BIOLOGIA PLANTARUM (PRAHA)

7 (2) : 86—92, 1965

Adaptability in Enzymes

KRISHNA BAHADUR and INDRA SAXENA

Chemistry Department, University of Allahabad, Allahabad, India

Received August 20, 1964

Abstract: In studying inhibitors and activators of enzymes it has often been found that inhibitors act as activators when in very low concentrations and that certain activators (in suitable concentrations) show inhibition. Similar results were obtained in the present work in studying urease from soya bean in the presence of lead acetate. Lead acetate is normally an inhibitor of urease. It was found, however, that in small concentrations of lead acetate increase in the activity of urease occurs. On prolonging the time of the reaction inhibition develops and still later increased enzymatic activity again occurs. The results were elaborated statistically and are discussed in relation to the use of "adaptation" of urease in unfavourable conditions in reaction mixtures by the action of lead acetate.

Adaptability is one of the essential properties of life and so far this has not been observed in a non-living system, though modern computers do have a certain degree of the capacity of learning. The problem of origin of the property of adaptability thus still remains unsolved. It is interesting to investigate whether the adaptability originated only when some system became alive, or the property of adaptability is inherent in matter and is only best displayed in a system which is capable of duplication and which has its self-energy release system.

We have repeatedly observed that when enzymic reactions are studied in their natural environment, i.e. when the enzyme is not purified and made free from other proteinous matter with which it occurs in the tissue, it does retain a certain degree of adaptability. If the enzymes are studied under these conditions, it is a matter of common observation that the reactivity of the enzyme decreases with increased period of reaction and it has been explained to be due to the formation of inhibitors in the mixture (BAHADUR, CHANDRA 1961). However, we (BAHADUR, CHANDRA 1959) have found that this decrease in reactivity with increasing period of reaction is not uniform and the enzyme once or twice is reactivated before it finally loses all its enzymic property. Whatever may be the explanation for such activation after a definite decrease in activity, it certainly can be explained by the simple process of feed back, and it can definitely be called an effort of the enzyme to counteract the unfavourable conditions created by the inhibitory substances present in the field of enzymic reactions.

During our study of the inhibitors and activators of enzymes it has been repeatedly observed that an inhibitor acts as an activator when present in very small quantities, and in appropriate concentrations certain activators show inhibition. In case of inhibition after a certain period of lag the enzyme will show another maximum of activation before being finally inhibited by the inhibitor.

Such conditions have been statistically investigated and will be reported in this paper. Urease from soyabean has been used as the source of urease enzymes, and urea as the substrate. The reaction has been studied at pH = 6.6 using lead acetate as an inhibitor (BAHADUR, CHANDRA 1959).

Methods and Results

Urea solution: 4% solution of urea of A.R. quality was prepared by dissolving it in redistilled water prepared from all glass distilling apparatus.

Urease solution: 0·6% of soyabean powder in redistilled water was prepared. It was shaken well and allowed to stand for $^1/_2$ an hour and filtered through a cotton plug. The filtrate was used.

Buffer solution: Phosphate buffer of 0·2 M potassium dihydrogen phosphate (B.D.H.) and 0·2 M sodium hydroxide was used.

Lead acetate solution: 0·4620% solution of lead acetate $Pb(CH_3COO)_2 . 3H_2O$ (A.R., B.D.H.) in boiled distilled water was prepared.

The reaction was carried out in pyrex tubes. Two sets of experiments were performed side by side, i.e. one as control and other to study the influence of lead acetate on the activity of urease against urea. For each set a series of five parallel experiments were performed, whose statistical average is tabulated below.

Table 1 (Fig. A & B)

Decomposition of urea without and with 0·7 ml. and 1·4 ml. of 0·4620 % concentration of lead acetate solution

S. No.	Difference between the titre values at	0.20 mts	20—40 mts	40—60 mts	60—80 mts	80—100 mts
1	Control (Statistical average)	1·54±0·081	1·00±0·010	1·82±0·022	1·00± 0·010	0·30±0·010
	With 0·7 ml. of lead acetate sol^n (Statistical average)	1·82±0·037	1·90±0·070	0·38±0·037	1·96±0·012	0·84±0·012
	Statistical Remark	Significant	Significant	Significant	Significant	Significant
2	Control (Statistical Average)	1·22±0·040	1·36±0·040	1·52±0·020	1·12±0·020	0·48±0·037
	With 1·4 ml. of lead acetate sol^n (Statistical average)	1·24±0·071	1·40±0	0·82±0·037	0·18±0·020	0·12±0·020
	Statistical Remark	Insignificant	Insignificant	Significant	Significant	Significant

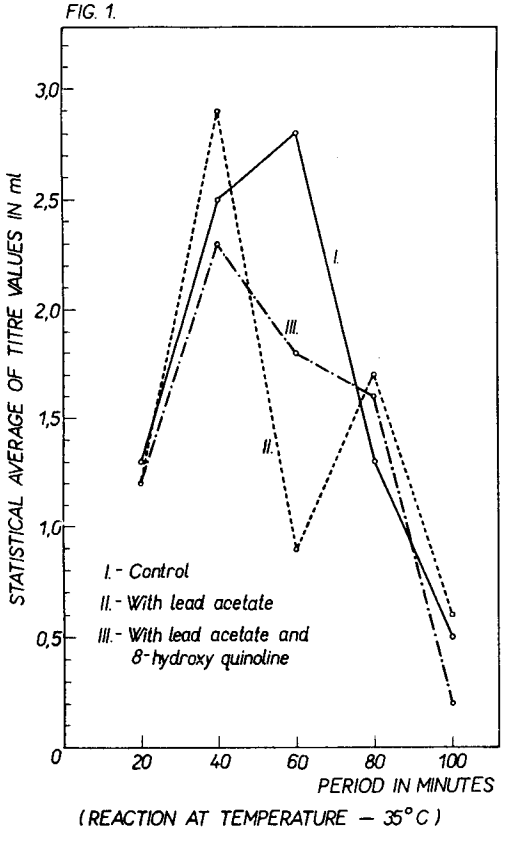
Table 2

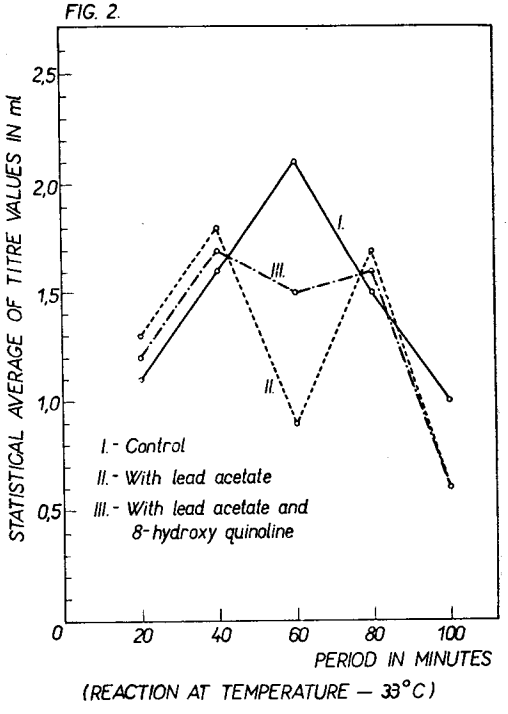
S. No.	Difference between the titre values at	0—20 mts	20—40 mts	40—60 mts	60—80 mts	80—100 mts
I 35° C	1) Control (Statistical average)	1·36±0·330	2·56±0·033	2·86±0·066	1·36±0·088	0·50±0·057
	(2) With lead acetate (Statistical average)	1·20±0	2·96±0·033	0·93±0·066	1·73±0·068	0·66±0·088
	(3) With lead acetate + + 8-hydroxy quinoline after 40 mts (Statistical average)	1·20±0·057	2·33±0·087	1·86±0·066	1·66±0·066	0·20±0
	Statistical Remark 1 & 2	Insignificant	Significant	Significant	Insignificant	Significant
	Statistical Remark 1 & 3	Insignificant	Insignificant	Significant	Significant	Significant
II 33° C	(1) Control (Statistical average)	1·10±0	1·82±0·047	2·10±0·040	1·50±0·040	1·02±0·022
	(2) With lead acetate (Statistical average)	1·30±0·057	2·07±0·045	0·95±0·028	1·75±0·050	0·60±0·014
	(3) With lead acetate + + 8-hydroxy quinoline after 40 mts (Statistical average)	1·20±0·024	1·80±0·057	1·52±0·024	1·60±0·057	0·62±0·047
	Statistical Remark 1 & 2	Significant	Significant	Significant	Insignificant	Insignificant
	Statistical Remark 1 & 3	Significant	Insignificant	Significant	Insignificant	Significant
III 30° C	(1) Control (statistical average)	1.4±0	1.52±0.047	1.72±0.047	1.50±0.04	1.40±0.05
	(2) With lead acetate (Statistical average)	1·35±0·050	1·80±0·040	0·92±0·024	1·60±0·040	1·67±0·047
	(3) With lead acetate + + 8-hydroxy quinoline after 40 mts (Statistical average)	1·40±0·040	1·60±0	1·30±0·040	1·37±0·007	1·25±0·080
	Statistical Remark 1 & 2	Insignificant	Significant	Significant	Insignificant	Significant
	Statistical Remark 1 & 3	Insignificant	Insignificant	Significant	Significant	Insignificant

The reaction mixture contains 16·6 ml urea, 6·6 ml urease, 6·6 ml buffer, and redistilled water, while in another case together with all the above the requisite amount of lead acetate was also added. The total volume of the sets was made equal with the help of redistilled water.

The reaction was carried out at pH 6·6 and at 35° C temperature. The samples were withdrawn at 20 minutes intervals. The liberated ammonia from the decomposition of urea was titrated against N/25—H_2SO_4.

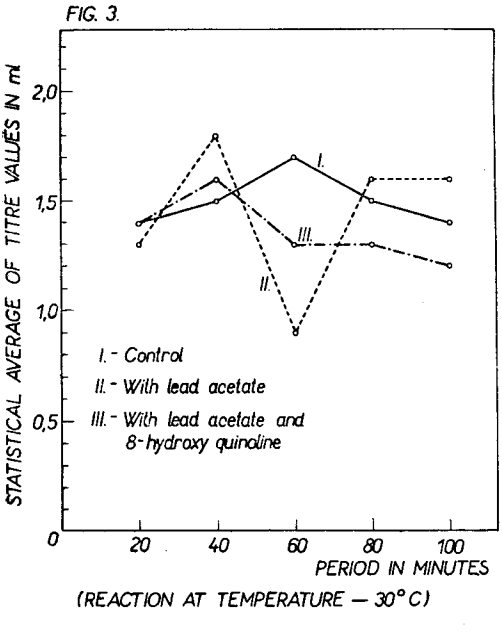
The reaction was carried out in three sets, i.e. 1st set was control, 2nd set with 0.7 ml of lead acetate and in the 3rd set 2 ml of 8-hydroxy quinoline was added after 40 minutes to the reaction mixture containing 0·7 ml of lead acetate initially. The reaction was carried out at pH 6·6 and at three temperatures, i.e. 35° C, 33° C and 30° C. The samples were withdrawn at intervals of 20 minutes. The liberated ammonia from the decomposition of urea was titrated against N/25—H_2SO_4. For each set at different temperatures a series of 4 parallel experiments were performed whose statistical average is tabulated In the Table 2.

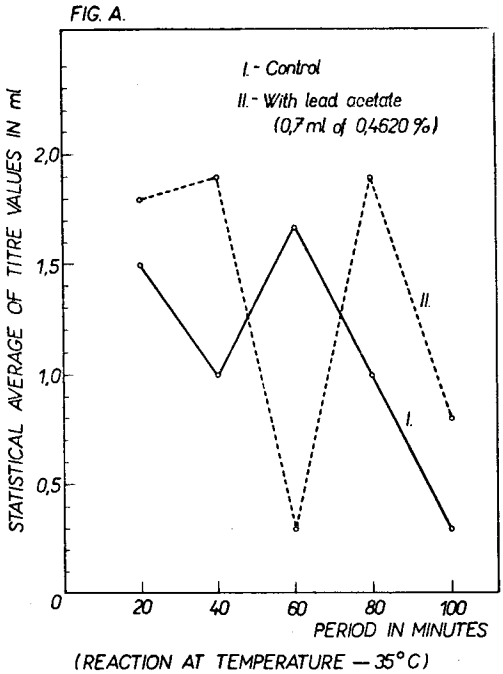




Discussion

Lead acetate in concentrations higher than studied here acts as inhibitor for urease from soyabean. In small concentration of lead acetate, i.e. when 0·7· ml of the lead acetate solution is present per 38·8 ml of the enzyme substrate mixture, lead acetate acts as a definite activator during the first 40 minutes of the reaction. During the next 20 minutes, i.e. between 40 to 60 minutes, lead acetate acts as a definite inhibitor. But the enzyme again adapts itself to its unfavourable condition caused by the presence of lead acetate, and the urease is modified to work faster than the control sample. This activation observed between 60 to 80 minutes is not permanent, after this period lead acetate acts as inhibitor again. This time the urease is unable to counteract the unfavourable influence caused by the presence of lead acetate and is permanently inhibited. These changes in the enzymic activity of urease have been observed in all the five similar mixtures and on statistical consideration these changes are significant, as shown in Table 2.





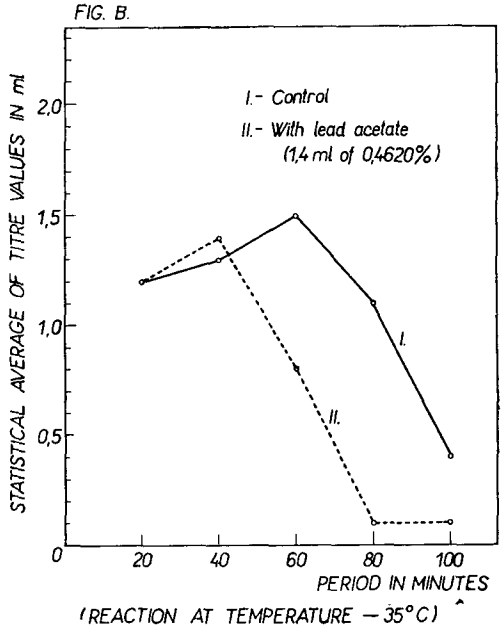
On statistical considerations it is further indicated that if a little higher concentration of lead acetate is used, i.e. if 1·4 ml of the lead acetate solution is present per 38·8 ml of the reaction mixture, the inhibitory influence of lead acetate is more pronounced and the enzyme shows only mild adaptability (Fig. B).

However, if a very small quantity of lead acetate is present in the reaction mixture and the experiments are performed keeping a number of similar

mixtures and the results are considered statistically to decrease any possibility of error, even then the adaptability of the enzyme is observed. Thus, if only 0·7 ml of the lead acetate is present per 38·8 ml of reaction mixture, the enzyme conveniently adapts itself to its unfavourable influence, and in its efforts to adapt to this unfavourable influence it becomes a better enzyme than the control one in urea decomposition (Fig. A).

In order to see whether the activation in the enzymic property caused by the lead acetate is permanent or it is only when lead acetate is present in the reaction mixture, 8-hydroxy quinoline was added in the reaction mixture after 40 minutes to remove the excess of lead acetate present in the mixture. It was observed that the activation of the enzyme is continued for the next 20 minutes and this effect is best observed at 33° C (Fig. 2), while at 30° C (Fig. 3) and at 35° C (Fig. 1) it is not so pronounced. This shows that the enzyme is modified during its effort to adapt to the unfavourable condition caused by the presence of lead acetate in the mixture, and this modified enzyme remains active even after the lead acetate is removed from the mixture.

FIG. B.



Each experiment was performed in a series of five similar mixtures and statistical calculations show a definite significance of the results. This behaviour of the enzyme is very similar to the process of adaptability in the living systems and the adaptability is an inherent property of matter which can be observed in bigger molecules if suitable reactions for their study are known.

References

BAHADUR, K., CHANDRA, V.: Inhibition of urease by lead-acetate. Enzymologia XX : 355—358, 1959.

BAHADUR, K., CHANDRA, V.: Influence of enzyme and substrate concentration on the decomposition of urea by commercial urease and urease obtained from glycine soyabeans in phosphate buffer. Enzymologia XXIII : 117—122, 1961.

BAHADUR, K., ATREYA, B. D.: Influence of aminoacids and amines on the enzymic activity of papain. Proc. Natl. Acad. Sci. (India) **30** : 126—140, 1961.

K. BAHADUR, I. SAXENA, Chemické odd. University v Allahabadu, Indie: **Přizpůsobivost enzymů.** — Biol. Plant. 7: 86—92, 1965.

V průběhu studia inhibitorů a aktivátorů enzymů bylo často zjištěno, že inhibitor působí jako aktivátor, jestliže je ve velmi malých koncentracích a jisté aktivátory (ve shodných koncentra-

cích) vykazují inhibici. Podobné výsledky jsme získali při studiu ureázy ze sojových bobů v přítomnosti octanu olovnatého. Octan olovnatý je normálně inhibitor ureázy. Bylo však zjištěno, že v malých množstvích octanu olovnatého nastává zvýšení aktivity ureázy. Prodloužením doby reakce nastává inhibice a ještě později opět zvýšení enzymatické ativity. Výsledky jsou statisticky zpracovány a jsou diskutovány ve vztahu k určité „adaptaci" ureázy na nepříznivé podmínky v reakční směsi způsobené octanem olovnatým.

К. Бахадур, И. Саксена, Химическое отделение Аллахабадского университета, Аллахабад, Индия: **Приспособительность энзимов.** — Biol. Plant. **7** : 86—92, 1965.

При изучении ингибиторов и активаторов энзимов часто наблюдается, что ингибиторы — присутствуя в малых количествах — действуют в качестве активаторов, и определенные активаторы (в подходящих концентрациях) оказывают ингибицию. Подобные результаты мы получили изучая уреазу из сои в присутствии ацетата свинца. Ацетат свинца нормально является ингибитором уреазы. Установлено, однако, что в малых количествах ацетата свинца имеет место повышение активности уреазы. В результате удлинения времени реакции наступает ингибиция и еще позже опять повышение энзиматической активности. Результаты статистически обработаны и обсуждаются в связи с определенной «адаптацией» уреазы на неблагоприятные условия реакционной смеси, вызванные ацетатом свинца.