Alkaline unfolding and salt-induced folding of bovine liver catalase at high pH

Shashi PRAJAPATI1, Vinod BHAKUNI1, Kodali Ravindra BABU1 and Swatantra Kumar JAIN2

1 Division of Membrane Biology, Central Drug Research Institute, Lucknow, India
2 Department of Biochemistry, Jamia Hamdard University, New Delhi, India

(Received 17 December 1997/27 March 1998) — EJB 97 1770/3

We have studied the alkaline unfolding of bovine liver catalase and its dependence on ionic strength by enzymatic activity measurements and a combination of optical methods like circular dichroism, fluorescence and absorption spectroscopies. Under conditions of high pH (11.5) and low ionic strength, the native tetrameric enzyme dissociates into monomers with complete loss of enzymatic activity and a significant loss of α-helical content. Increase in ionic strength by addition of salts like potassium chloride and sodium sulphate resulted in folding of alkaline-unfolded enzyme by association of monomers to tetramer but with significantly different structural properties compared to native enzyme. The salt-induced tetrameric intermediate is characterized by a significant exposure of the buried hydrophobic clusters and significantly reduced α-helical content compared to the native enzyme. The refolding/reconstitutio studies showed that the salt-induced partially folded tetrameric intermediate shows significantly higher efficiency of refolding/reconstitution as compared to alkaline-denatured catalase in the absence of salts. These studies suggest that folding of multimeric enzymes proceeds probably through the hydrophobic collapse of partially folded multimeric intermediate with exposed hydrophobic clusters.

Keywords: bovine liver catalase; alkaline unfolding; monomer; salts; refolding.

The extent of unfolding of denatured states of proteins under different conditions has long been of interest because of the possible relevance of their conformations to the protein folding pathways. It has been demonstrated that residual structural preferences, ranging from local clusters of side chains to highly ordered side chains to highly ordered subdomains, persist in denatured states of proteins [1–6]. Hence, there has been a growing recognition of the importance of the compact denatured and partially folded states of proteins, as characterization of these structures and the factors involved in their stability would provide important insight into the interactions responsible for their formation as well as their role in protein folding.

pH is known to influence the stability of a protein by altering the net charge on the protein. Many proteins denature at extreme pH because of the presence of destabilizing repulsive interactions between like charges in the native protein [7, 8]. The exact behavior of a given protein at low or high pH is a complex interplay between a variety of stabilizing and destabilizing forces, some of which are sensitive to the environment. Salts have been known to affect the physico-chemical properties of proteins like their solubility [9], stability [10] and pK a [8, 11] to a great extent. Salts affect mainly the electrostatic and hydrophobic interactions in the protein molecules. In the presence of salts a conformational transition at acidic or alkaline pH from a largely unfolded state to an intermediate conformational state have been reported for several small globular proteins [12–17], but very few reports on the effects of salts on the subunit assembly of multimeric enzymes are available.

Catalase is a highly active ubiquitous enzyme which occurs in almost all aerobically respiring organisms and in part serves to protect the cells from the toxic effects of hydrogen peroxide. Bovine liver catalase, molecular mass 240 kDa, contains four identical 57-kDa subunits each equipped with a high-spin Fe(III) protoporphyrin IX [18]. We have investigated the changes in the structural and functional properties associated with the alkaline denaturation of bovine liver catalase and also studied its dependence on ionic strength.

EXPERIMENTAL PROCEDURES

Materials. Crystalline bovine liver catalase was prepared according to the earlier reported method [19]. The purity of the protein was checked by SDS/PAGE followed by silver staining. The catalase preparation was found to be about 99% pure. All the chemicals used were purchased from Aldrich Chemical Company and were of the highest purity available.

Spectrophotometry. The absorption spectra of catalase solutions were measured with Schimadzu UV-240 double-beam spectrophotometer. The concentrations of catalase solutions were determined spectrophotometrically based on ε nm = 13.5 at 405 nm, as determined previously [19] and also by the method of Lowry et al. [20] using bovine serum albumin as a standard. The concentration of denatured proteins was determined by the method of Lowry et al. [20] only.

Alkaline denaturation of catalase. For preparation of alkaline-denatured catalase two methods were tried. Bovine liver catalase (10 mg/ml) was dissolved in 0.025 M sodium phosphate pH 7.0 and used as stock. In the first method the stock solution of enzyme was diluted by addition of 50 µl enzyme stock to 950 µl of pH 7.0 buffer and the pH of the solution was raised to 11.5 by the addition of 0.5 M sodium hydroxide. In the second method, 50 µl stock enzyme (pH 7.0) was added to the 950 µl

---

Correspondence to V. Bhakuni, Division of Membrane Biology, Central Drug Research Institute, Lucknow 226 001, India
Fax:+91 522 223405
E-mail: root@cscdri.ren.nic.in

Abbreviation. ANS, 8-anilino-1-naphthalenesulphonic acid.

Enzyme. Bovine liver catalase, H 2 O 2 :H 2 O 2 oxidoreductase (EC 1.11.1.6).
A protein sample was loaded onto the column equilibrated with the Soret band absorption or tryptophan fluorescence emission. Circular dichroic measurements. CD measurements were made with a Jasco J500 spectropolarimeter calibrated with ammonium (+)-10-camphorsulfonate. The results are expressed as the mean residue ellipticity \([\Theta]\), which is defined as \(\Theta = 100 \times \Theta_{\text{ave}} / (l c)\), where \(\Theta_{\text{ave}}\) is the observed ellipticity in degrees, \(c\) is the concentration in mol residue \(\cdot 1^{-1}\), and \(l\) is the length of light path in centimeters. The CD spectra were measured at a protein concentration of 0.3 \(\mu\)M with a 1-mm cell.

Tryptophan fluorescence. Fluorescence spectra were recorded with Perkin-Elmer LS 5B spectrophotometer meter in 5 mm path-length quartz cells. All the samples were equilibrated for 2 h at 25°C before recording. Protein concentration was 0.33 \(\mu\)M for all experiments and the measurements were made at 25°C. The excitation wavelength was 290 nm and the spectra were recorded between 300–400 nm.

8-Anilino-1-naphthalenesulphonic acid (ANS) fluorescence measurements. Fluorescence spectra were recorded with Perkin-Elmer LS 5B spectrophotometer meter in 5-mm path-length quartz cells. The aliquots of protein with a final concentration of 0.7 \(\mu\)M were equilibrated in the desired buffer with or without salts for 2 h at 25°C. They were then mixed with concentrated stock solution of ANS dissolved in the same buffer. The excitation wavelength was 365 nm and the emission was recorded either over 400–500 nm or at fixed wavelength at 480 nm. The ANS concentration was 60 \(\mu\)M. The values were normalized by subtracting the baseline recording for the probe alone under similar conditions.

Crosslinking of native and alkaline unfolded catalase. To 50 \(\mu\)g native and pH-11.5 treated catalase, an aliquot of 25% (mass/vol.) glutaraldehyde was added so as to make a final concentration of 1% glutaraldehyde. This sample was incubated for 4 min at 20°C followed by quenching the cross-linking reaction by adding freshly prepared solution of 2 \(\mu\)M sodium borohydride dissolved in 0.1 \(\mu\)M sodium hydroxide. After a 20-min incubation, 20 \(\mu\)L 10% aqueous sodium deoxycholate was added. The pH was lowered to 2–2.5 by addition of trichloroacetic acid which results in precipitation of cross-linked protein. The precipitate was redissolved in 0.1 M Tris/HCl pH 8.0, 1% SDS and 50 mM dithioerythritol and heated at 90–100°C. SDS/PAGE was run on 6% acrylamide gels as described [22].

Size-exclusion chromatography. Gel-filtration experiments were carried out on a BioGel 0.5 M column (manufacturer’s exclusion limit 500 kDa for proteins) attached to a peristaltic pump (Pharmacia) for controlling the flow rate of the column. 100 \(\mu\)L protein sample was loaded onto the column equilibrated with the buffer in which the samples were incubated (flow rate 28 mL/h, detection 280 nm). The column was run at 4°C.

Refolding/reconstitution studies. Refolding of solubilized proteins is usually performed by dilution or dialysis. The bovine liver catalase solution at pH 11.5, in the presence or absence of salts (prepared by the method described above), was incubated for 30 min at 4°C. Reconstitution/refolding of subunits of alkaline-denatured catalase (pH 11.5, both in presence and absence of salt) by dialysis against pH 7.0 buffer, resulted in the precipitation of the protein, so a rapid dilution technique for reconstitution was tried. The alkaline-denatured protein samples were diluted tenfold with 0.025 M sodium phosphate pH 7.0 and stirred vigorously for 3 h at 25 ± 1°C. A study of the time dependence of recovery of alkaline-denatured catalase on refolding showed that maximum recovery of enzymic activity can be obtained within 2 h (data not shown). Hence, for all the studies, a reconstitution time of 3 h was used. The reconstituting/renaturation was monitored by recovery of enzymic activity as a percentage of that of the native enzyme.

RESULTS

Changes in some molecular properties of bovine liver catalase on alkali-induced unfolding. The pH-dependent changes in the structural and functional properties of bovine liver catalase at alkaline pH as studied by monitoring the changes in tryptophan fluorescence, far-ultraviolet CD and enzyme activity are summarized in Fig. 1. The values are presented relative to those obtained for native catalase at pH 7.0. No significant alterations in the structural properties of catalase was observed in the pH range 7–10 as monitored by CD ellipticities at 222 nm, tryptophan fluorescence, Soret absorption, and tryptophan fluorescence emission. However, above pH 10, a sharp decrease in CD ellipticities at 222 nm, tryptophan fluorescence, Soret absorption, and a red shift of wavelength of both Soret band absorption and tryptophan fluorescence emission were observed. In contrast, the enzymic activity was found to be rapidly reducing from pH 9.5 onwards with a sharp decrease between pH 9.5–11.0 and a complete loss of activity from pH 11 onwards. Based on the enzymic activity results, the pH of half-transition was found to be 10.2. These results indicate that significant structural and functional changes are associated with alkaline unfolding of catalase at pH-11.5 buffer. The pH of the solution was checked and, if found altered, was adjusted to 11.5. For size-exclusion and structural studies, the enzyme samples were incubated for 2 h at the desired temperature for achieving equilibrium. No significant difference in the properties studied was observed from the samples prepared by the above-mentioned methods.

Catalase activity. The catalase activity was measured by the rate of decomposition at 0.03 M hydrogen peroxide (H2O2). The decomposition of H2O2 was followed directly by the decrease in absorbance at 240 nm. The difference in absorbance (A260) for unit time is a measure of the catalase activity [21].
(pH 11.5) catalase at 4°C. For both single peaks but with different retention volumes were observed, indicating the presence of single but different species under both conditions. For native and alkaline-denatured catalase retention volumes of 13.7 ml and 18.9 ml respectively were observed, indicating a vastly decreased hydrodynamic radius for catalase on alkaline denaturation. 64 kDa (BSA) showed a retention volume of 18.6 ml on the BioGel 0.5 M column, close to the retention volume of 18.9 ml observed for alkaline-denatured catalase at pH 11.5. As the molecular mass of catalase monomer has been reported to be 57 kDa, the above observations indicate that alkaline-denatured catalase at pH 11.5 is present as a monomer. This was further confirmed by the cross linking experiment using glutaraldehyde which showed the presence of a single band with slightly higher mobility than the molecular mass marker of 64 kDa (BSA) on SDS/PAGE (Fig. 2C). These observations demonstrate beyond doubt that alkaline unfolding of bovine liver catalase at pH 11.5 leads to dissociation of tetrameric native enzyme into monomers.

Salt-induced folding of alkaline denatured catalase at high pH. Effect of KCl and Na₂SO₄ on the subunit assembly. The effect of addition of salt on the subunit assembly of alkaline-denatured catalase at pH 11.5 was studied by gel-permeation experiments. Fig. 2, A and B, show profiles of gel-permeation experiments on a BioGel 0.5 M column for native, alkaline-denatured (pH 11.5) catalase and on addition of increasing KCl or Na₂SO₄ to alkaline-denatured catalase, at 4°C. For alkaline-denatured catalase to which a low concentration of KCl (1 or 2 M) or very low Na₂SO₄ concentration (0.2 M) was added, two distinct peaks (A and B) with retention volumes of 13.7 ml and 18.9 ml, respectively, were observed. Since the retention volumes of peaks A and B correspond to that of native and alkaline-denatured catalase, respectively, the observation presented above indicates that, on addition of up to 2 M KCl or 0.2 M Na₂SO₄ to catalase at pH 11.5, both the mononeric and tetrameric species of catalase molecules are present although in varying percentages. With increase in KCl concentration to 3 M or Na₂SO₄ to 0.5 M, a single peak corresponding to the tetrameric enzyme is observed. These observations indicate beyond doubt that the monomers of catalase obtained on alkaline denaturation at pH 11.5 undergo salt-induced reassociation to form tetramers at higher salt concentration.

Structural properties of salt-induced tetramer. The structural properties of salt induced tetrameric catalase at pH 11.5 were studied by monitoring the Soret band absorption, far-ultraviolet CD and ANS binding. Fig. 3A shows the effect of addition of KCl on the Soret band absorption of alkaline-denatured catalase at pH 11.5. For native catalase, the Soret band absorption is observed at 405 nm. The monomeric form of catalase obtained on alkali denaturation at pH 11.5 showed a shift in Soret band absorption to 410 nm with a significant decrease in absorption. Addition of 3 M KCl to the alkaline-denatured catalase resulted in a slight enhancement in intensity with no change in absorbance maxima as compared to alkaline-denatured catalase. These observations suggest that the haem environment in the salt-induced tetrameric state of alkaline-denatured catalase is significantly different as compared to native tetrameric enzyme. Fig. 3B shows the far-ultraviolet CD spectra of native and alkali-denatured catalase (pH 11.5) and, on addition of 3 M KCl, to alkaline-denatured catalase. A significant decrease in ellipticity at 222 nm as compared to native catalase was observed for KCl-induced tetrameric intermediate of catalase.

Solvent accessibility of buried hydrophobic clusters in salt-induced tetramer. The solvent exposure of non-polar clusters present on the alkali denaturation of catalase in the presence and
Fig. 4. Salt-dependent aggregation of alkaline-denatured catalase at pH 11.5. (A) Changes in the fluorescence intensity of ANS on incubation with bovine liver catalase at pH 11.5 in the presence of increasing concentration of KCl (●) and Na₂SO₄ (○). The samples were prepared as described in Fig. 3 except that samples were incubated at 25°C. (B) Effect of increasing concentration of KCl (●, ○) and Na₂SO₄ (▲, ▼) on formation of scattering particles as monitored by the absorbance at 540 nm at 4°C (●, ▲) and 25°C (○, ▼). The samples were prepared as described in Fig. 3 except that they were incubated at 4°C and 25°C. After incubation, they were transferred to a cuvette and the absorbance at 540 nm was monitored in a double-beam spectrophotometer equilibrated at 4°C and 25°C. No absorbing species are present at this wavelength, so the light beam is attenuated by scattering from the aggregated protein particles. (C) Salt concentration dependence of the soluble catalase solution at pH 11.5 and 25°C. The catalase solution (2.0 mg/ml) at pH 11.5 was incubated at the desired KCl (●) or Na₂SO₄ (○) for 2 h at 25°C. The solution was then centrifuged at 10000 rpm and the protein estimated by the method of Lowry et al. [20].

Absence of 3 M KCl was studied by ANS binding. The fluorescent probe binds to solvent-accessible clusters of non-polar groups in proteins and the fluorescence emission of the probe is known to increase binding to hydrophobic clusters of a protein [24]. Fig. 3C shows the fluorescence spectra on binding of ANS to native and alkali-denatured catalase and on addition of 3 M KCl. At pH 7.0 and 11.5, a very weak fluorescence was observed, suggesting a very low binding of ANS molecules to native tetrameric or alkali-denatured monomeric catalase. However, for alkali-denatured catalase, addition of 3 M KCl results in a significant enhancement in fluorescence intensity with the emission maximum at 480 nm, indicating a significant exposure of buried hydrophobic clusters under these conditions [25].

The results presented above imply the presence of repulsive charge interaction within the alkali-induced monomers of catalase. It seems that addition of salts leads to neutralization of these charges resulting in association of monomers to tetramers. However, the salt-induced tetrameric intermediate has significantly different structural properties as compared to native tetramer of catalase.

Salt-induced aggregation of partially folded tetramer of catalase. Fig. 4 shows the relative effectiveness of KCl and Na₂SO₄ in stabilizing hydrophobic interactions in alkaline-denatured catalase as studied by ANS binding and aggregation studies. The effect of increasing salt concentration on the ANS fluorescence of alkaline-denatured catalase is shown in Fig. 4A. Irrespective of the salt studied, an increase in ANS fluorescence intensity...
Fig. 5. Effect of alkaline denaturation in the presence and absence of salts on the refolding efficiency of bovine liver catalase denatured at pH 11.5. Refolding of solubilized proteins is usually performed by the rapid dilution method. The bovine liver catalase solution at pH 11.5, with the addition of KCl (●) or Na₂SO₄ (○), was prepared as described in Fig. 2 with the only modification that total incubation time at 4°C was kept constant at 30 min. The samples were diluted to 10 vol. 0.025 M sodium phosphate pH 7.0 and stirred vigorously for 3 h 25 ± 1°C. The refolding/reconstitution was monitored by recovery of enzyme activity as a percentage of the native enzyme. With increasing salt concentration was observed. However, the enhancement in ANS fluorescence intensity with increasing sodium sulfate was far steeper as compared to that observed for KCl. For a similar amount of enhancement in ANS fluorescence intensity, a significantly higher (about 10 times) concentration of KCl compared to Na₂SO₄ is required, indicating that the latter is more effective in stabilizing the hydrophobic interactions in alkali-denatured catalase compared to KCl. As the ANS studies suggest that addition of salts to the alkali-denatured catalase results in significant exposure of buried hydrophobic clusters, the possibility of salt-induced aggregation of this intermediate was also studied. The salt-induced formation of protein aggregates of alkali-denatured catalase at pH 11.5 was monitored by the turbidity measurements at 540 nm. Fig. 4B summarizes the increase in absorbance at 540 nm for bovine liver catalase at pH 11.5 on addition of increasing KCl and Na₂SO₄ concentration at 4°C and 25°C. The size of scattering particles was found to increase dramatically with increasing salt concentration but, for a similar change in absorbance, a significantly lower concentration of Na₂SO₄ as compared to KCl was required. These observations were in accordance with the ANS observations suggesting a more efficient stabilization of hydrophobic interactions by Na₂SO₄, as compared to KCl. Furthermore, in the presence of 3 M KCl, the effectiveness of aggregation of alkali-denatured catalase was found to be dependent on temperature as a significantly higher turbidity was observed for measurements at 55°C as compared to those at 25°C. As the aggregation was found to be significantly enhanced at higher temperature, where the hydrophobic interactions are predominant, and very low at low temperature, where the hydrophobic interactions are weakened [26, 27], hydrophobic interactions seem to play a major role in the salt-dependent aggregation of alkali-denatured bovine liver catalase. Further conformation of salt-dependent aggregation of alkali-denatured catalase came from the precipitation studies. Fig. 4C shows the percentage of bovine liver catalase at pH 11.5 in solution on addition of increasing concentrations of KCl and Na₂SO₄ at 25°C. It can be seen that, in the presence of even 2 M KCl, no precipitation of protein was observed as all the protein was found in the supernatant. However, in the presence of increasing concentrations of Na₂SO₄, a significantly higher loss of protein due to precipitation was observed and at higher Na₂SO₄ concentrations almost all the protein was found to be precipitated. These observations indicate that the presence of high concentrations of Na₂SO₄ in the solution of bovine liver catalase at pH 11.5 leads to aggregation resulting in precipitation of protein.

Reconstitution of alkali-induced monomers and salt induced tetrameric intermediate of catalase. The subunits of alkali-denatured catalase were reconstituted by a rapid-dilution method. The protein solution at pH 11.5 was diluted tenfold in 0.025 M sodium phosphate pH 7.0 and stirred vigorously for almost 3 h. The extent of renaturation was judged by the recovery of enzymic activity, which, on refolding, was found to be diminished on prolonged exposure at pH 11.5 with a maximum decrease of about 90% in recovery of enzymic activity observed after denaturation for 3 h. These observations suggest that extent of renaturation of catalase on denaturation at alkaline pH depends on the length of time it is denatured, in accordance with earlier reported results [28]. Alkaline-denatured catalase (pH 11.5 for 30 min) showed a maximum recovery of about 28% on refolding. However, a significant enhancement in recovery of native catalase on refolding was observed in presence of increasing concentration of KCl or Na₂SO₄. Fig. 5 shows the recovery of native catalase on refolding from alkali-denatured state in the presence and absence of salts. A linear enhancement with a maximum of about 3.5-fold in the recovery of native enzyme on refolding was observed for alkali denaturation in presence of increasing KCl concentration.

**DISCUSSION**

It is generally recognized that the activity of an enzyme is strongly dependent on its conformational integrity. But several
studies on the metal enzymes and multi-subunit enzymes, such as creatin kinase, yeast alcohol dehydrogenase and aminocoylase, have shown that during the denaturation of these enzymes with urea, guanidine hydrochloride and SDS, inactivation of the enzyme occurs before noticeable conformational changes in the enzyme molecule as a whole can be detected [29–31]. In the present study, pH-dependent inactivation and conformational change in bovine liver catalase during alkaline denaturation (Fig. 1) shows that inactivation of enzyme starts from pH 9.5 onwards but no significant changes in the structural features, as studied by change in secondary structure, Soret band absorption or tryptophan environment, compared to native enzyme were observed up to pH 10.0. The observations presented above show that, during alkaline unfolding of catalase, inactivation occurs at a much lower pH than significant changes in the enzyme molecule were detected, thus supporting the theory of flexibility at the active site of enzymes suggested by Tsou [32, 33] according to which the enzyme active sites are formed by relatively weak molecular interactions and hence may be conformationally more flexible than the intact enzyme.

Based on the results presented in this paper, the effect of alkaline unfolding of bovine liver catalase and its dependence on ionic strength can be summarized as shown in Fig. 6. At pH 11.5 and above, the native tetrameric catalase molecule dissociates into monomers as observed by the size-exclusion chromatography and cross-linking studies (Fig. 2). Refolding of alkaline-denatured catalase (pH 11.5) by diluting 10 times in a pH 7.0 buffer shows a very low (maximum of ~80%) recovery of native enzyme activity. Studies on the effect of salts on native catalase showed that addition of high salts (3 M KCl) to native catalase brings about slight exposure of buried hydrophobic clusters as observed by ANS binding, resulting into aggregation at elevated temperatures as observed by light-scattering studies (Fig. 7). Refolding of these samples after incubation for 3 h at 4°C results in total recovery of native enzyme activity (data not shown). Furthermore, increase in pH of native catalase containing 3 M KCl from pH 7.0 to pH 11.5 results in formation of partially folded tetramer with exposed hydrophobic clusters, as observed by size-exclusion chromatography and ANS binding (data not shown).

Based on these observations, it seems that the high net charge and the effective increased size of the charged residue, due to the presence of the associated counter ion on the enzyme molecule, stabilize the intermediate conformational state of catalase and prevent the formation of the native tertiary structure. Reconstitution/refolding studies showed that a maximum of about 25% recovery of native enzyme activity can be obtained on reconstitution of monomers obtained on alkaline denaturation of catalase, whereas about 80% recovery of native catalase can be obtained on refolding of salt-induced partially folded tetrameric intermediate.

Mechanism of the salt-dependent conformational transition. The main forces unfolding the protein under extreme pH conditions are the repulsive forces between charged groups on the protein molecule. Under these conditions, the presence of higher concentrations of salts will have two effects on the protein conformation. The Debye-Huckel screening effect, i.e. the counter ion presence around the charged groups will weaken the repulsion, thus permitting the other forces favoring folding to become relatively strengthened and resulting in folding of the protein. In addition to this nonspecific effect of salts, specific effects of salts on protein folding will also come into play. The stabilizing or destabilizing effects of salts on proteins arise either by effects on water structure, and hence hydrophobic interactions or by specific interactions with charged groups. It is well established that the effects of anions in stabilizing protein structure follow the Hofmeister series [34]. The representative series is sulphate > phosphate > fluoride > chloride > bromide > iodide > perchlorate > thiocyanate. In this series the ions to the left of chloride stabilize the native structure of the protein, whereas the anions to the right of chloride destabilize the native structure of proteins. Arakawa and Timasheff [35] have shown that preferential hydration of proteins occur in the presence of salts such as NaCl, sodium acetate and Na_2 SO_4, and that the resulting unfavorable free energy of the unfolded state is related to the stabilizing effects of these salts on the proteins. If the salt-induced conformational transition of alkali-denatured catalase reported in this study results from the changes in the water structure, then the effects of various ions should follow Hofmeister series. According to this series Na_2 SO_4 is much more effective than KCl in stabilizing hydrophobic interactions and in increasing the stability of protein. Studies on ANS binding and temperature-dependent aggregation of alkaline-denatured catalase in the presence of salt as reported above showed that indeed Na_2 SO_4 was much more effective than KCl in stabilizing hydrophobic interactions in alkaline-denatured catalase.

Salt-dependent conformational transition and subunit association at alkaline or acidic pH may be a general property of multimeric proteins. However, the concentration of salt required to bring about the transition will depend on the particular protein and the condition employed. The stability of the salt-induced
REFERENCES


