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CHAOTROPIC IONS AND THEIR INTERACTIONS WITH PROTEINS[1]

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INTRODUCTION

In this contribution, I would like to discuss the effects of anions on the solubility of small nonelectrolytes and on stability and resolution of large enzyme complexes derived from the mitochondrial inner membrane. Although this has been a topic for decades, it is only recently that ion–solute interactions have been studied in detail with the use of homologous series of ions such as the trihaloacetates and the chloroacetates (1,2), and in aqueous media of different isotopic compositions (2). The results of these studies, together with a wide variety of earlier data, provide suggestive evidence that many important interactions of anions with macromolecules and membranous material are of an indirect nature, i.e., mediated by the aqueous solvent rather than originating from direct binding. Specifically, it has been proposed that the effect of ions on the structure of water is directly responsible for changes in the solubility of nonelectrolytes as well as in the strength of hydrophobic interactions in biopolymers and membrane systems (3). Especially, the latter aspect is of obvious relevance for hydrophobic and affinity chromatography. Indeed, one of the earlier applications of this technique involved the use of salts containing large, monovalent anions, i.e., "chaotropic" salts, for the elution of an antibody from a column containing covalently attached antigen (4).

THE ROLE OF THE STRUCTURE OF WATER IN SALT EFFECTS AND HYDROPHOBIC INTERACTIONS

The term "chaotropic" as a qualification for a certain type of salt or ion is relatively new (5). However, it has been known for a long time that not all

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TABLE 1. **Concentration of Sodium Salts Necessary to Induce Turbidity in Solutions of Egg Globulin**[a]

Sodium Salt	Concentration (mol/liter)
Citrate	0.56
Tartrate	0.78
Sulfate	0.80
Phosphate	0.83
Chromate	1.13
Acetate	1.69
Chloride	3.69
Nitrate	5.42
Chlorate	5.52
Bromide	[b]
Iodide	[b]

[a] Modified from ref. (7).
[b] No precipitation at the highest possible concentration.

salts are alike, even at constant ionic strength, normality, and concentration. From the earliest systematic studies of the effects of salts on proteins and biopolymers (6–9), it was evident that in the original Hofmeister series two groups of ions could be distinguished. In protein precipitation studies (Table 1), the position of acetate marks the dividing line between anions which salt out effectively, and those which have little or no capacity to cause protein precipitation. Similarly, in a Hofmeister series of ions ordered according to their effect on gelatine,

$$\text{sulfate, tartrate, citrate} < \text{acetate} < \text{chloride} < \text{bromide},$$

$$\text{nitrate} < \text{iodide} < \text{thiocyanate},$$

ions at the left side of chloride are able to induce shrinking of gelatine disks, while ions at the right side of acetate cause swelling (8,9). Much later, in their studies on the denaturation of DNA, Hamaguchi and Geiduschek found that certain large anions lower the melting points, i.e., the midpoints of the irreversible thermal denaturation, by between 16° and more than 68°C. These ions can be ordered in a "chaotropic" series,

$$\text{trichloroacetate} \gg \text{trifluoroacetate} > \text{thiocyanate} >$$

$$> \text{perchlorate} > \text{iodide},$$

according to their tendency to disorder DNA (5). Studies of the effects of chaotropes on biomembranes were initiated in Hatefi's laboratory as part of a search for rational techniques in the resolution of membrane-derived enzyme complexes (1–3,10–16).

In pilot experiments it was found that the most chaotropic ions in the series of Hamaguchi and Geiduschek, and the anions most distant from the salting-out end of the Hofmeister series, were able to solubilize up to 40% of the protein from a variety of membrane materials (3). Remarkably, the order of effectiveness of anions in these experiments parallels that found for a variety of other processes such as denaturation, depolymerization, and solubilization of biopolymers, as well as the effects of ions on the solubility of nonelectrolytes such as riboflavin, adenine, 2-methylnaphthoquinone, and even small, nonpolar molecules such as benzene, oxygen, and hydrogen (3). Obviously, in these extremely diverse systems, the only common denominator is the aqueous medium. Indeed, ions ranked according to their effect on water alone form an order very similar to the Hofmeister and the chaotropic series.

The properties of water influenced by the presence of salts are all manifestations of the structure of water, and include self-diffusion, surface tension, surface potential, proton chemical shift, and, most importantly, the entropy of ions and the entropy of hydration of ions (3). The large increase of the entropy values in the series

$$\text{sulfate} < \text{fluoride} < \text{chloride} < \text{bromide} < \text{iodide} < \text{perchlorate}$$

was interpreted by Frank and Evans as an effect of these ions on the structure of water in the direction of more disorder (17). The details of the structure of water are not known in terms of a precise and realistic model (18). However, as a consequence of the quadrupolar structure of hydrogen oxide, water is known to be a highly associated liquid with a boiling point equal to a hydrocarbon five times its molecular weight (Table 2), and some 260–350°C higher than unassociated substances of similar molecular weight, such as methane or neon. Important consequences of the strong molecular interactions in liquid water are the low solubility of nonpolar solutes and the hydrophobic interactions in biopolymers and membranes.

It is seen in Table 3 that the transfer of nonpolar molecules from a lipophilic surrounding such as liquid hydrocarbon or the interior of a

TABLE 2. **Molecular Weight of Compounds with Similar Boiling Points**

	Boiling point (°C)	Molecular weight
Water	100.0	18.02
1-Propanol	97.4	60.11
2-Butanol	99.5	74.12
n-Heptane	98.4	100.21

TABLE 3. **Thermodynamics of Transfer from Apolar Surroundings into Water**[a]

	$\Delta\mu^0$ (kJ/mol)	ΔH^0 (kJ/mol)	$-T\Delta S^0$ (kJ/mol)
Liquid hydrocarbon to water			
Ethane	16.3	−10.5	26.2
Propane	20.5	−7.1	27.4
Butane	24.7	−3.3	28.7
Benzene	17.0	0	17.5
Dodecyl sulfate micelle to water			
Ethane	14.4	−8.4	22.8
Propane	17.7	−4.2	21.8
Butane	21.5	0	21.5

[a]Modified from refs. (19) and (20).

detergent micelle into water is energetically unfavorable ($\Delta\mu^0 > 0$).This is entirely due to a decrease in entropy ($-T\Delta S > 0$), which overcomes a usually favorable enthalpy term. Since there are little, if any, changes in the internal energy of the solute molecules, the decrease in entropy must reflect an increased order in the water phase. A similar thermodynamic situation is assumed to be the basis for hydrophobic interactions, which, according to Kauzmann (20), originate from an entropic barrier against the transfer of hydrophobic groups into the aqueous medium, more than from van der Waals interactions between apolar groups themselves.

Thus, with respect to the structure of water, aqueous solutions of chaotropic salts and those of apolar atoms, molecules, or groups have effects of opposite sign. Therefore, we have proposed that chaotropic ions weaken hydrophobic bonds and increase the solubility of nonelectrolytes by a common mechanism involving a more positive transfer entropy (3). This hypothesis rests on several assumptions and qualitative analogies. In order to obtain more precise and quantitative information about the system ion-water-nonelectrolyte, a detailed study using haloacetates as salts and both light and heavy water as aqueous media was initiated (1,2).

THE CHAOTROPIC PROPERTIES OF HALOACETATES

The classical homologous series of anions in the study of salt effects, viz., F^-, Cl^-, Br^-, I^-, is unsuitable for our type of study with derivative

preparations of the mitochondrial inner membrane. This is because fluoride reacts with iron porphyrins, and bromide and iodide are apt to participate in a variety of radical and redox reactions. Instead of the halogens, we have used two homologous series composed of halogen substituted acetates. Successive chlorine substitution converts acetate, an anion with moderate salting-out properties, into chloro-, dichloro-, and trichloroacetate, one of the most powerful chaotropic ions available. At this point, another series branches off, which includes, in addition to the latter anion, trifluoro- and tribromoacetate. In both series, deviations from the ideal spherical shape due to the presence of the carboxyl groups are common to all derivatives and should not interfere in qualitative and quantitative comparisons.

The salt effect of these ions was studied in three reactions or equilibria:

1. The solubility of 2-methylnaphthoquinone, a nonelectrolyte of low polarity and meager solubility.
2. The resolution of complex I (NADH: ubiquinone oxidoreductase) of the mitochondrial respiratory chain yielding a soluble NADH dehydrogenase with a low molecular weight and enzymatic properties that are different from those of the parent enzyme complex (10).
3. Structural destabilization of complex I, resulting in lipid oxidation (11,12).

Figure 1 shows the effect of several sodium acetates and two inorganic sodium salts on the solubility of 2-methyl naphthoquinone. It is seen that acetate and bromide salt out to about the same moderate extent as dichloroacetate and trifluoroacetate salt in. In contrast, trichloroacetate has a strong salting-in effect. At 3.6 M trichloroacetate, the solubility of

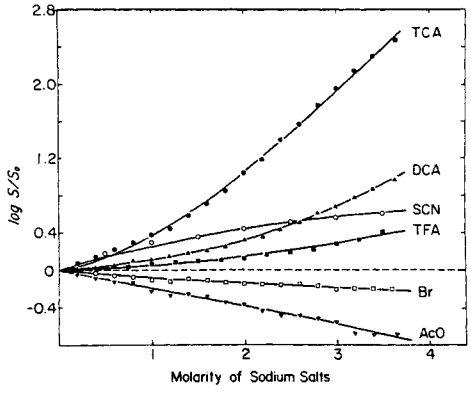

FIG. 1. Solubility of 2-methylnaphthoquinone in aqueous salt solutions at 25°C. For symbols, see Eq. (1). From ref. (1).

2-methylnaphthoquinone is increased by a factor of 300. It is also seen that most curves are not linear, i.e., the Setschenow equation [Eq. (1)] is obeyed only at low salt concentrations:

$$\log S_0/S = k_S C_S \tag{1}$$

where S_0 is the solubility in water alone, S is the solubility in aqueous salt solutions at the concentration C_S, and k_S is the Setschenow constant, negative or positive, for salting in or salting out, respectively.

Similar results are seen in the resolution of complex I. The appearance of a soluble NADH dehydrogenase follows first-order kinetics. A plot of the rate constants as a function of type and concentration of the salt used is shown in Fig. 2. Tribromoacetate and monochloroacetate are also included, because they are sufficiently stable over the short period of time necessary for the resolution experiments. The curves are characterized by a high degree of parabolic curvature, which may be due to the cooperative nature of the resolution of this multicomponent system.

In the third test, lipid oxidation is taken as a parameter of the destabilization of the mitochondrial membrane induced by chaotropic salts. In complex I this reaction appears to be initiated by radicals produced during the destriction of iron-sulfur centers, which in turn is a consequence of chaotrope-induced membrane perturbation (11,12). Thus, although the object under study is the same as in the dissociation of NADH dehydrogenase (Fig. 2), the sequence of events leading to the measured reaction, i.e.,

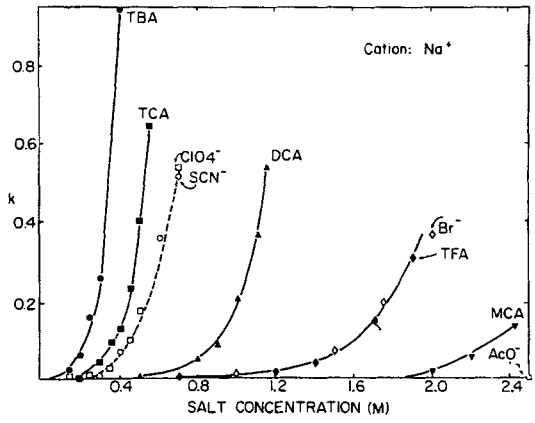

FIG. 2. Resolution of complex I by various chaotropic salts. The ordinate shows k (min^{-1}), the first-order rate constant of the resolution reaction. From ref. (1).

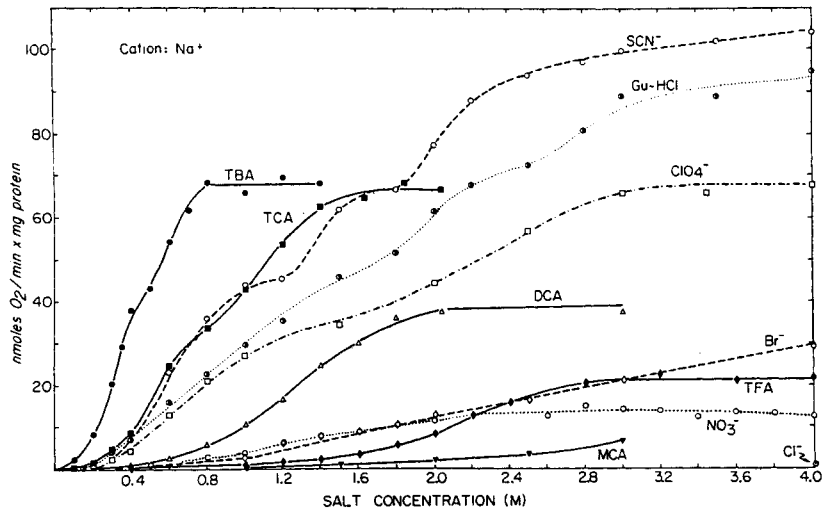

FIG. 3. Membrane destabilization by chaotropic salts as measured by lipid oxidation in complex I. From ref. (1).

the uptake of oxygen, is of course quite different. Figure 3 shows again that the effectiveness of membrane destabilization increases in the order

$$CH_3COO^- \ll ClCH_2COO^- < F_3CCOO^- < Cl_2CHCOO^- < Cl_3CCOO^- < Br_3CCOO^-.$$

Similar to Fig. 2, the rate of lipid oxidation is an obviously complicated function of the salt concentration. Thus, while the solubilities shown in Fig. 1 can be described by an extended Setschenow equation, that is,

$$\log S_0/S = k_S^0 C_S + aC_S^2 + bC_S^3 \tag{2}$$

the parameters necessary to describe the curves in Fig. 2 by a power series approach the experimental points in number and are therefore less valuable. Obviously, the complexity of the curves in Fig. 3 defies any mathematical treatment in the context of this study. For these data, as a substitute for single parameters such as k_S or k_S^0 in Eqs. (1) and (2), respectively, it is useful to compare the reciprocal concentrations of a salt eliciting a standard response. This type of procedure is widely used in studies of relationships between biological response and substitutent constants (21).

In Fig. 4, parameters derived in this manner for resolution and membrane destabilization are compared with solubility data. It is seen that there is a good correlation not only between resolution and lipid oxidation in complex I, but also, and more importantly, between the effect on the solubility of 2-methylnaphthoquinone and the former two reactions. These

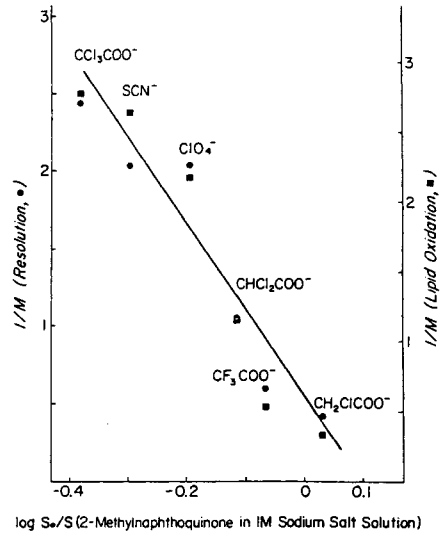

FIG. 4. Correlation between resolution and lipid oxidation in complex I, and the solubility of 2-methylnaphthoquinone. The left ordinate shows the reciprocal of the salt concentration necessary to elicit resolution of complex I with a rate constant of 0.13 min^{-1}. The right ordinate shows the reciprocal of the salt concentration necessary to induce lipid oxidation at a rate of 6.8 nmol O_2/min · mg mitochondrial protein. The correlation coefficients for lipid oxidation vs. solubility, resolution vs. solubility, and lipid oxidation vs. resolution are 0.95, 0.96, and 0.99, respectively. From ref. (1).

quantitative relationships can be used to analyze the chaotropic properties of haloacetates in terms of a theory of the salting-in effect developed by Bockris et al. (22). In this theory, salting in occurs when the coulombic salting-out effect is smaller than the effect of dispersion forces. For 1 : 1 salts with a common cation, the Setschenow constant k_s [Eq. (1)] or a similar parameter can be expressed in a simplified equation:

$$k_S = A(1/r) - B(R/r^3) + C \qquad (3)$$

where the first and the second terms at the right side represent the effect of electrostatic and dispersion forces, respectively, and the third term is

characteristic for the common cation. The terms r and R are radius and molecular refraction of the anion, respectively.

There is, of course, a statistical problem with data sets containing only five points each, i.e., data obtained with five acetate derivatives, to be fitted with an equation already containing three adjustable parameters. However, because of the linear correlations shown in Fig. 4, it is possible to normalize all data sets and analyze them simultanously. The normalized data are then a function of radius and molar refraction, as are the original data. This function, $f(r, R)$, replaces k_S in Eq. (3). The data used consisted of 20 points and were contained in four sets: two sets of solubility data, i.e., k_S^0 [Eq. (2)] and $k_1 = k_S^0 + a + b$, the experimental $\log S_0/S$ at 1 M salt concentration; and two sets of membrane perturbation data, i.e., $1/M_{res}$, the reciprocal of the salt concentration necessary to elicit the resolution of complex I with a rate constant of 0.13 min^{-1}; and $1/M_{ox}$, the reciprocal of the salt concentration necessary to induce lipid oxidation at a rate of 6.8 nmol $O_2/min \times mg$.

Normalization with respect to $1/M_{ox}$ and least squares analysis of the data resulted in Eq. (4):

$$f(r/R)_{calc} = 77.18(1/r) - 31.34(R/r) - 3.10 \quad (4)$$

The contributions of the coulombic salting-out term, A/r, and the dispersion term leading to salting in, $B(R/r^3)$, are shown in Table 4. It is seen that increasing chloro substitution decreases the dispersion term (column 3). However, this decrease is smaller than the strong decrease in the coulombic term (column 2) due to the increase in ionic radius. The relatively weak salting-in effect of trifluoroacetate can be traced to the relatively small size of this molecule and the correspondingly large coulombic term. Thus, the

TABLE 4. **The Contributions of Electrostatic and Dispersion Forces to the Chaotropic Potency of Haloacetates**[a,b]

	A/r	$B(R/r^3)$	$f(r, R)_{calc}$
CH_3COO^-	30.79	25.83	1.87
CH_2ClCOO^-	26.39	23.69	−0.39
$CHCl_2COO^-$	23.32	21.95	−1.12
CCl_3COO^-	22.15	21.82	−2.76
CF_3COO^-	27.40	24.85	−0.55
CCl_3COO^-	22.15	21.83	−2.76
CBr_3COO^-	20.84	23.28	−5.54

[a] From ref. (1).

[b] The expression $f(r/R)_{calc}$ is equivalent to the Setschenow constant [Eq. (1)]. The dimension of all entries is liter/mol. Positive and negative values indicate salting out and salting in, respectively.

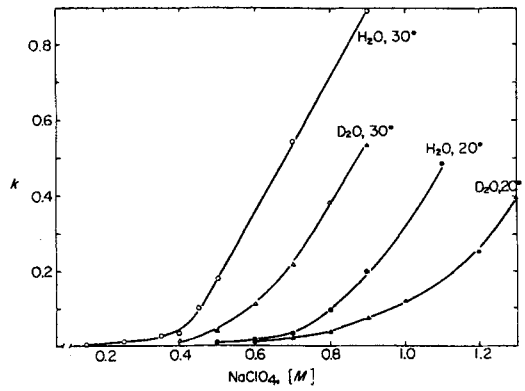

FIG. 5. Solvent isotope effect in the resolution of complex I. From ref. (2).

chaotropic properties of polyhalogen substituted acetates appear to be mainly due to decreased coulombic salting out.

The linear correlation between the effects of salts on small nonelectrolytes and on large membrane assemblies (Fig. 4), and the importance of low coulombic salting-out terms for the chaotropic properties of salts, are quantitative results that are entirely compatible with the hypothesis that chaotropes interact with solutes mainly indirectly, i.e., through their effect on the structure of water.

SOLVENT ISOTOPE EFFECTS AND CHAOTROPIC PROPERTIES

The role of water in the interactions of chaotropes with proteins and membranes can also be studied from a different angle, namely, with resolution experiments carried out in light and heavy water. D_2O is a medium with an apparently higher degree of structure than H_2O (23–25). The solubility of nonelectrolytes is generally lower, and the stability of hydrophobic bonds appears to be greater than in ordinary water (2,26). Figure 5 shows that the resolution of complex I requires a higher chaotrope concentration or a higher temperature in D_2O in order to occur at the same rate as in H_2O. Because of the highly nonlinear nature of the resolution curves, the ratio k_H/k_D is not constant, and is therefore not useful as a measure of the solvent isotope effect. As discussed above for the data in Figs. 2 and 3, it is advantageous to compare the concentrations of salt necessary to elicit a standard response. From an inspection of Eq. (1), it can be easily seen that the ratio C_D/C_H of the salt concentrations in both isotopic solvents cor-

TABLE 5. **Solvent Isotope Effect in the Resolution of Complex I in the Presence of Sodium Perchlorate**[a]

k $(min^{-1})^b$	C_D/C_H[c]	
	at 20°C	at 30°C
0.03	1.14	1.27
0.07	1.18	1.26
0.10	1.20	1.29
0.20	1.24	1.33
0.27	1.25	1.33
0.38	1.23	1.31
0.53	—	1.30
av ± SD	1.21 ± 0.04	1.30 ± 0.03

[a] From ref. (2).
[b] First-order rate constant of the resolution of Complex I.
[c] C_H and C_D: Molar concentrations of $NaClO_4$ in H_2O and D_2O, respectively.

responds to the ratio k_S^H/k_S^D of Setschenow constants obtained in light and heavy water. Table 5 lists C_D/C_H values for rate constants ranging over a factor of nearly 20. Surprisingly, the values are essentially independent of the rate constant and vary only a few percent at a given temperature. These results allow some conclusions about the mechanism of chaotrope-induced resolution of complex I, which may be derived as follows.

The rate constant k of the resolution reaction can be represented by a power series in the salt concentration C [Eq. (5)], irrespective of the mechanism of the salt effect:

$$k_H = \sum_{i=1} (a_i C_H)^i, \quad \text{in } H_2O \tag{5a}$$

$$k_D = \sum_{i=1} (b_i C_H)^i, \quad \text{in } D_2O \tag{5b}$$

At $k_H = k_D$,

$$\sum_{i=1} (a_i C_H)^i = \sum_{i=1} (b_i C_D)^i \tag{6}$$

Experimentally, under the conditions of Eq. (6), we find that the ratio $C_D/C_H \equiv F$ is constant at a given temperature (Table 5). Therefore

$$C_D^i \equiv (FC_H)^i \tag{7}$$

Combining Eqs. (6) and (7), one obtains

$$a_i = F \cdot b_i \tag{8}$$

Because the activity coefficients of sodium perchlorate up to 1.4 M are the same within less than 2% (27) in light and heavy water, Eqs. (7) and (8) reflect changes in the enzyme complex imposed by the two isotopic solvents. These changes have the same magnitude in all coefficients of the concentration polynomial [Eq. (5)]. If the mechanism of resolution involved direct ion-protein interaction in a large number of binding equilibria of different strength, the coefficients a_i and b_i would be functions of sums and products of the corresponding equilibrium constants (28,29). Because different equilibria will exhibit qualitatively and quantitatively different solvent isotope effects, the coefficients a_i and b_i are not likely to change all in a similar manner when H_2O is replaced by D_2O. However, Eq. (8) is compatible with the following two-step hypothesis.

1. At low concentrations, the effect of sodium perchlorate on the degree of order in the structure of water is an essentially linear function of the salt concentration, differing by the factor F in H_2O and D_2O. The solubility of nonelectrolytes and nonpolar groups is a linear function of the solvent structure (for quantitative measures of the latter, see ref. 30).
2. The rate of resolution of complex I is a direct function of the "solubility" of nonpolar groups in proteins, i.e., the strength of individual hydrophobic interactions. Because of the number and different strengths of the latter, the resolution process is a distinctly nonlinear function of the solvent structure and therefore the concentration of the chaotropic ion.

Thus, the quotients C_D/C_H in Table 1 are constant because they are not only formally, but also intrinsically, equivalent to the quotient k_S^H/k_S^D of Setschenow constants determined in H_2O and D_2O, respectively.

PREPARATIVE USE OF CHAOTROPIC SALTS

Because of the importance of hydrophobic bonds in the structure of proteins, enzyme complexes, biomembranes, and nucleic acids, a wide applicability of the use of chaotropic ions can be expected. However, as in the case of detergents, a generally applicable method for dissociation, resolution, or solubilization of membrane components cannot be given. In every instance, the experimental procedure will have to be tailored to the particular strength of hydrophobic interactions, the stability of products and starting material, and other factors such as temperature dependence and pH

characteristics. Nevertheless, some general rules are apparent which, together with selected examples, might be useful as a guide.

Among a number of established chaotropic ions, there are several which are more suitable than others. The strongest chaotrope known at present, i.e., tribromoacetate, has a tendency to decarboxylate and produce bromoform, $CHBr_3$, a solvent that will interfere wherever chloroform is not innocuous. Because of its stability and ready availability as sodium salt, trichloroacetate is probably the most useful among the very strong chaotropes. In the class of the moderately strong chaotropic salts, sodium perchlorate is the most preferable one. It may be obtained in high purity, is very soluble in water (>8 M), and is unreactive under all conditions of biological research. Thiocyanate is a chaotrope of similar strength, but has the disadvantage of being a fairly reactive nucleophile (31) and of forming complexes with transition metals. If weak chaotropic effects are desired, or if both hydrophobic and ionic bonds appear to be involved (32), bromide or nitrate should be employed rather than chloroacetate because of the alkylating properties of the latter.

There appear to be three distinct, if overlapping, concentration ranges in which sodium perchlorate and thiocyanate are active:

0.001–0.4 M:	Effect on enzyme activities (33–36)
0.1–1 M:	Dissociation and solubilization of proteins (3,10,13,15,16,37)
1–6 M:	Isolation of RNA from animal tissue (38)
	Denaturation of DNA (5)
	Dissociation of antigen-antibody complexes (4)

In exploratory experiments, it is also useful to work at different temperatures in order to distinguish reactions (equilibria) with high and low activation energies (reaction enthalpies). For instance, the resolution of complex I is an essentially irreversible process, which has an exceptionally high activation energy of 155 kJ at 0.47 M $NaClO_4$ (Fig. 2; refs. 2,39). In contrast, the resolution of complex II (succinate:ubiquinone reductase) yields water-soluble succinate dehydrogenase in a reversible process characterized by a low enthalpy term (15).

At present, the most thoroughly studied resolution processes are those yielding low-molecular weight, water-soluble dehydrogenases derived from the mitochondrial inner membrane, i.e., NADH dehydrogenase (Figs. 2, 5; refs. 10,40,41) and succinate dehydrogenase (13,15,16,42). Figure 6 shows the effect of several chaotropes on rate and extent of the resolution of complex II. As in Fig. 4, it is seen that trichloroacetate is a very strong chaotropic ion, while urea is nearly ineffective at the concentration used. It is important to note that the activity of the solubilized succinate

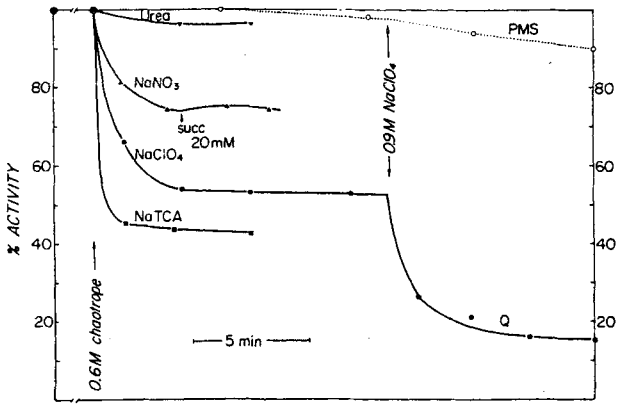

FIG. 6. Resolution of complex II induced by various chaotropic agents at 0°C. From ref. (15).

dehydrogenase is not irreversibly affected by 0.6 M $NaClO_4$ (dotted line, PMS reductase activity), although 85% of the ubiquinone reductase activity is abolished. The further resolution of succinate dehydrogenase into an iron-sulfur flavoprotein (MW 70,000) and a smaller iron-sulfur protein (MW 27,000) requires freeze-thaw steps in the presence of chaotropic salts (14) or treatment with sodium dodecyl sulfate (see below; Fig. 8).

Figure 7 shows that the resolution is an equilibrium process, which is influenced only to a small degree by changes in the temperature ($\Delta H =$

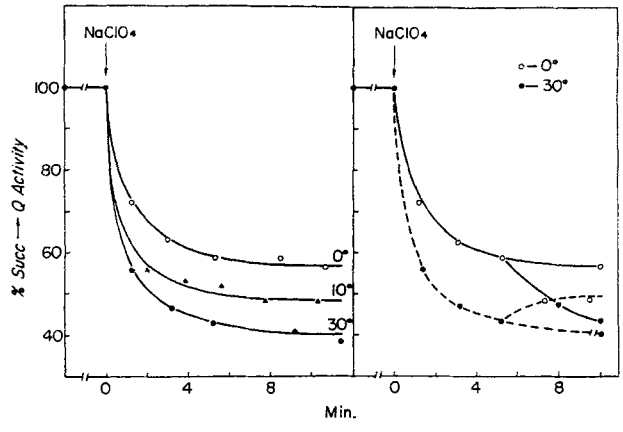

FIG. 7. Effect of temperature on the resolution of complex II. From ref. (15).

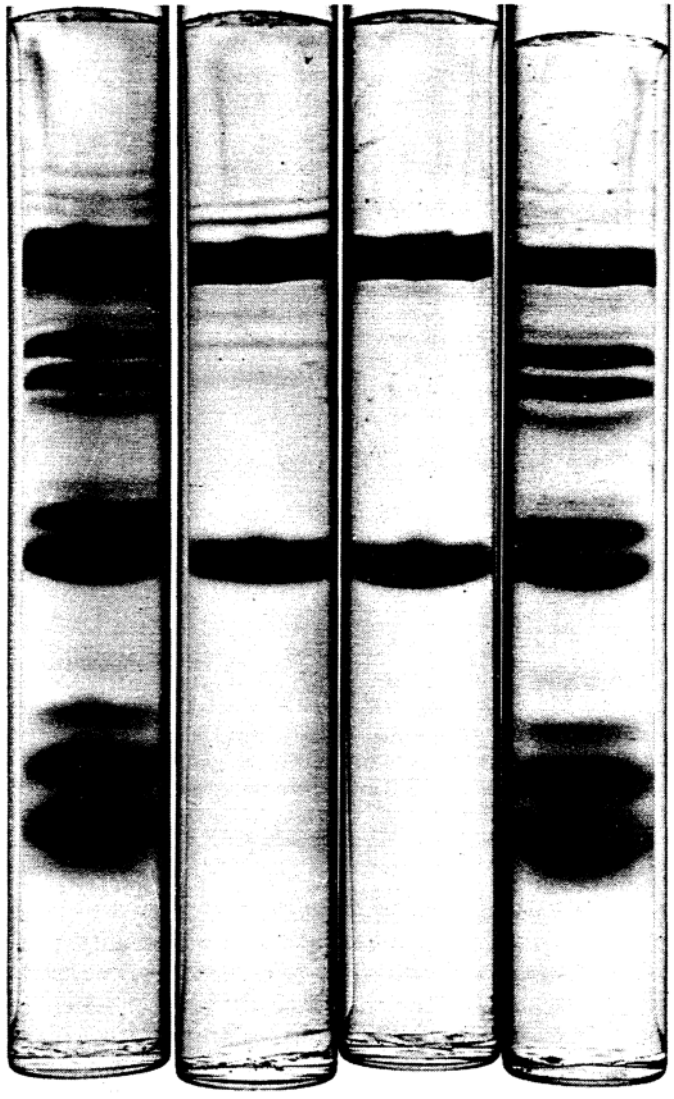

FIG. 8. Purification of succinate dehydrogenase from complex II by stepwise resolution at 0.4 M and 0.75 M $NaClO_4$. For details, see text. From ref. (13).

17 kJ/mol). Incubation at 0°C results in over 60% resolution of complex II under conditions where complex I remains completely intact.

Figure 8 shows the results of a preparative resolution of complex II (13). In the first gel from the left, the polypeptide pattern of the starting material is

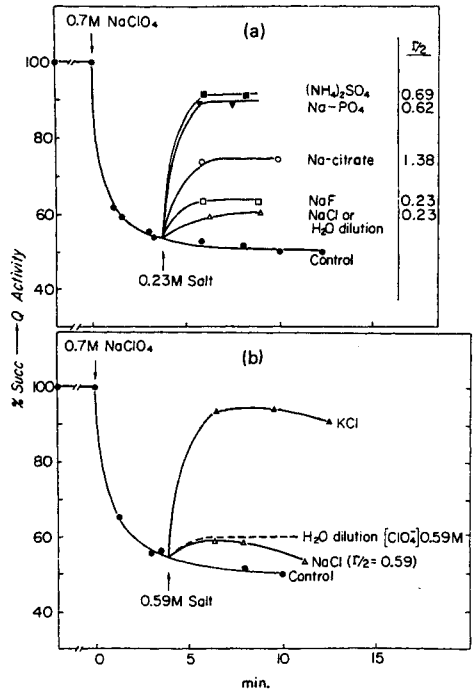

FIG. 9. Reconstitution of partially resolved complex II by antichaotropic salts (a), and removal or dilution of perchlorate (b). From ref. (15).

seen. The second gel demonstrates that a succinate dehydrogenase prepared in one step from complex II is already quite pure. The third gel shows pure succinate dehydrogenase prepared by reextraction of the once-extracted pellet with $NaClO_4$, centrifugation, and differential precipitation with ammonium sulfate. The remaining pellet is seen in the fourth gel. This stepwise technique leads to a preparation of succinate dehydrogenase in a purity and activity not possible before the application of chaotropic salts. In a similar two-step technique, succinate dehydrogenase from *R. rubrum* was isolated in a purity of 70% directly from the chromatophores (16).

Chaotropic salts are, of course, to some degree denaturing agents. If the product of a chaotrope-induced process is unstable under the reaction conditions, it may be necessary to abolish the chaotropic effect faster than is possible with the usual desalting procedures. With sodium perchlorate as the chaotropic salt, this can be readily achieved by precipitation of the anion

with potassium salts or, in a more general procedure, by the addition of counteracting, "antichaotropic" anions.

The dramatic effects of antichaotropic salts in reversing the resolution of complex II are illustrated in Fig. 9a. Most effective are sulfate, phosphate, and citrate, i.e., ions from the salting out end of the Hofmeister series (see Table 1). Significantly, the antichaotropic effectiveness of anions at a given concentration is independent of the respective ionic strengths. Figure 9b shows that dilution of the chaotrope has a similar, but weaker effect, which results in partial reconstitution of complex II. The addition of KCl (Fig. 9b) causes precipitation of $KClO_4$ and nearly quantitative removal of free perchlorate. The resulting reconstitution demonstrates again the complete reversibility of this resolution process. These results also illustrate that in reversible processes the components must be separated before abolishing the chaotropic effect.

REFERENCES

1. HANSTEIN, W. G., DAVIS, K. A., and HATEFI, Y. (1971) Arch. Biochem. Biophys. 147 : 534.
2. HANSTEIN, W. G., DAVIS, K. A., and HATEFI, Y. (1974) Arch. Biochem. Biophys. 163 : 482.
3. HATEFI, Y., and HANSTEIN, W. G. (1969) Proc. Natl. Acad. Sci. U.S.A. 62 : 1129.
4. DANDLIKER, W. B., ALONSO, R., DE SAUSSURE, V. A., KIERSZENBAUM, F., LEVISON, S. A., and SCHAPIRO, H. C. (1967) Biochemistry 6 : 1460.
5. HAMAGUCHI, K., and GEIDUSCHEK, E. P. (1962) J. Am. Chem. Soc. 84 : 1329.
6. LEWITH, S. (1888) Arch. Exp. Pathol. Pharmacol. 24 : 1.
7. HOFMEISTER, F. (1888) Arch. Exp. Pathol. Pharmacol. 24 : 247.
8. HOFMEISTER, F. (1888) Arch. Exp. Pathol. Pharmacol. 25 : 13.
9. HOFMEISTER, F. (1891) Arch. Exp. Pathol. Pharmacol. 28 : 210.
10. DAVIS, K. A., and HATEFI, Y. (1969) Biochemistry 8 : 3355.
11. HATEFI, Y., and HANSTEIN, W. G. (1970) Arch. Biochem. Biophys. 138 : 79.
12. HANSTEIN, W. G., and HATEFI, Y. (1970) Arch. Biochem. Biophys. 138 : 84.
13. DAVIS, K. A., and HATEFI, Y. (1971) Biochemistry 10 : 2509.
14. HANSTEIN, W. G., DAVIES, K. A., GHALAMBOR, M. A., and HATEFI, Y. (1971) Biochemistry 10 : 2517.
15. DAVIS, K. A., and HATEFI, Y. (1972) Arch. Biochem. Biophys. 149 : 505.
16. HATEFI, Y., DAVIS, K. A., BALTSCHEFFSKY, H., BALTSCHEFFSKY, M., and JOHANSSON, B. C. (1972) Arch. Biochem. Biophys. 152 : 613.
17. FRANK, H. S., and EVANS, M. W. (1945) J. Chem. Phys. 13 : 507.
18. NARTEN, A. H., and LEVY, H. A. (1969) Science 165 : 447.
19. TANFORD, C. (1973) The Hydrophobic Effect, Wiley, New York, pp. 18, 38.
20. KAUZMANN, W. (1959) Advan. Prot. Chem. 14 : 1.
21. HANSCH, C. (1968) Accts. Chem. Res. 2 : 232.
22. BOCKRIS, J. O'M., BOWLER-REED, J., and KITCHENER, J. A. (1951) Trans. Farad. Soc. 47 : 184.
23. HOLTZER, A., and EMERSON, M. F. (1969) J. Phys. Chem. 73 : 26.

24. ARNETT, E. M., and MCKELVEY, D. R. (1969) *In* Solute-Solvent Interactions, COETZEE, J. F., and RITCHIE, C. S. (eds.), Marcel Dekker, New York, p. 343.
25. LAUGHTON, P. M., and ROBERTSON, R. E., ibid. p. 399.
26. KRESHEK, G. C., SCHNEIDER, H., and SCHERAGA, H. A. (1965) J. Chem. Phys. 69 : 3132.
27. BONNER, O. D. (1971) J. Chem. Thermodyn. 3 : 837.
28. SCATCHARD, G. (1949) Ann. N.Y. Acad. Sci. 51 : 660.
29. KLOTZ, I. M. and HUNSTON, D. L. (1975) J. Biol. Chem. 250 : 3001.
30. BEN-NAIM, A. (1978) J. Phys. Chem. 82 : 874.
31. KOSOWER, E. M. (1968) Physical Organic Chemistry, Wiley, New York, p. 80.
32. MARQUARDT, H., WILSON, C. B., and DIXON, F. J. (1973) Biochemistry 12 : 3260.
33. FRIDOVICH, I. (1963) J. Biol. Chem. 238 : 592.
34. WARREN, J. C., and PETERSON, D. M. (1966) Science 152 : 1245.
35. JACOBSEN, D. W., and HUENNEKENS, F. M., Biochem. Biophys. Res. Commun. 37 : 793.
36. SEIDEL, J. C. (1969) J. Biol. Chem. 244 : 1142.
37. GERSHMAN, L. C., and DREIZEN, P. (1970) Biochemistry 9 : 1677.
38. SCOTT, J. F., and KUHNS, V. L. (1972) Anal. Biochem. 47 : 471.
39. HATEFI, Y., and HANSTEIN, W. G. (1974) Methods Enzymol. 31 : 770.
40. HATEFI, Y., and STEMPEL, K. E. (1969) J. Biol. Chem. 244 : 2350.
41. DOOIJEWAARD, G., SLATER, E. C., VAN DIJK, P. J., and DE BRUIN, G. J. M. (1978) Biochim. Biophys. Acta 503 : 405.
42. ACKRELL, B. A., KEARNEY, E. B., and COLES, C. J. (1977) J. Biol. Chem. 252 : 6963.