A CASE OF SPECIFIC ADSORPTION OF ENZYMES

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In this journal I have recently given an account of experiments on the anti-tryptic action of charcoal.¹ The process by which trypsin was rendered inactive could be divided into two consecutive stages: the mere adsorption or taking up of the trypsin by the charcoal, and the fixation of the same. The amount of trypsin adsorbed could be represented by the difference between the digestive power of the trypsin solution before the treatment with charcoal and that of the filtrate after the treatment. In addition it was found that the action of charcoal was very much like that of the tryptic antibody in the serum, and it was suggested that the effect was brought about in the same way in both cases.

I have extended these investigations, as far as the mere adsorption is concerned, to the proteolytic enzymes of the spleen, at the same time trying the influence of another solid substance, namely, kieselguhr. That kieselguhr may be capable of taking up enzymes, has been shown by Dauwe.² Previously I have found that the spleen of the ox contains at least two proteolytic enzymes, the one acting principally in an alkaline medium (α -protease) and the other in an acid (β -protease).³ In addition, the spleen (and the serum) contains an antibody which decidedly checks the digestion with alkali, whilst it has practically no effect upon the digestion with acid.⁴ Since this antibody is destroyed by the action of weak acetic acid, the spleen substance shows a stronger effect with alkali after previous treatment with acetic acid than it does without treatment with acid. After previous treatment with acid the spleen-pulp gave,

1. *Bio-Chemical Journ.*, I, 484, 1906.

2. *Hofmeisters Beiträge*, Vol. VI, p. 427, 1906.

3. *Journ. of Physiol.*, XXX, p. 155, 1903.

4. Hedin: 'An explanation of the influence of acid and alkali on the autolysis of organs' (*Hammarstens Festschrift*, Upsala, 1906).

200 c.c. of 2·5 per cent. casein in 0·25 per cent. Na_2CO_3 solution was taken to each sample. In the case of the β -protease, acid reaction was effected by adding 5 c.c. of 20 per cent. acetic acid to each specimen. The estimation of the digestion which had taken place was carried out by determining the nitrogen in equal volumes of the tannic acid filtrates, as described in previous papers. The figures given represent cubic centimetres of decinormal acid required for neutralising the ammonia obtained.

		With alkali	With acid
10 c.c. Protease sol. + 10 c.c. H ₂ O ...	...	12.2	12.9
20 c.c. „ ...	...	28.85	24.1

In accordance with what I have found before,¹ the effect with alkali shows a tendency to increase more rapidly than the amount of enzyme. On the other hand, the effect with acid is very nearly proportional to the amount of enzyme.

The charcoal used was animal charcoal, prepared from bones and purified with HCl. The kieselguhr contained only traces of substances soluble in water and the watery extract of the same gave a perfectly neutral reaction.

In order to effect adsorption of the enzymes equal volumes of enzyme mixtures, prepared as above, were heated with equal weights of charcoal and of kieselguhr *in a neutral medium* at 37° for about two hours. In the same way a control was heated without any adsorbing substance. Then the charcoal and the kieselguhr were filtered off and equal volumes of the control and of the filtrates were taken for digestion with casein, as above.

The results obtained with two different enzyme mixtures were as follows :—

I				Effect with acid	
		With alkali	With acid	<hr/> Effect with alkali	
Without adsorption ...	...	22·8	31	1·36	
After adsorption by charcoal	...	4·2	6·05	1·44	
„ „ kieselguhr	...	9·3	27·7	3·0	

II					
Without adsorption ...	...	11·5	19·6	1·7	
After adsorption by charcoal	...	3·6	6·3	1·75	
„ „ kieselguhr	...	6·0	17·7	2·95	

Since the same weight of charcoal and of kieselguhr was used, charcoal in this case exercises a more powerful adsorption than does kieselguhr. Both enzymes are adsorbed equally readily by charcoal, the ratio between the effect with acid and with alkali being the same after the adsorption as before.

On the other hand, the effect of the kieselguhr is different inasmuch as a pronounced reduction in the digestion with alkali takes

1. *Hammarstens Festschrift.*

place, whilst the digestion with acid is very little affected. In fact the slight diminution in the digestion with acid might depend upon adsorption of the α -protease, since this enzyme, although acting principally with alkali, might be able to exercise some slight action with acid too. Kieselguhr therefore evidently adsorbs a considerable amount of α -protease, whilst it must be left undecided whether a slight adsorption of the β -protease takes place or none at all.

In the above experiments it was not proved that the process of adsorption had come to an end when the casein was added, and therefore the results obtained do not necessarily correspond to the final distribution of the enzymes. The results show that the *rate of adsorption* by charcoal is approximately the same for both enzymes, whilst the *rate of adsorption* by kieselguhr is higher for the α -protease than for the β -protease.

The experiments bear out the fact that the adsorption of enzymes can be, to a large degree, specific.

In this connection it is interesting to note similar experiments carried out by Bayliss,¹ who found that filter paper adsorbs congo-red and acid fuchsin in the same proportions, like charcoal does the two spleen enzymes, whereas gelatine adsorbs a larger proportion of congo-red than it does of acid fuchsin, thus forming a parallel to the kieselguhr in the case of the spleen enzymes.

The amounts of spleen enzymes adsorbed by charcoal in the above experiments are rather insignificant, as compared with the enormous amounts of trypsin adsorbed by the same amount of charcoal in my investigations on the adsorption of this enzyme.² On the whole, charcoal seems to possess quite an exceptional aptitude for adsorbing trypsin. This might, perhaps, depend upon some specific relationship.

It must, however, be borne in mind that the trypsin solutions used in my experiments were nearly free from proteins, whilst the solutions of the spleen enzymes held large amounts of proteins, which could not be digested away lest the enzymes were too much weakened. In the case of trypsin, it was found that the addition of

1. *This Journal*, I, p. 212, 1906.

2. *This Journal*, I, p. 484, 1906.

casein prevents some trypsin from being adsorbed by the charcoal; in fact, casein is capable of extracting trypsin already adsorbed by the charcoal. Therefore the large amounts of proteins present might account for the spleen enzymes being less readily adsorbed by charcoal than trypsin. On the other hand, the different behaviour of the two spleen enzymes to kieselguhr cannot be accounted for by a different amount of proteins, because they were acted upon in the same fluid.

In conclusion, I wish to call attention to rather an important conclusion which can be drawn from the above experiments, namely, that juices prepared with kieselguhr, as proposed by Buchner in the case of yeast, do not necessarily contain the enzymes in the same proportions as in the cells used. The enzymes might be adsorbed by the kieselguhr, or perhaps by the solid remains of the cells, and consequently some of them might, perhaps, be completely retained in the press-cake.

SUMMARY

The α and β -protease in the spleen of the ox, are adsorbed in the same proportions by charcoal, whilst kieselguhr adsorbs the α -protease much more readily than the β -protease, and it seems doubtful whether the latter be adsorbed at all. This fact might account for the observation that juice pressed from the spleen after grinding and mixing with kieselguhr contains relatively less α -protease than does the organ itself.