3047

On the unity of castor lipase.

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The peculiar activation of castor bean lipase by acids and the changes that this lipase undergoes during germination have attracted the interest of investigators for a long time. The contradictory views on the nature of the activation of castor lipase by acids are partly due to the different character of the two kinds of castor lipase preparations commonly used. Castor lipase in the form of "lipase cream" is in no way altered by water, while the lipase preparation known as "defatted seed" is easily destroyed by it.

In our experiments lipase cream and defatted seed were prepared according to Willstätter and Waldschmidt-Leitz[1]; the lipase estimations were carried out as described by Willstätter,[2] olive oil being used as substrate.

When prepared from resting seed, lipase cream and defatted seed both show that castor lipase has an exceptionally sharp pH optimum around pH 4.7 and no activity in a neutral medium; the lipase is not injured by dilute alkali and its synthesizing power is practically *nil.* If we, however, subject lipase cream to a treatment with aqueous reagents of a pH of 4.7 (dilute HCN, acetate buffer, citrate buffer), it becomes evident that we are dealing now with a lipase which has properties quite different from what they were before such treatment. The former sharp pH optimum at pH 4.7 has disappeared, and castor lipase is now more uniformly active over a much wider range of the pH and active even in a neutral medium. Further, castor lipase thus treated has become easily destructible by dilute alkali and gained considerable synthetic power. It is important to lay stress upon our finding that the characteristic alterations of lipase cream by aqueous reagents of pH 4.7 take place, while the total lipolytic activity is fully pre-

[1] Willstätter, R., and Waldschmidt-Leitz, E., *Ztschr. f. physiol. Chem.*, 1924, cxxxiv, 161.

[2] Sandberg, M., and Brand, E., *J. Biol. Chem.*, 1925, lxiv, 59.

served* These alterations of the lipase, therefore, may be characterized as an "activation" of castor lipase.

If we, however, subject lipase cream to a treatment with aqueous reagents of a pH lower than pH 4.7, or if we treat lipase cream with pepsin solutions, then the activation of castor lipase is complicated by a simultaneous destruction of the enzyme. As far as the characteristic activation of castor lipase by acids is concerned, the action of *watersoluble* acids on lipase cream is not different for different hydrogen ion concentrations, but the same for any pH lower than pH 4.7. It appears to be immaterial, whether HCN, HCl, water soluble fatty acids, amino acids, acid buffer solutions, or pepsin solutions inactivated by heating are used, and whether the acid is formed by autolysis or by pepsin hydrolysis of proteins.

In order to more closely scrutinize the activation of castor lipase, it seemed advisable to use as the enzyme preparation defatted seed instead of lipase cream. We have, however, to recall the fact that the lipolytic activity of defatted seed is easily destroyed by water. If we now let buffer solutions of pH 4.7 act on defatted seed, we expect to find that the lipase is, on the one hand activated by the acid, and on the other hand is partially destroyed by water. But we know already from our experiments with lipase cream that activation of castor lipase and lipase destruction are two separate processes, which may occur simultaneously, but which are not necessarily associated.

An activated lipase powder can be obtained with a destruction of only 35 per cent by treating defatted seed for five minutes with 0.5 N acetate buffer (pH 4.7), centrifuging, washing three times with water for one minute, and drying.[3] Twenty gr. defatted seed of a phyto lipase value of 0.25, containing 501 phyto lipase units, yield 8.6 gr. activated lipase powder of a phyto lipase value of 0.37 containing 321 phyto lipase units (yield 64 per cent).

Activated lipase powder splits fats not only in a neutral medium, but to a certain extent also at an alkaline pH. We find the pH optimum of activated castor lipase around pH 5.6.

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* Such experiments may be carried out under conditions similar to those described by Willstätter and Waldschmidt-Leitz[1] in table 20, p. 211.

[3] Tanaka, Y., *J. Coll. Engin.*, Imp. Univ. Tokyo, 1910, v, 25; *Cf. Chem. Zentralbl.*, 1910, ii, 1637.

The following considerations may offer a tentative explanation of the process concerned in the activation of castor lipase by aqueous reagents of pH 4.7. According to Jacques Loeb, when we have Na gelatinate and add acid, the gelatine salt will give off its Na until the isoelectric point of gelatin is reached, where no more Na is combined with gelatine. Our experiments seem to indicate that castor lipase, as it exists in the resting seed, is combined with a seminal protein on the alkaline side of the isoelectric point (pH 4.7) of that protein, involving the lipolytically active group of the lipase. When we have lipase cream or defatted seed and add acid, castor lipase is given off from its combination, involving the active group of the lipase, until the isoelectric point of the seminal protein is reached where no more lipase is combined. A re-combination of the activated lipase with the seminal protein seems to take place under certain experimental conditions[4] and such re-combination may explain why activated lipase cream is sometimes found to be inactive at a slightly alkaline pH.

It should be noted, however, that when castor lipase is liberated by acid from its inactive combination with a seminal protein, we unfasten only the first of the many ties by which the lipase is linked to the protein material of the seed. Moreover, it becomes evident that the capacity of castor lipase for activation and the primary location of the pH optimum at pH 4.7 are characteristic only of the way in which the lipase is stored in the resting castor bean, but do not constitute a property of the enzyme itself.

As regards the activation of castor lipase during germination, it seems to take place in a way similar to the *in vitro*-activation of the lipase by acid as Connstein[5] pointed out long ago; for it is well known that there occurs in the first stages of germination a distinct shifting of the pH to the acid side. Our knowledge of germination, however, is too limited to understand the details of lipase activation and lipase destruction in the germinating seed.

We should like to mention that activated castor bean lipase cream is especially suitable for use in lecture experiments, when

[4] Willstätter and Waldschmidt-Leitz,[1] experiment 6 on p. 219.

[5] Connstein, W., Hoyer, E., and Wartenberg, H., *Ber. deutsch. chem. Ges.*, 1902, xxxv, 3988.

it is desirable to demonstrate to a large audience within on hour the synthesizing as well as the hydrolyzing power of one and the same enzyme preparation.

3048

L. acidophilus and L. bulgaricus as influenced by surface tension.

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Following the suggestive report of Albus and Holm[1] on the effect of surface tension on lactobacilli, studies were undertaken with some of the same organisms used by them, and we employed their technic, as described in an unpublished paper generously placed at our disposal. Sodium ricinoleate was used as a depressant, for which we are indebted to Dr. W. P. Larson of the University of Minnesota, who very kindly supplied us with a pure product.

Six strains each of *L. bulgaricus* and *L. acidophilus* were tested. While no growth of the former took place in media depressed below 42 dynes, the growth of *L. acidophilus* was abundant at this point and considerably below. *L. acidophilus* was inhibited at 35 dynes as measured by the drop-weight method.

The results, therefore, are in close agreement with those of Albus and Holm, and it appears that surface tension may well be considered an effective criterion for differentiating the very closely allied *L. acidophilus* and *L. bulgaricus*.

[1] Albus, W. R., and Holm, G. E., PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 337-338.