THE STABILITY OF ALKALINE PHOSPHATASE IN SODIUM DODECYL SULFATE

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1. Introduction

While sodium dodecyl sulfate (SDS) is a most effectieve detergent for solubilizing membrane proteins, this detergent is also an effective protein denaturant [1–4]. At appropriate detergent concentrations and after equilibrium dialysis, the SDS–protein complexes formed contain approximately 1.4 g of SDS bound/g protein [1,2]. By raising the ionic strength and the SDS concentrion, the amount of detergent bound can be increased to about 2 g/g protein [3]. However with most enzymes which have been studied activity is completely lost after treatment with SDS.

Alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1) was released from milk fat globule membrane (MFGM) in a soluble, active form by treatment of the membrane with SDS. Enzyme activity was recovered after electrophoresis in polyacrylamide gels containing SDS. Four other mammalian alkaline phosphatases and the enzyme from *E. coli* retained activity over a wide range of detergent concentrations.

2. Experimental procedure

MFGM was prepared at room temperature from fresh milk [5]. Alkaline phosphatases from *E. coli* (12 units/mg, Type III-S, referred to here as preparation B), calf intestine (520 units/mg, Type VII), beef liver (9.6 units/mg, Type IX) and hog intestine (0.87 units/mg, Type IV) were obtained from Sigma Chemical Co., St. Louis, Missouri. Human placenta alkaline phosphatase (81 units/mg, B grade) was from Calbiochem. Highly purified *E. coli* alkaline phosphatase (preparation A) was a gift from Dr. L. G. Butler, Purdue University. The specific activity of this preparation was 56.6 IU under the conditions of assay [6].

Enzyme activity was assayed spectrophotometrically by following the release of 4-nitrophenol from 4-nitrophenyl phosphate (2.5 mM) at 400 nm. The enzyme was incubated in glycine–NaOH buffer (100 mM, pH 9.8) at 37°C in the presence of $MgCl_2$ (1.5 mM). The purified *E. coli* enzyme (preparation A) was assayed according to Sperow and Butler [6]. The unit of activity used is the international unit.

Membrane samples were incubated in SDS (5:1, SDS: protein, w/w) and mercaptoethanol (10 mM) for 30 min at 37°C before electrophoresis. Gel electrophoresis was conducted according to Weber and Osborn [4] in polyacrylamide gels (10%, w/v) containing methylenebisacrylamide (0.135%, w/v) and mercaptoethanol (10 mM).

After electrophoresis, enzyme activity was detected by incubating the gels in glycine–NaOH buffer (300 mM, pH 9.8) containing 4-nitrophenyl phosphate (2.5 mM) at 30°C. A discrete yellow band of 4-nitrophenol became visible after about 15 min in gels containing alkaline phosphatase activity. Gels were washed once in glycine buffer and scanned at 400 nm with a Beckman gel scanner in an Acta III spectrophotometer.

3. Results

Extraction of alkaline phosphatase from MFGM

Table 1
Extraction of alkaline phosphatase from milk fat globule membrane with ionic and non-ionic detergents*

Fraction	Detergent		
	Sodium dodecyl sulfate	Deoxycholate	Triton X-100
Experiment I			
Supernatant	53	33	5
Pellet	23	18	0
Experiment II			
Supernatant	44	42	2
Pellet	22	12	0

Milk fat globule membrane (2.5 mg) was suspended in 50 mM Tris–HCl, pH 7.5 and incubated with either SDS (5 mM), deoxycholate (5 mM) or Triton X-100 (0.5%, v/v) in a final volume of 5 ml at 37°C. After 15 min the suspensions were centrifuged at 100 000 *g* for 1 hr and the supernatants and pellets were collected. Enzyme activity was assayed in the presence of detergent at the concentration used for the initial extraction. Values are expressed as the percent of enzyme activity present relative to the activity of untreated whole membrane taken as 100%.

with a variety of detergents gave unexpected results (table 1). In contrast to other enzymes, alkaline phosphatase activity was highest in the SDS supernatants, with substantial activity remaining in pellets after centrifugation. Under identical conditions xanthine oxidase, 5′-nucleotidase and adenosine triphosphatase activities in MFGM were completely inactivated by SDS solutions (unpublished). Triton X-100, generally considered to be a mild detergent for enzyme extraction, caused substantial inactivation of MFGM alkaline phosphatase in both soluble and particulate fractions. Deoxycholate was less effective than SDS in solubilizing and preserving alkaline phosphatase activity.

Alkaline phosphatase activity was localized in polyacrylamide gels containing SDS (0.1%, w/v) after electrophoresis of solubilized MFGM (fig. 1). This enabled direct determination of the molecular weight of MFGM alkaline phosphatase using crude membrane fractions. The apparent value obtained from seven determinations was 185 000 ± 10 000.

Further experiments showed that the stability of alkaline phosphatase in SDS was not restricted to the enzyme from MFGM. Alkaline phosphatase from four other mammalian sources and from *E. coli* retained activity over a wide range of SDS concentrations, both

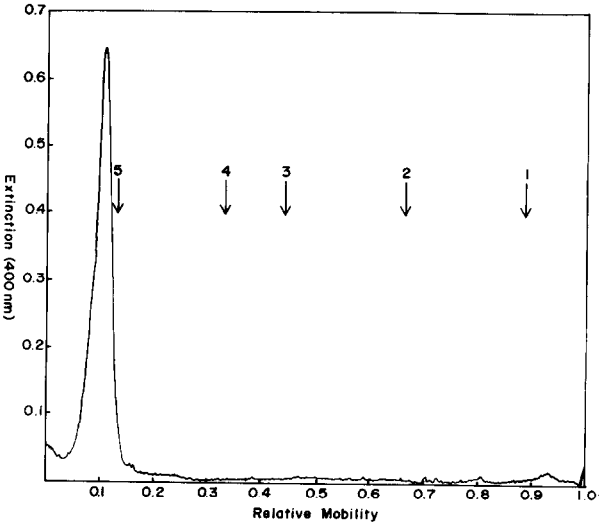

Fig. 1. Activity of milk fat globule membrane alkaline phosphatase on a polyacrylamide gel containing sodium dodecyl sulfate (0.1%, w/v). MFGM (1 mg/ml), solubilized in SDS and mercaptoethanol, was applied to the gel in sucrose solution (10%, w/v). 50μg of membrane protein (about 0.01 units) was in the sample. The numbers and arrows locate the positions of standards used for molecular weight determination. 1, cytochrome *c*; 2, cytochrome *c* dimer; 3, ovalbumin; 4, bovine serum albumin; 5, β-galactosidase.

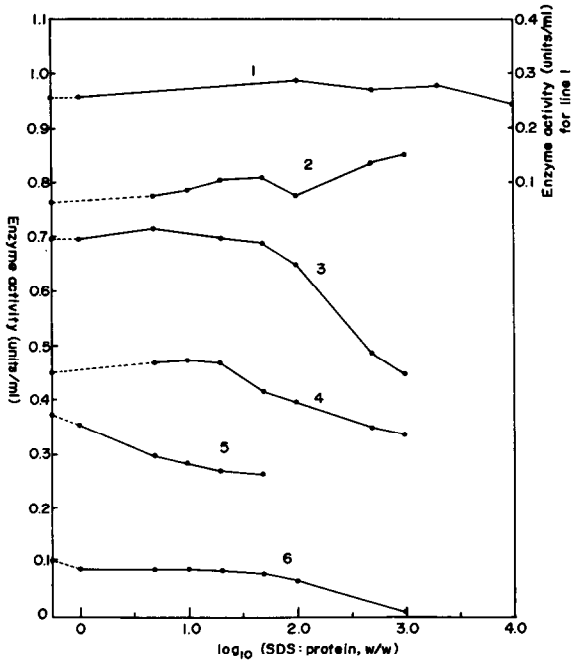

Fig. 2. Stability of alkaline phosphatases in sodium dodecyl sulfate. Enzyme was incubated with SDS, at the SDS to protein weight ratios indicated, in 50 mM Tris–HCl, pH 7.0 (2 ml) at 37°C. Samples were taken after 15 min and tested for activity in the presence of SDS at the concentrations used for incubation. Activity values for untreated controls are given on the ordinate. 1, *E. coli* (preparation A), 1 μg/ml; 2, *E. coli* (preparation B), 100 μg/ml; 3, calf intestine, 5 μg/ml; 4, beef liver, 100 μg/ml; 5, hog intestine, 1 mg/ml; 6, human placenta, 10 μg/ml.

below and above the critical micelle concentration [7] (fig. 2). The enzyme from *E. coli* was most stable. Preparation A, which was highly purified, retained 95% of the control activity at a weight ratio of 10^4 :1, SDS:protein. Experimentally this amounts to nearly full activity with enzyme (5 μg/ml) in a 5.0% w/v solution of SDS (173 mM). The mammalian enzymes lost some activity, particularly at weight ratios between 10^2

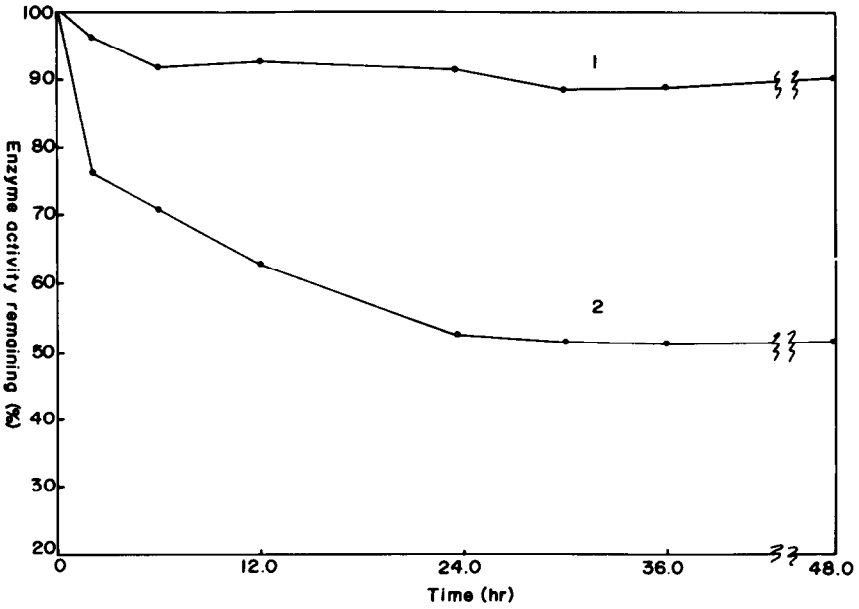

Fig. 3. Stability of *E. coli* and calf intestine alkaline phosphatase as a function of time. Enzyme was incubated with SDS (50:1, SDS: protein, w/w) in 50 mM Tris–HCl, pH 7.5 in a final volume of 2 ml at 37°C. Samples (100 μl) were taken at the times indicated and assayed in the presence of the same concentration of SDS. The curves are: 1, *E. coli* (preparation B), 100 μg/ml; 2, calf intestine, 5 μg/ml. Activity values are expressed as the amount (%) of enzyme activity remaining to control samples containing no SDS.

and 10^3:1, SDS:protein. None of the enzymes tested were completely inactivated.

Time course experiments with the *E. coli* enzyme (preparation B) and the calf intestine enzyme are summarized in fig. 3. After 2 days in SDS solution at 37°C the *E. coli* enzyme was still 90% active compared with untreated controls. Under similar conditions the calf intestine enzyme retained about 50% of the control activity.

4. Discussion

This study has shown that alkaline phosphatases from five mammalian sources and *E. coli* are unusually stable in the presence of the protein denaturant SDS. Enzyme preparations used ranged from whole membrane to a highly purified *E. coli* alkaline phosphatase.

Localization of enzyme activity in polyacrylamide gels containing SDS enables the direct determination of the molecular weight of alkaline phosphatase using crude membrane fractions. The values obtained for the molecular weight of MFGM alkaline phosphatase by this method (185 000 ± 10 000) are in close agreement with other values in the literature (180 000 ± 18 000 [8] and 190 000 ± 10 000 [9]). However, the method may not be applicable to other alkaline phosphatases since many of the mammalian enzymes are glycoproteins. Glycoproteins often migrate anomalously in molecular sieves [8].

The reason for the stability of alkaline phosphatases in SDS is not known. The *E. coli* enzyme was found to retain activity in SDS (0.1%, w/v) under certain conditions [10]. The dimer was stable but the monomer was inactivated. Other enzymes are also known to be relatively stable in oligomeric form in SDS. The tetramer form of catalase binds little SDS and retains full activity whereas the monomer form binds more of the detergent and is inactivated [3]. Uricase is resistant to denaturation by both SDS and guanidinium chloride and is difficult to separate into subunits [11]. Some monomeric enzymes are also uncommonly stable in the presence of SDS, eg. papain, pepsin and an alkaline protease from *Acremonium kiliense* [12]. It has been suggested that pepsin may repel SDS because of its high negative charge [3]. The isoelectric point of pepsin is below 1.0.

The molecular basis for the stability of alkaline phosphatase in SDS solutions is under investigation.

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