BBA 63282

Lysozyme adsorption and activity in urea solutions

Being aware of the scarcity of information relating the surface properties of enzymes to their biological activity[1], we decided to study adsorption of an enzyme, lysozyme of hen's egg white (EC 3.2.1.17), at the air–water interface and to attempt to relate adsorption to enzymic activity. This enzyme was chosen because of the advanced state of knowledge about its structure[2,3] and the amount of interest in its surface chemistry[4–6].

Lysozyme was obtained 3× crystallized from Pierce Chemical Co. Other chemicals were of reagent grade. Assay was made by following the change in transmittance of a suspension of *Micrococcus lysodeikticus* cells (from Pierce Chemical Co.), at a final concentration of 0.25 mg/ml produced by 5.04 μg of lysozyme in 0.0155 M phosphate buffer (pH 5.90). Measurements were made at 540 mμ in a Beckman DK-2A Spectrophotometer utilizing a time-drive attachment. Enzymic activity was taken as the slope of the usually linear curve of $\%T$ between 1 and 3 min with temperatures regulated between 24 and 26° (see ref. 7).

Adsorption was measured by the pendant drop technique using the method of the selected plane[8]. The experimental apparatus has been described[9].

Surface tensions of the enzyme solutions at concentrations of 0.252 mg/ml were measured by forming drops which were aged for 15 min and then caused to shrink by withdrawing solution into the capillary supporting them. The films were compressed and static values of the surface tension were attained by this desorption process within 15 min after compression instead of after 30–60 min by adsorption onto a fresh surface. These static values of the tensions are reported in Table I along with the activities of the diluted lysozyme solutions.

Treatment with the various agents, urea, guanidine hydrochloride or bisulfite was carried out for at least 3 h at room temperature with the enzyme at a concentration of 0.252 mg/ml in phosphate buffer. Subsequently the enzyme was diluted with phosphate buffer alone to the 5.04 mg/ml concentration for assay. The surface tension of the controls was 55.3±0.7 dyne/cm for 3 samples; activities were 2.48±0.32 $\%T$/min for 15 determinations. The other data reported are the means of several experi-

TABLE I

SURFACE TENSION AND ACTIVITY

Solution	*Molarity*	γ *(dyne/cm)*	π *(dyne/cm)*	*Activity (%T/min)*
Control		55.3	16.7	2.48
Urea	1.5	54.5	17.5	3.65
	3.0	52.9	19.1	4.55
	6.0	53.0	19.0	4.39
	8.0	47.8	24.2	0.38
Guanidine	1.0	51.5	20.5	0.15
	4.0	53.7	18.3	0.05
Bisulfite	0.1	45.5	26.5	0.0

ments. In column 3 of Table I is listed the surface pressure, π. This value is computed from the relationship $\pi = \gamma_0 - \gamma$ where γ_0 is the surface tension of the clean surface (here about 72.0 dyne/cm) and γ is the surface tension of the film-covered layer. The magnitude of the surface pressure serves as an indicator of the closeness of packing or degree of spreading of molecules in the surface.

The data indicate an apparent increase in enzymic activity resulting from pretreatment with urea solutions of 1.5–6.0 M, a considerable loss of activity with 8.0 M urea or guanidine hydrochloride and an essentially complete loss of activity in bisulfite solutions. Accompanying the changes in enzymic activity are slightly enhanced surface pressure resulting from treatment with urea (below 8.0 M), and guanidine solutions and greater surface pressures with 8.0 M urea and bisulfite treatment. Dialysis to completely remove the denaturant essentially reverses the activation or inactivation due to urea or guanidine hydrochloride and results in enzymic activities slightly below the level of the control. Following dialysis the surface pressures of solutions which had been treated with urea or guanidine were lowered to values nearly characteristic of the control. Neither the inactivation or large increase in surface pressure associated with bisulfite treatment could be removed by dialysis.

Such results are consistent with the picture of lysozyme as a compact protein which does not readily spread or denature at surfaces[4]. Because of its relatively low molecular weight and rather high degree of covalent cross-linking due to 4 intrachain disulfide bonds, it has been suggested that lysozyme should be resistant to denaturation[5]. Increased activity in urea solutions rather than the expected inactivation has already been reported[10,11]. Guanidine hydrochloride, generally considered to act in a similar way to urea, in this case was found to produce a reversible inactivation of lysozyme despite a report that guanidine at 3 M could not denature the enzyme[12].

LÉONIS[11] has suggested that the activation by urea at moderate concentrations was due to its swelling action upon the substrate used to assay lysozyme, the bacterial cell walls. He postulated exposure of sensitive parts of the cell wall or increased adsorption of enzyme upon the wall due to the action of urea. Our experiments employed a different preparation of the admittedly ill-defined substrate. They indicate that activation by urea results from changes induced in the conformation of the enzyme and not directly from effects upon the cell walls.

Overnight exposure of a *M. lysodeikticus* suspension to 0.5 M urea (50× the urea concentration of the mixture when lysozyme treated with 1.5 M urea was diluted for assay) resulted in an apparent decrease in enzymic activity when the cells were used as substrate. An attack by the 0.5 M urea during long contact with cell walls seemed likely as the suspension showed increased turbidity and developed a yellow color. The result of this action upon the cells was a decreased rate of clearing by lysozyme and, hence, a lower rate of activity. Exposure of cells to 0.5 M urea for 1 h prior to assay with the urea concentration maintained constant produced no change in enzymic activity. This indicates that lengthy exposure of enzyme to relatively concentrated urea solutions is the important factor in producing activation and that contact between cells and dilute urea during the 10 min required for assay is without effect.

The variations in surface tension or surface pressure resulting from the various treatments of lysozyme show that such properties can serve as sensitive but nonspecific measures of small conformational changes in the enzyme. Minor elevations in

the surface pressure result at urea concentrations of 1.5–6.0 M, the range in which we observed the activating effects of urea. With 8.0 M urea or 0.1 M bisulfite there are rather large increases in surface pressure and marked inactivation of the enzyme. The action of urea or sulfite to unfold or expand lysozyme molecules spread in monolayers at the air–water interface has been reported[5,6]. However, treatment with guanidine hydrochloride at 1.0 or 4.0 M also induces moderate increases in surface pressure but nearly complete inactivation of the enzyme. Hence it seems that moderate and apparently reversible unfolding of lysozyme can either enhance or greatly reduce activity depending upon the agent employed. Greater unfolding such as is produced apparently by 8.0 M urea or reduction with bisulfite is associated with inactivation. It would be desirable to confirm the effects of urea or other denaturants by assaying with a better-defined substrate such as an oligosaccharide of *N*-acetylglucosamine.

Support under N.S.F. Undergraduate Research Participation Program grant GY-2093 is gratefully acknowledged.

Department of Chemistry,
Lafayette College,
Easton, Pa. (U.S.A.)

L. K. JAMES, JR.
D. A. HILBORN

1 L. K. JAMES AND L. G. AUGENSTEIN, *Advan. Enzymol.*, 28 (1966) 1.
2 P. JOLLÈS, *Angew. Chem. Intern. Ed. Engl.*, 3 (1964) 28.
3 D. G. PHILLIPS, *Sci. Am.*, 215 (1966) 78.
4 T. YAMASHITA AND H. B. BULL, *J. Coll. Interface Sci.*, 24 (1967) 310.
5 K. HAMAGUCHI, *J. Biochem. Tokyo*, 42 (1955) 449.
6 K. HAMAGUCHI, *J. Biochem. Tokyo*, 43 (1956) 355.
7 R. M. PARRY, R. C. CHANDAN AND K. M. SHAHANI, *Proc. Soc. Exptl. Biol. Med.*, 119 (1965) 384.
8 J. M. ANDREAS, E. A. HAUSER AND W. B. TUCKER, *J. Phys. Chem.*, 42 (1938) 1001.
9 L. K. JAMES AND J. N. LABOWS, *J. Phys. Chem.*, 68 (1964) 1122.
10 S. R. DICKMAN AND C. M. PROCTOR, *Arch. Biochem. Biophys.*, 40 (1952) 364.
11 J. LÉONIS, *Arch. Biochem. Biophys.*, 65 (1956) 182.
12 B. JIRGENSONS, *Arch. Biochem. Biophys.*, 41 (1953) 333.

Received August 28th, 1967

Biochim. Biophys. Acta, 151 (1968) 279–281

BBA 63286

Inorganic pyrophosphatase activity of human placental alkaline phosphatase

Alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1) has been reported to be capable of inorganic pyrophosphate phosphohydrolase activity[1–5]. In other studies with alkaline phosphatase pyrophosphatase activity has not been observed, and consequently, there is uncertainty whether the enzyme reacts with inorganic pyrophosphate[6–8]. The present studies which were conducted with a highly purified human placental alkaline phosphatase demonstrate both inorganic pyrophosphate phosphohydrolase and phosphotransferase activities as properties of this enzyme.

The procedures for purification of placental alkaline phosphatase have been