium chloride was added to the liver homogenate alone, or before or after the addition of chondroitin sulphate.

The mixtures were allowed to stand for 10 min at room temperature before enzyme activity was measured with methylumbelliferyl-β-*O*-galactoside (2 mM) as substrate at pH 4.0 (0.05 M acetate). The fluorescence of the methylumbelliferone was measured after zero, 10, 20 and 30 min incubation at 37°C by diluting the enzyme mixture 60 times with 0.1 M diaminoethane, pH 10.5.

The technique of electrofocusing and isoenzyme detection has been described previously [5].

3. Results and discussion

As mucopolysaccharides can be precipitated from urine by cetylpyridinium chloride (CPC) [6] I examined whether the addition of cetylpyridinium chloride could counteract the inhibition brought about by the previous addition of the chondroitin sulphate. Therefore CPC was added either to a liver homogenate or to a mixture of liver homogenate and chondroitin sulphate. In addition chondroitin sulphate was also added to a previous mixture of liver homogenate and CPC (fig. 1). The addition of chondroitin sulphate alone produced an almost immediate reduction of the β-galactosidase activity and linearity of the reaction was lost after a few minutes of the enzyme reaction. Addition of CPC alone produced a complete inactivation of the β-galactosidase activity. However, when CPC was added to the mixture of liver and chondroitin sulphate, there was a complete restoration of the activity. In contrast the addition of chondroitin sulphate to a mixture of liver and CPC failed to restore the activity significantly. I presume that the inhibition of the enzyme activity by chondroitin sulphate is due to the formation of a stable complex

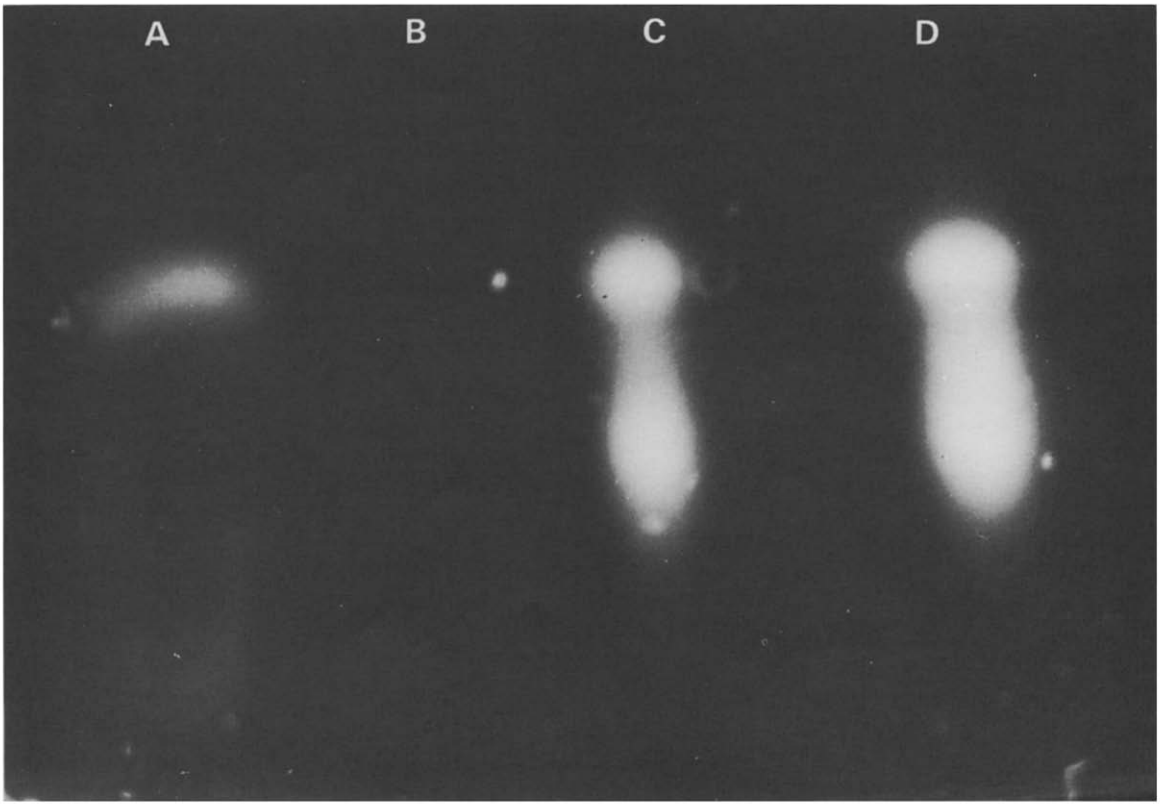

Fig. 3. Isoelectric focusing in polyacrylamide gel of β-galactosidase of liver: A = 50 μl of a mixture (1:1) of liver homogenate (10%) and chondroitin sulphate (5 mg/ml); B = 50 μl of a mixture (1:1) of the same liver homogenate and CPC (5 mg/ml); C = 75 μl of a mixture (1:1:1) of the same liver homogenate chondroitin sulphate (5 mg/ml) and CPC (5 mg/ml) – the last being added 1 hr after the mixing of the first two; D = 25 μl of a 10% liver homogenate. The electrofocusing was started about 15 min after sample application. A pH-gradient from pH 3.0 (bottom of the photograph) to pH 5.0 (top of the photograph) was used.

and that this complex can be dissociated by CPC. On the other hand CPC is a strongly irreversible denaturating agent for the enzyme alone and this denaturation cannot be reversed by subsequent addition of chondroitin sulphate.

Complex formation between chondroitin sulphate and β-galactosidase is a rapid reaction and an almost immediate inhibition is seen when one mixes liver homogenate and chondroitin sulphate, reaching its maximum after about 30 min.

This effect of chondroitin sulphate is suppressed by about the same amount – on a weight for weight basis – of CPC (fig. 2). In these experiments the enzyme is measured alone and in the presence of three different amounts of chondroitin sulphate. In each experiment total activity was restored to 100% only when the same amount of CPC was present. If the amount of chondroitin sulpahte was higher than the amount of CPC there was a non-linear reduced enzyme activity and when the amount of chondroitin sulphate was lower than that of CPC the reaction rate dropped almost to zero.

Assuming that one sulphate molecule is attached to every disaccharide-unit of the mucopolysaccharide chain, one may conclude that the antagonistic action proceeds through an equimolecular association of 1 CPC molecule (mol. wt. 358) and 1 sulphated dissaccharide unit (mol. wt. 411).

The complex between chondroitin sulphate and β-galactosidase cannot be dissociated by isoelectric focusing in polyacrylamide gel. The isoenzyme distribution is grossly altered by adding chondroitin sulphate

to the liver homogenate, the fractions with the more acid isoelectric points disappear while the neutral isoenzyme apparently is not affected. However, after adding CPC to the mixture of liver and chondroitin sulphate the isoenzyme distribution recovers completely, while adding CPC to liver homogenate alone, again gives complete denaturation (fig. 3).

All these data are compatible with the following hypothesis:

1. chondroitin sulphate binds reversibly but strongly to the more acidic β-galactosidase isoenzymes from human liver with concomitant inactivation of the enzyme activity;
2. CPC is an irreversible denaturating agent for β-galactosidase, probably by unfolding the polypeptide chain;
3. chondroitin sulphate has a higher affinity for CPC than for β-galactosidase, and the compounds interact stoichiometrically.

The strong interaction between two components – mucopolysaccharides and β-galactosidase – which are present in normal lysosomes raises the question whether the phenomenon described here may have any physiological significance regarding some lysosomal function.

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