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BIOSPECIFIC OR NON-SPECIFIC ADSORPTION OF AMYLASE ON STARCH IN STARKENSTEIN'S EXPERIMENTS (1910)?

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CONTENTS

1. INTRODUCTION

From the end of the 19th century it has widely been accepted that enzyme specificity is dependent on the steric configuration of the enzyme and substrate (Emil Fischer, Abderhalden, Oppenheimer, etc.). An analogy with the specific interaction between an antigen and an antibody and with Ehrlich's theory for the explanation of this phenomenon was considered.

According to Henri[1] (quoted by Starkenstein from a monograph) "every actual splitting activity of an enzyme is preceded by the first phase in which a labile chemical compound is formed between the enzyme and substrate at the site of their specifically acting atom groupings."

In order to prove and clarify these concepts, efforts were undertaken to demonstrate the binding of the enzyme to the substrate. Such an adsorption was reported, *e.g.*, by Hedin[2,3]; Michaelis and Ehrenreich[4] objected that this did not prove a specific binding since enzymes can also be adsorbed by adsorbents which are not their substrates. Starkenstein[5] did not share this objection, since, for example, antibodies are adsorbed specifically by the respective microorganism, though they are also adsorbed non-specifically by a number of other adsorbents.

1.1. Starkenstein's experiments on the adsorption of liver amylase on starch

At the 6th International Symposium on Bioaffinity Chromatography, Prague, 1985, the 75th anniversary of the publication of a paper by Starkenstein[5] (on enzyme activity and the influence of neutral salts on it) was recorded and a poster concerning this subject and Starkenstein's scientific and literary achievements was displayed. Part

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of its contents has appeared in the symposium volume[6], thus we will not deal here with the remarkable personality of the great pharmacologist (born December 18, 1884 in Poběžovice, died in the concentration camp at Mauthausen, November 11, 1942).

In line with the scientific interests of his supervisor, Privat-Dozent W. Wiechowski (Starkenstein was a "Volontär", demonstrator and, after graduating in 1909, Assistent at the Institute of Pharmacology), some compounds of biochemical interest were studied: inositol, inositol phosphate, glycogen and amylase.

The present essay is devoted to Starkenstein's second paper[5] of the series [7] of studies on liver amylase Both the full paper[5] and the oral version[8–10] were presented in conjunction with the communication of Weil[11] on the mechanism of the complement binding by the complexes of corpuscular or soluble antigen and the respective antibody (amboceptor). The enzyme preparation used in this study[5] was prepared according to Wiechowski[12] by air-drying of ground liver and resuspension of the powder. Fat can then be removed with toluene. Remarkably, this paper, essentially the result of the scientific activity of a student of medicine, is generally accepted as being the first to describe biospecific sorption[13,14].

Starkenstein[5] speculates. "The study of the way of binding between enzyme and substrate has been made generally difficult by the fact that the enzyme has not been sufficiently inactivated to prevent immediate splitting of the substrate after the binding has taken place Separation of these phases is possible with diastase." Since amylase (diastase) requires chloride as its activator and is therefore inactive after dialysis (Starkenstein quotes six papers dating from 1899[15,16] to 1908[17–20]), he decided to test whether the "compound" formed between the enzyme and its corpuscular substrate could be isolated in the absence of electrolytes. (In the preceding paper[7] on liver amylase he had mentioned, on p. 202, the adsorption of the enzyme on insoluble rice starch.) Corpuscular starch adsorbed amylase from the dialysed solution, the complex could be washed with distilled water and, after activation by the addition of chloride, amylase could be identified. Specificity of this sorption was suggested by the lack of adsorption of the uric acid-degrading (oxidative) enzyme on corpuscular starch; further proteins were not tested as an additional check of specificity.

If this had been the end of the story, the reader would have concluded that the formation of the specific enzyme–substrate complex ("compound") was thus confirmed. However, Starkenstein was not satisfied. He wrote "If this phenomenon had to be interpreted as a specific chemical binding between substrate and enzyme, this binding should take place even with dissolved starch " He incubated the amylase preparation with a solution of soluble starch in the absence of chlorides, shook it with solid starch and centrifuged Even in the presence of soluble starch, amylase was completely adsorbed on corpuscular starch and no enzyme activity was detectable in the supernatant after addition of sodium chloride. Starkenstein arrived at a rather rash conclusion: "It follows from these experiments that a chemical binding between the enzyme and substrate does not take place. The adsorption of the enzyme by the corpuscular starch is thus a purely physical phenomenon and does not in any way depend on enzymic activity."

Starkenstein's results and conclusions parallelled those of Weil[11] in the Institute of Hygiene at the same Faculty (Weil's disease bears his name). Starkenstein and

Weil quote each other in their respective articles and lectures For example, Starkenstein[5] wrote: "A similar process has been observed by E. Weil when he was studying the binding of complement by dissolved bacterial substance and immune bodies, and he arrives also at the conclusion that both substances are present side by side without producing a common compound; he points to certain analogies between these processes and the enzyme effect, which are substantiated by the experiments reported above".

1.2. Adsorption of amylase on starch in the experiments of Ambard and other workers

Ambard[21–23] later studied biospecific adsorption of amylase from various animal sources on starch and elaborated a purification and routine analytical method for amylase based on these phenomena Amylase is adsorbed on corpuscular starch and desorbed ("defixed") by dissolved starch or glycogen! What is the essence of the contradiction between Ambard[23] and Starkenstein[5]? It is possibly due to the rôle of chlorides in assisting the formation of the enzyme–substrate complex. In their absence the complex between amylase and soluble starch is not produced or only to a limited extent, as noted by Ambard[23] on p. 61· "glycogen solution, boiled and repeatedly reprecipitated by ethanol, showed defixation of 4% whereas in the presence of salts it was 98% " He then drew attention to the hypothesis put forward by Henri and Bierry[24] as early as 1905, namely that the condition for the diastatic effect is the formation of the enzyme–substrate complex and that the formation of this complex requires the intervention of some electrolytes. Ambard believes that his "defixation" experiments proved Henri's hypothesis* which had been formulated speculatively on the basis of an analogy with dyeing processes. On the other hand, Ambard did not confirm Henri's hypothesis that the reaction (pH) also affects the amylase activity by influencing the formation of the enzyme–substrate complex.

If chloride were required for enzyme–substrate binding, how can we explain that in Starkenstein's experiments an insoluble complex between corpuscular starch and the dialysed amylase preparation was obtained? The likely explanation is that the corpuscular starch contained traces of chloride sufficient for the binding of the low amount of amylase present in the mixture, but allowing only a "just perceptible" demonstration of the hydrolytic effect. (Starkenstein commonly used Wohlgemuth's[19,20] method of dilution series and iodine staining, but in the adsorption experiments he followed up the splitting of starch by a reduction test.) The content of amylase in the liver must be very low. Some authors (including Kamarýt[26]) could not detect any amylase in the liver. Among those who confirmed its presence are Wohlgemuth[17,18] and Tiger and Simmonet[27].

Further papers[28–37] on the adsorption of amylase on starch have been reviewed by Porath and Sundberg[13] and by Nishikawa[14]. Various kinds of amylase may thus be differentiated[32]

Exoamylase (α), which is adsorbed, may be separated from endoamylase (β) which is not adsorbed[30,36]; a concentration gradient of soluble starch was used in this separation[36,37]. The name of F. Chodat[28] (adsorption of malt amylase on starch)

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* The problem is evidently complex. According to Levitzki and Steer[25], chloride is the activating effector of pig pancreatic amylase which increases k_{cat} towards starch 30 times, but does not change K_M

reminds chromatographers of Tswett, among whose botany professors in Geneva was Robert Chodat (1865–1934).

I will leave out of discussion the point that the methods used by Starkenstein for testing the enzyme would not differentiate amylase from enzymes degrading starch by phosphorolytic routes, if phosphate had been present. De la Haba[38] used starch powder for preliminary purification of glycogen phosphorylase of rabbit muscle. (Yet phosphorylase does not depend on chloride in the way as amylase.)

1.3. Notes on the life and scientific activity of E. Starkenstein

A number of articles[6,39–43] have been devoted to the life and scientific and teaching activities of the great pharmacologist (and also historian of science) in Czechoslovakia, in his Dutch exile (from 1939) and in German prisons (from 1941). A carefully compiled and nearly complete bibliography of Starkenstein's publications (243 entries) is to be found in the article by Senius[43]. Although the series on glycogen and amylase comprised seven full papers and a number of brief reports, Starkenstein did not return to the problem of sorption of the enzyme on its substrate, not even in the last paper of the series, which again dealt with the influence of chloride on the activity of amylase[44]

It may also be of interest that Starkenstein, though deeply involved in many research and literary projects in his own discipline of pharmacology, and active in several fields of cultural life, retained an interest in chemistry. He was, together with the biochemist Felix Haurowitz, among those workers of the German University of Prague who, in the thirties, used to attend seminars organized by Jaroslav Heyrovský, the inventor of polarography, in his department of Physical Chemistry in the Faculty of Natural Sciences, (Czech) Charles University[45].

2 CONCLUSIONS

We see that Starkenstein, whose main scientific work eventually centered on pharmacology, especially of iron and drug combinations, is remembered[13,14] as the first to describe clearly biospecific sorption, though, at the end of the article[5], he dismissed this interpretation of his findings, basing his point of view on his additional experiments with soluble starch. In this respect, I share the opinion of Turková, who argued that Starkenstein's experiments did actually demonstrate biospecific sorption, irrespective of whether he himself tended toward an alternative interpretation.

3 SUMMARY

A paper by Starkenstein on the influence of chloride on the enzymatic activity of liver amylase, including a description of the adsorption of the enzyme on starch, is discussed with reference to the early concepts of enzyme–substrate binding and to later work on specific adsorption of amylase on starch.

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