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Review

Hydration and partial compressibility of biological compounds

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Abstract

We review the results of compressibility studies on proteins, nucleic acids, and systematically altered low molecular weight compounds that model the constituents of these biopolymers. The model compound data allow one to define the compressibility properties of water surrounding charged, polar, and nonpolar groups. These results, in conjunction with compressibility data on proteins and nucleic acids, were used to define the properties of water that is perturbed by the presence of these biopolymers in aqueous solutions. Throughout this review, we emphasize the importance of compressibility data for characterizing the hydration properties of solutes (particularly, proteins, nucleic acids, and their constituents), while describing how such data can be interpreted to gain insight into role that hydration can play in modulating the stability of and recognition between biologically important compounds.

Key words: Partial compressibility; Hydration; Charged, polar, and nonpolar atomic groups; Globular proteins; DNA duplexes

1. Introduction

Knowledge of the structures of biological molecules has provided us with substantial insights into how such molecules express their biological functions. This structural information primarily has been gleaned from crystallographic and NMR studies. However, the chemistry of life occurs in a predominantly aqueous milieu, and, consequently, water undoubtedly plays an important role in modulating the structures as well as the recognition events of biological molecules.

Based on crystallographic studies on nucleic acids, the Dickerson and Berman laboratories independently have emphasized the central role that solvent might play in dictating the detailed features of DNA duplex structures [1,2]. Over a similar period of time, the Breslauer group has emphasized the need and demonstrated the value of complementing microscopic structural studies on nucleic acids with parallel macroscopic thermodynamic studies [3,4]. With regards to proteins, the role of hydration in modulating structural stability and functional activity has been recognized for an even longer period of time [5]. In addition to a significant number of structural studies, numerous thermodynamic characterizations of proteins in aqueous solutions have been reported (particularly noteworthy are the calorimetric studies of the Privalov, Sturtevant, and,

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more recently, Freire groups). Taken together, these studies are beginning to provide us with insight into protein hydration and its role in modulating protein properties, including biological function [6–8].

It is becoming increasingly clear that microscopic and macroscopic studies should be conducted in parallel to enable one not only to identify specific intra- and intermolecular contacts (the microscopic result), but also to characterize their relative importance (the macroscopic data). The latter assessment is required for the rational engineering of biological molecules to produce substances with predictably altered structures, stabilities, and biological functions. The importance that such microscopic and macroscopic studies also include characterizations of hydration properties (solute–solvent interactions) recently has been underscored by the results of several protein–nucleic acid studies. Specifically, it has been shown that recognition between a protein and nucleic acid can occur 'indirectly' through a hydration interface rather than directly through contact between the atomic groups of the two biopolymers [9–11].

A review of the literature reveals that macroscopic characterizations of hydration have not kept pace with X-ray and NMR microscopic studies of hydration. This deficiency, in part, reflects an inherent feature of macroscopic measurements in which it is difficult to resolve an overall observable into the hydration contribution of a particular solute domain. Moreover, relatively few macroscopic observables are sensitive to solute hydration in dilute aqueous solutions. However, as explained below, compressibility is a macroscopic observable which is sensitive to solute–solvent interactions and therefore can and has been used to characterize hydration properties of solutes in dilute solutions. Furthermore, recent technical advances in acoustic techniques now make it possible to conduct high precision compressibility measurements on small volumes of dilute solutions of biological samples over a range of temperatures and pressures, thereby facilitating the resolution of this macroscopic observable into solute and solvent contributions.

2. Background

The compressibility of a fluid is equal to the isothermal or adiabatic pressure derivative of the volume and, consequently, is a second pressure derivative of the Gibbs free energy. Since compressibility, like volume, is determined by the composite effect of intra- and intermolecular interactions, its value can be used to gain insight into these interactions.

When studying solute–solvent interactions, and, in particular, hydration, it is convenient to deal not with the total compressibility of a solution, but rather with the apparent molar or specific compressibility of the solute. The apparent compressibility of a solute is determined by (i) interatomic interactions within the solute molecule itself (the intrinsic compressibility of a solute molecule); (ii) solute–solute interactions, if any occur under the experimental conditions; and (iii) solute–solvent interactions (hydration in the case of aqueous solutions). The contribution of solute–solute interactions to the value of the apparent compressibility of a solute diminishes with decreasing solute concentration, and at infinite dilution becomes equal to the partial compressibility of the solute. In such cases, the partial compressibility of a solute solely reflects contributions from the intrinsic compressibility and the hydration of the solute. Significantly, various atomic groups (e.g., charged, polar, aliphatic, aromatic) when solvated, differ not only with respect to their contributions to the total partial compressibility of the solute, but also with respect to the temperature dependences of these contributions [12–14]. In principle, knowledge of such functional dependences can be used for two complementary purposes: (i) to calculate the contribution of hydration to the partial compressibility of complex molecules (e.g., biopolymers) from knowledge of their surface accessible groups [15,16]; and/or conversely (ii) to derive information about the nature of the accessible surface atomic groups from knowledge of the absolute values and temperature dependences of the partial compressibility. Such assessments require an empirical data base of partial compressibilities

for a wide range of compounds in which contributions from the substituent atomic groups have been resolved.

In recognition of this need and towards this end, a number of laboratories in the 1970s, 1980s, and 1990s have extensively studied the volumetric properties (e.g., volume, expansibility, compressibility) of biological substances and have explored relationships between these macroscopic properties and the different types of microscopic solute–solvent interactions [12,17–21]. Based on this body of work, it has become clear that measurements of the partial molar (or specific) volumes as well as adiabatic and/or isothermal compressibilities of solutes provide a powerful means of characterizing the hydration properties of biologically interesting solutes [12,17–28]. Reviews of partial volume studies of biological compounds can be found in refs. [26–28].

This review will focus on recent compressibility measurements on biological molecules and related model system compound studies, with an emphasis on how such data can be interpreted in terms of hydration properties. Such compressibility studies are distinct from investigations concerned with the influence of high hydrostatic pressure on association and dissociation processes, studies which also have been interpreted in terms of hydration properties [29,30].

The development of new high precision acoustic methods [31–33] has enabled accurate evaluation of adiabatic compressibilities on samples of greatly reduced volumes. This technical advance has made it possible to study dilute solutions of biological compounds which are not available in large quantities or which exhibit low solubilities. The existing volumetric data base primarily has been derived from partial compressibility measurements on low molecular weight model compounds and some biopolymers. The low molecular weight compounds studies, including alcohols [34–37], carboxylic acids [38,39], carbohydrates [40], amino acids [13,18,23,41], peptides [24,42–45], nucleobases, nucleosides, nucleotides [14,46–50], as well as some lipids [51–53], have been widely used to derive the contributions of different atomic groups to the partial molar compressibility of solutes. Compressibility studies of biopolymers themselves are much fewer in number and primarily have been limited to several studies of proteins [15–17,21,54,55] and DNAs [25,56]. In the aggregate, these results have begun to establish the requisite data base, although a great deal of additional volumetric measurements are needed to define the hydration contributions that different atomic groups make to the partial compressibilities of solutes, including biopolymers, and to interpret the partial compressibility data in terms of hydration.

A number of publications previously have reviewed partial compressibility data on biological compounds, particularly as they relate to hydration [12,20,57]. Consequently, to avoid duplication this review is not intended to be all inclusive. Instead, we focus on studies which address the influence of charged, polar, and nonpolar atomic groups on the compressibility properties of surrounding water molecules, and discuss how such model system data can be used to describe the hydration properties of proteins and nucleic acids. As just noted, this review is not designed to be comprehensive but rather emphasize our most recent results, as well as related studies from select other laboratories. Furthermore, we intentionally do not include technical descriptions of the instruments and methods of measurement since they can be found in the literature [12,26,58, and references cited therein], but rather focus on the resulting experimental compressibility data and how such data can be interpreted in terms of hydration properties. We begin by defining the partial molar adiabatic compressibility of a solute, we then describe how this parameter is measured, and finally we explain how its value can be used to gain insight into hydration properties.

3. Partial molar adiabatic compressibility

3.1. Definitions

The apparent molar adiabatic compressibility of a solute is described by the expression

$$\phi K_S = \left(\beta_S V - N_1 \beta_{S0} \bar{V}_0\right)/N_2, \qquad (1)$$

where V is the volume of a solution containing N_1 moles of solvent and N_2 moles of solute; $\bar{V}_0$ is the partial molar volume of the solvent; and β_S and β_{S0} are the coefficients of adiabatic compressibility of the solution and the solvent, respectively. (If the coefficients of isothermal compressibility of the solution, β_T and the solvent, β_{T0}, are used instead of β_S and β_{S0}, Eq. (1) transforms into an expression for the apparent molar isothermal compressibility of the solute, ϕK_T). The coefficient of adiabatic compressibility of a medium is related to its density, ρ, and sound velocity, U, through the Laplace equation

$$\beta_S = -V^{-1}(\partial V/\partial P)_S = (U^2\rho)^{-1} \quad (2)$$

where S is the entropy; and P is the pressure.

For dilute solutions, the apparent molar adiabatic compressibility, ϕK_S, of a solute is related to its apparent molar volume, $\phi V = (V - N_1\bar{V}_0)/N_2$, by the expression [59,60]

$$\phi K_S = \beta_{S0}(2\phi V - 2[U] - M/\rho_0), \quad (3)$$

where M is the molecular weight of a solute; ρ_0 is the density of the solvent; $[U] = (U - U_0)/(U_0 C)$ is the relative molar increment of sound velocity; U and U_0 are the sound velocities in the solution and the solvent, respectively; and C is the molar concentration of a solute. The apparent molar volume, ϕV, is determined from density measurements [61]. Thus, the apparent molar adiabatic compressibility, ϕK_S, can be calculated directly from density and sound velocity measurements. The apparent molar isothermal compressibility, $\phi K_T = -(\partial \phi V/\partial P)_T$, may be calculated from the value of ϕK_S using [62]

$$\phi K_T = \phi K_S + \left(T\alpha_0^2/\rho_0 C_{P0}\right) \times (2\phi E/\alpha_0 - \phi C_P/\rho_0 C_{P0}), \quad (4)$$

where T is the absolute temperature; $\alpha_0 = V_0^{-1}(\partial V_0/\partial T)_P$, is the coefficient of thermal expansion of the solvent; C_{P0} is the specific heat capacity of the solvent; $\phi E = (\partial \phi V/\partial T)_P$, is the apparent molar expansibility of a solute; and ϕC_P is the apparent molar heat capacity of a solute. Although less accurate, another way of determining ϕK_T is to derive it directly from the pressure dependence of the apparent molar volume, ϕV [62–66].

In the range of concentrations typically used in biochemical studies (1–10 mg/ml), the difference between the partial molar compressibility, $\bar{K}$, and the apparent molar compressibility, ϕK, for most organic compounds, is a linear function of concentration, C [18,20],

$$\phi K = \bar{K} + S_K C, \quad (5)$$

where S_K is a coefficient of proportionality, which, in general, is different for different solutes.

Partial molar adiabatic or isothermal compressibilities usually are evaluated by measuring the concentration dependence of ϕK and extrapolating to zero solute concentration. In the sections that follows, we shall use both definitions (partial and apparent compressibilities) depending on the subject under consideration. Our discussions will predominantly concentrate on the partial (or apparent) molar adiabatic compressibilities, $\bar{K}_S$ (or ϕK_S), of biological compounds, since the partial (or apparent) molar isothermal compressibility data, K_T (or ϕK_T), are far more scarce and, usually, have been determined with less precision.

3.2. Compressibility of bulk water

The compressibility of water, like many of its other thermodynamic parameters, manifests 'abnormal' properties as compared with other liquids. For example, as shown in Fig. 1, the temperature dependences of the coefficients of both the adiabatic, β_S, and isothermal, β_T, compressibilities of water exhibit extrema (as do density, specific heat capacity, and sound velocity). Note, that β_S and β_T exhibit minima at 64 and 46.5°C, respectively [67,68]. The microscopic basis for such temperature dependent macroscopic behavior generally is rationalized in terms of the influence of temperature on the structural and dynamic properties of the hydrogen bonding networks in water. At low temperatures, the compressibility of water actually decreases with increasing temperature (unlike other more 'normal' liquids, e.g. benzene or aniline [69]), while at higher temperatures (beyond the minima), water begins to behave more 'normally', exhibiting a

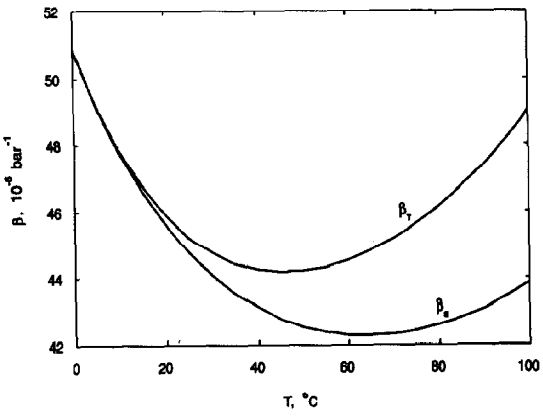

Fig. 1. Temperature dependences of the coefficients of adiabatic, β_S, and isothermal, β_T, compressibilities of water.

positive temperature derivative of compressibility (see Fig. 1).

In compressibility studies, the coefficient of adiabatic compressibility of water, β_S, usually is determined by ultrasonic techniques. In such studies, the β_S value measured at a specific frequency, f, can be represented as the sum of the adiabatic compressibility, measured at infinite frequency, β_∞ (when the structural degrees of freedom are 'frozen out'), and a relaxation component of compressibility, β_r [70]:

$$\beta_S = \beta_\infty + \beta_r \tag{6}$$

where $\beta_r = \beta_{r0}/(1 + i2\pi f\tau)$; β_{r0} is a real number equal to the difference between the adiabatic compressibility measured at zero-frequency and β_∞. The parameter τ in this expression is the relaxation time, which for water is on the order of 1 ps.

A positive relaxation contribution to the compressibility of water results from periodical shifts in the dynamic equilibrium between hydrogen bonded water structures due to changes in temperature and pressure in an ultrasonic wave field. The compressibilities β_∞ and β_r have been evaluated by Leyendekkers [71] as a function of temperature. The 'abnormality' of the temperature dependence of water compressibility is due to the presence of the relaxation contribution, β_{r0}, which is as large as 65% of the total compressibility at 0°C, and reduces to 25% at 100°C [71,72]. The 'normal' component of water compressibility is represented by the high frequency compressibility, β_∞, which increases with increasing temperature, like the compressibility of other liquids [71,72].

The 'abnormal' temperature dependence of water compressibility observed at atmospheric pressure, ceases at higher pressures. Specifically, at 2 kbar, the compressibility increases with temperature, displaying no extrema. Moreover, at pressures higher than 3.5–4 kbar, the temperature dependence of water compressibility becomes almost linear (the second temperature derivative of compressibility becomes equal to 0) [73]. Thus, the 'normal' or 'abnormal' nature of the temperature dependence of water compressibility depends on the pressure range under consideration.

4. Hydration of atomic groups

4.1. Compressibility and hydration

Interpretation of apparent molar adiabatic compressibility data in terms of hydration makes use of the following simple relationship [74]:

$$\phi K_S = K_{SM} + \Delta K_h = K_{SM} + n_h(\bar{K}_{Sh} - \bar{K}_{S0}), \tag{7}$$

where K_{SM} is the intrinsic adiabatic compressibility of a solute molecule; ΔK_h is the compressibility effect of hydration; $\bar{K}_{S0}$ is the partial molar adiabatic compressibility of the solvent; $\bar{K}_{Sh}$ is the partial molar adiabatic compressibility of water in the hydration shell of a solute; n_h is the 'hydration number', which is the number of water molecules in the hydration shell of a solute. The term 'hydration shell' as defined here refers to those water molecules which due to the presence of the solute exhibit altered physico-chemical characteristics compared with bulk water.

For low molecular weight substances (when all the atomic groups are accessible to the solvent), the partial molar compressibility and partial molar volume of a solute can be treated in the framework of an additive model as the sum of

contributions from the constituent atomic groups [13,75,76]. In this model, deviations from additivity are ascribed to the presence of intra- or intermolecular interactions, and therefore can be used as a tool for studying such interactions.

4.2. Hydration shell dimensions

Recall that we have defined the hydration shell of a solute as encompassing those solvent molecules which in the presence of a solute exhibit altered physico-chemical properties compared with bulk water. Various estimates based on both experimental data and theoretical considerations show that the changes in properties of water around solute molecules extend to a distance which corresponds to no more than 2 layers of water molecules [12,16,19,22,24,57,77–79]. One way to estimate how far solute-perturbed solvent extends out from atomic groups is to determine the minimum distance within a single molecule at which two groups no longer interact with each other via the solvent (i.e. the distance at which the hydration shells of two atomic groups no longer overlap). This approach requires a system in which the distance between the two groups of interest can be systematically varied, and a method for detecting an observable that is sensitive to direct and/or solvent-modulated interactions between the groups.

As previously described by us and others [12–14,57], and elaborated on below, compressibility measurements allow one to detect interactions between two atomic groups. Furthermore, the distance between two groups can be varied in a systematic manner by studying homologous series of compounds. We recently have employed this approach to investigate interactions between the oppositely charged amino and carboxy termini in an homologous series of α,ω-amino acids ($^{+}NH_3$–$(CH_2)_n$–COO^{-}) [22] and oligoglycines ($^{+}NH_3$–CH_2–$(CONHCH_2)_nCOO^{-}$) [24]. Our results are summarized in Figs. 2a and 2b which show how the partial molar compressibilities of the α,ω-amino acids and the oligoglycines vary with the number of methylene groups [22] and the number of peptide groups [24], respectively, at 18, 25, 40, and 55°C. Inspection of these figures reveals an intriguing similarity in the dependence of $\bar{K}_S$ on intercharge distance for both classes of molecules. Specifically, at every temperature studied, the dependence becomes linear when the oppositely charged amino and carboxy termini are separated by 5 or more $-CH_2-$ groups (an intercharge distance equal to ~ 7 Å) for the α,ω-amino acids (Fig. 2a), or by 2 or more peptide groups (an intercharge distance equal to ~ 9 Å) for the oligoglycines (Fig. 2b). These results suggest that when the charged terminal groups are separated by more than 7–9 Å, the incremen-

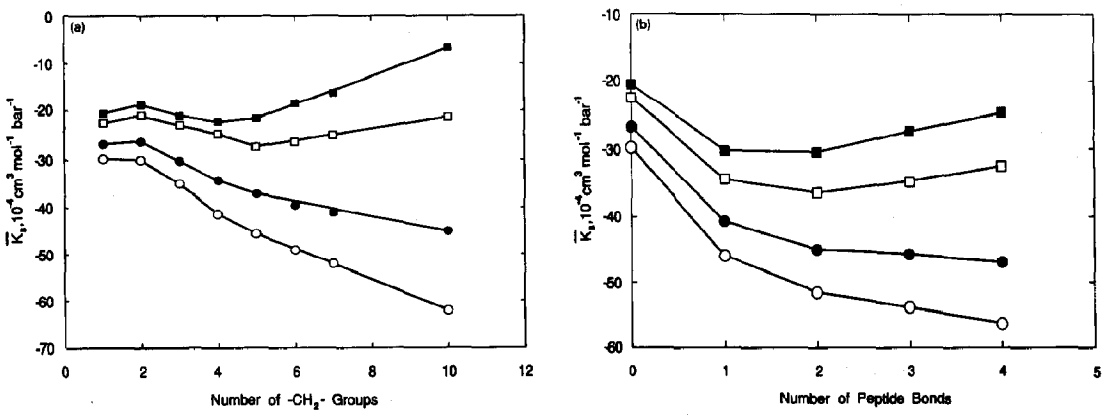

Fig. 2. The dependences of the partial molar adiabatic compressibilities at 18°C (○), 25°C (●), 40°C (□), and 55°C (■) of: (a) α,ω-aminocarboxylic acids on the number of methylene groups [22]; and (b) oligoglycines on the number of peptide groups [24].

tal change in hydration caused by each new spacer link is the same and is determined by the independent hydration of each added link [22,24]. In other words, the charged termini cease to interact both with each other and with the added links when separated by a distance of 7–9 Å. We therefore propose that 7–9 Å corresponds to the double 'thickness' of the hydration shell of a charged atomic group (assuming similar hydration shell 'thickness' for both charged groups). Thus, even for strong solute–solvent interactions, like those between water molecules and charged atomic groups, the solute-perturbed solvent appears to extend to a distance no more than 3–4 Å. This distance corresponds to 1–1.5 water molecule diameters if one reasonably takes the effective diameter of a water molecule to be equal to 2.75 Å [80].

Based on the foregoing discussion, the hydration shell of a solute can be envisioned as being comprised mainly of those water molecules which directly contact the solute molecule; in other words, water in the first coordination sphere. Thus, as we previously have discussed [22,24] the hydration number, n_h, for a solute molecule can be calculated as the ratio of its accessible surface area, S_M, to the effective cross section of a water molecule, S_W, so that $n_h = S_M/S_W$. We assume that the number of water molecules within the first coordination sphere, n_h, should not depend strongly on temperature. More strictly speaking, the value of $n_h^{-1}(\partial n_h/\partial T)$ should be on the order of the coefficient of thermal expansion which for most liquids is in the range of 10^{-4}–10^{-3} K^{-1}, and, therefore, as a first approximation can be neglected.

4.3. Hydration of charged groups

Electrostatic interactions represent one of the significant forces which stabilize the structures of both proteins [81–84] and nucleic acids [85,86]. In fact, numerous properties of these biopolymers can be altered significantly by modest changes in pH and/or salt concentration. Such results suggest that electrostatic interactions may provide a means for modulating biopolymer structure and function. For example, solution pH influences the degree of ionization of charged atomic groups, particularly, on the surface of biopolymers, while local salt concentration can modulate the extent of interactions between charged groups. Clearly, knowledge of how such charged groups are hydrated and how this hydration influences intra- and intermolecular interactions is essential for understanding biopolymer properties and how these properties relate to function. Simple low molecular weight organic compounds containing ionizable amino and/or carboxyl groups (e.g., carboxylic acids, aminoalkanes, amino acids, peptides), as well as 1–1 electrolytes provide convenient model systems for studying the hydration properties of charged groups.

Charged groups influence adjacent water molecule dipoles by causing strong electrostatic contraction [54]. This phenomenon, which usually is called electrostriction, causes a partial loss in the mobility of hydrating waters ([84] and references cited therein), which results in a diminution in the partial molar volume of the solvating water and a reduction in the relaxation part of its compressibility (which, in turn, leads to a decrease in the total compressibility). As a result, most 1–1 electrolytes (e.g., all the alkali halides [87]) and small organic zwitterionic molecules, such as α-amino acids [13,18,41,88], α,ω-amino acids [20,22,89], and peptides [24,42–45], manifest large negative partial molar adiabatic compressibilities. For example, at 25°C, the partial molar adiabatic compressibilities, $\bar{K}_S$, of the chlorides of alkali metals lie within the range of -50.5×10^{-4} to -36.6×10^{-4} $cm^3mol^{-1}bar^{-1}$ [90,91], and the values of $\bar{K}_S$ for α-amino acids are within the range of -36.2×10^{-4} to -23.3×10^{-4} $cm^3mol^{-1}bar^{-1}$ [13,18]. By contrast, the values of $\bar{K}_S$ for uncharged species, such as methanol, glycolamide, and ribose are equal to 12.5×10^{-4}, 2.74×10^{-4}, and -12.5×10^{-4} $cm^3mol^{-1}bar^{-1}$, respectively [20].

From the point of view of biological significance, the amino ($-NH_3^+$) and the carboxylate ($-COO^-$) groups are among the most important charged atomic moieties since they are found in a wide variety of biopolymers. As we previously have shown [22], amino and carboxyl terminal groups in α,ω-amino acids do not interact with

each other (their hydration shells do not overlap) in 5-aminopentanoic acid and longer homologues; in other words, when the charged termini are at least 6–7 Å apart. We proposed [22] that the water molecules contacting the hydrophobic $-CH_2-$ groups in 5-aminopentanoic acid are under the predominant influence of the charged termini, since water molecules which simultaneously contact a charged group and an aliphatic group are dominated by the former interaction. In short, the influence of the $-CH_2-$ groups on the hydration of 5-aminopentanoic acid is negligible and the formation of the hydration shell of this α,ω-amino acid is predominantly electrostatic in origin [22]. Thus, the total contribution of the independently hydrated amino and carboxylate groups can, to a first approximation, be taken to be equal to the partial molar adiabatic compressibility of 5-aminopentanoic acid, which is -34×10^{-4} cm^3 mol^{-1} bar^{-1} at 25°C [22].

Charged compounds (e.g., 1–1 electrolytes) characteristically exhibit strongly nonlinear temperature dependences of their partial molar compressibilities, with negative second temperature derivatives, and maxima in these dependences [92]. Fig. 3 shows the temperature dependence of the partial molar adiabatic compressibility, $\bar{K}_S$, of the 1–1 electrolyte NaCl [92], the amino acid glycine [13], the α,ω-amino acid 5-aminopentanoic acid [22], and the dipeptide diglycine

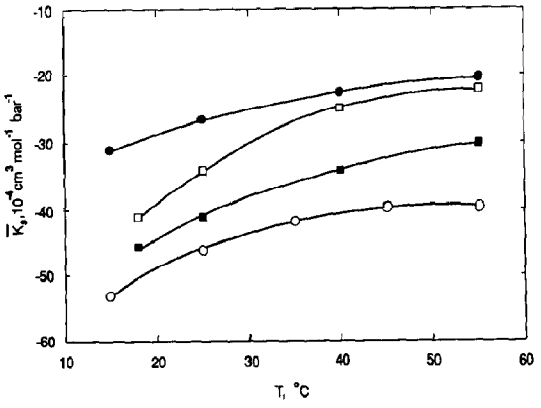

Fig. 3. Temperature dependence of the partial molar adiabatic compressibility of NaCl (○) [92], glycine (●) [13], 5-aminopentanoic acid (□) [22], and diglycine (■) [24].

[24]. The latter three molecules are zwitterionic, and as with NaCl, electrostatic solute–solvent interactions play the major role in the formation of their hydration shells. In fact, as seen in Fig. 3, the shape of the temperature dependences of $\bar{K}_S$ is qualitatively similar for NaCl and for the three zwitterionic organic molecules noted above.

For low molecular weight compounds, the intrinsic compressibility, K_{SM}, in Eq. (7) is determined mostly by the compressibilities of the covalent bonds and the external electron shells, both of which are very small and can be neglected [13]. Thus, for low molecular weight compounds, in particular for NaCl and glycine, the hydration effect, ΔK_h, provides the major contribution to the partial molar adiabatic compressibility so that Eq. (7) reduces to:

$$\bar{K}_S = \Delta K_h = n_h\left(\bar{K}_{Sh} - \bar{K}_{S0}\right). \tag{8}$$

As previously discussed [12,13,57], the nonlinear nature of the temperature dependences of $\bar{K}_S$ for charged compounds can be understood in terms of Eq. (8) if one makes a single plausible assumption; namely, that water molecules in the hydration shells of charged atomic groups are exposed to strong electrostatic fields which cause them to behave like water under high pressure (higher than 3.5–4 kbar) [93], thereby exhibiting a linear increase in the compressibility with increasing temperature. Thus, the temperature dependences of $\bar{K}_S$ of charged compounds represents a difference between a linear term ($n_h\bar{K}_{Sh}$) corresponding to water molecules in the hydration shell, and a nonlinear term ($n_h\bar{K}_{S0}$) corresponding to bulk water. As a result, the temperature dependence of $\bar{K}_S$ adopts a nonlinear form with an extremum, the position of which depends both on the absolute value of $\bar{K}_{Sh}$ and on its temperature slope, $\Delta\bar{K}_{Sh}/\Delta T$.

In the context of the assumption noted above (the linearity of the temperature dependence of the partial molar compressibility of water in the hydration shell around charged moieties), Eq. (8) can be differentiated to yield [13]:

$$\left(\partial^2\bar{K}_S/\partial T^2\right)_P = -n_h\left(\partial^2\bar{K}_{S0}/\partial T^2\right)_P. \tag{9}$$

Thus, the hydration number, n_h, can be derived if the second temperature derivative of the

partial molar compressibility, $(\partial^2\overline{K}_S/\partial T^2)_P$, is measured. Using this approach, a 'hydration number', n_h, for glycine (the hydration shell of which is formed predominantly by electrostatic solute–solvent interactions) has been calculated to be equal to 14 ± 2 [13,22]. This experimental value qualitatively agrees with 18, the number of water molecules one can calculate as directly contacting a glycine molecule based on its solvent accessible surface area [13]. This agreement lends credence to the two assumptions employed in our data analysis; namely, the linearity of the temperature dependence of the compressibility of water solvating charged atomic groups, and the confinement of the hydration shell of solutes to water molecules within the first coordination layer. We have used the approach implicit in Eq. (9) to calculate values of n_h for glycine, β-alanine, 4-aminobutanoic acid, and 5-aminopentanoic acid [22]. Armed with these values of n_h, we used Eq. (8) to calculate the coefficient of adiabatic compressibility, β_{Sh}, of water in the hydration shell of charged atomic groups [22]. The results of these calculations revealed the β_{Sh} value for water in the hydration shell of charged groups to be lower than the coefficient of adiabatic compressibility of bulk water by 15–20% [22].

Some of our most recent studies reveal that the hydration of charged sites (e.g., amino and carboxyl groups) depends on the nature of neighboring atomic groups [unpublished data from our laboratory]. The detailed description of the influence of the neighboring groups on the hydration of the amino and carboxy termini will be published elsewhere. Here we simply wish to note that this influence must be taken into account when the hydration of charged groups is analyzed within complex molecules.

4.4. Hydration of polar groups

Polar groups in a solute generally influence adjacent water molecules via solute–solvent hydrogen bond formation. In fact, Kharakoz [13] has defined relationships between the partial compressibility contributions of different polar groups and the hydration properties of these groups by analyzing data on a large number of related low molecular weight model compounds which contain different polar groups. As previously shown, the properties of water around a polar group, in general, strongly depend on the distance between the group and other polar moieties [13,57]. If polar groups within a solute molecule are situated sufficiently close to each other (separated by three or less covalent bonds (e.g., sugars and saccharic alcohols; α-amino acids having polar side chains such as serine or glutamine)) then it is postulated that each adjacent water molecule can, in principle, form simultaneously two hydrogen bonds with the neighboring polar groups [13]. Such a situation would lead to the partial 'immobilization' of water molecules within the solute hydration shell and, consequently, to a decrease in the relaxation part of their compressibility (see Eq. (6)). As a result, one would expect the compressibility of water in the hydration shell of closely situated polar groups to become less than that of bulk water. In addition, the second temperature derivative of the compressibility of the solvating water also should become less than that of bulk water (the temperature dependence becomes more linear). For these reasons, it is reasonable to expect that the contributions of such polar groups to the partial molar adiabatic compressibility, as well as to the second temperature derivative, would be negative. In fact, at 25°C, the contribution of closely situated polar groups to the partial molar adiabatic compressibility of a solute has been estimated to be -5.5×10^{-4} cm^3 mol^{-1} bar^{-1} [13]. While less pronounced, it should be noted that the influence of closely situated polar groups on the adjacent water molecules qualitatively resembles that of charged groups.

By contrast, the situation is qualitatively different for solute molecules with a single polar group or with several polar groups which are located sufficiently far apart (separated by 5 or more covalent bonds). Alcohols exemplify the former class of compounds since they generally contain only a 'single' polar –OH group. Water molecules in the hydration shell of such polar groups only can form one hydrogen bond with a solute molecule. The mobility of the water molecules bound to single polar groups and their ability to

form hydrogen bonds with other solvent molecules are similar to the corresponding properties of water molecules in the bulk state. Consequently, the relaxation part of the compressibility of water solvating single polar groups is almost the same as that of bulk water and can be even higher, if the hydrogen bonds between the single polar groups and water are stronger compared with the hydrogen bonds between bulk water molecules. As a result, the partial molar adiabatic compressibility contribution of a single polar group, as well as the second temperature derivative of this contribution, becomes positive (the temperature dependence of the compressibility becomes more nonlinear). In fact, at 25°C, the compressibility contribution of a single polar group has been estimated to be 3.7×10^{-4} cm^3 mol^{-1} bar^{-1} [13].

The contribution of 'intermediate' polar groups (those separated from each other by a distance of about 4 covalent bonds) to the partial molar adiabatic compressibility of a solute is in between the positive contribution of a single polar group and the negative contribution of closely positioned polar groups [13]. Thus, at 25°C, the compressibility contribution of 'intermediate' polar groups has been estimated to be equal to $-2 \pm 3 \times 10^{-4}$ cm^3 mol^{-1} bar^{-1} [13].

4.5. Hydration of nonpolar groups

Aqueous solutions of nonpolar molecules exhibit unusual thermodynamic properties. Specifically, at room temperature, dissolution of nonpolar molecules in water leads to large positive changes in heat capacity [94–97]. Large heat capacity changes imply strong dependences of both the enthalpy and entropy on temperature, an expectation that has been experimentally confirmed [97–100]. Interestingly, nonpolar aliphatic groups also exhibit an unusual temperature dependent contribution to the partial molar adiabatic compressibility of solutes [12,13,17,22,34,36]. Fig. 4 shows the temperature dependence of the compressibility contribution of the aliphatic $-CH_2-$ to the partial molar adiabatic compressibilities of α-amino acids with aliphatic side chains [13], α,ω-aminocarboxylic acids [22], and *n*-alcohols and α,ω-diols [34]. Note that the methylene group exhibits the same contribution to the partial molar adiabatic compressibilities of substances which belong to structurally diverse classes. Specifically, below ~ 30°C, the compressibility contribution of the methylene group is negative but becomes positive at higher temperatures, while exhibiting a negative second temperature derivative. The average contribution of the methylene group at 25°C is $-1.8 \pm 0.2 \times 10^{-4}$ cm^3 mol^{-1} bar^{-1}. It is clear from Eq. (9), that at low temperatures, water that solvates aliphatic groups is less compressible than bulk water, while at higher temperatures the opposite is found. To understand this behavior of water around nonpolar groups, one should focus on the entropy data, as discussed below.

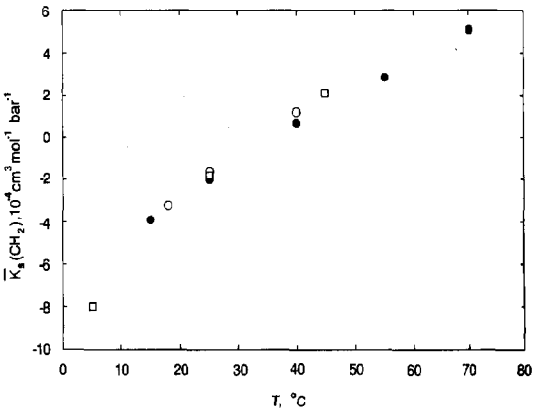

Fig. 4. The temperature dependence of the contribution of the $-CH_2-$ group to the partial molar adiabatic compressibility of different solutes: α,ω-amino acids (○) [22]; α-amino acids with the nonbranched aliphatic side chain (●) [13]; primary alcohols and α,ω-diols (□) [34].

The transfer of a nonpolar solute to water at room temperature generally results in a large decrease in entropy. This macroscopic observation usually is microscopically rationalized in terms of an increase in the order of water molecules in the proximity of nonpolar groups [97,101–105]. In this picture, water molecules surrounding nonpolar solutes do not interact directly with the solute molecules. As a result, these water molecules are forced to form their complement of solvent–solvent hydrogen bonds within a more limited space, thereby decreasing the total

entropy of the solution [82,106]. It should be noted here, that since the entropy of dissolution of nonpolar compounds in water is negative within the whole temperature range corresponding to liquid water (0 to 100°C), water molecules within the hydration shell of nonpolar solutes are always more ordered than bulk water. At low temperatures, these hydrogen bonds become so abundant, that water molecules around nonpolar groups adopt 'frozen patch' structures [78] rather than 'liquid' water structures, thereby manifesting reduced mobility. By extension, one also could assume that the relaxation part of the compressibility of such 'frozen patch' structures is greatly reduced, thereby causing the total compressibility of water near nonpolar groups to be less than that of bulk water at temperatures below 30°C. At temperatures above 30°C, the mobility of water molecules in the hydration shell of nonpolar groups increases, however the water molecules still maintain hydrogen bonds between each other. In this case, the 'frozen patch' state of waters contacting a hydrophobic group, probably shifts towards a 'liquid' state more akin to that of bulk water but at lower temperatures (below the temperature of the solution). However, as discussed above (see section 3.2), the relaxation part of water compressibility increases with decreasing temperature. As a result, at higher temperatures, the relaxation part of the compressibility and, consequently, the total compressibility of water solvating nonpolar groups becomes higher than that of bulk water.

We just noted that the altered relaxation compressibility, β_r, of water solvating nonpolar groups may be responsible for the absolute value and the temperature dependence of the partial compressibility of nonpolar groups. One also must consider the contribution to the overall partial compressibility from the void space surrounding nonpolar atomic groups, a feature which originates from the mutual thermal motion of the solute and solvent molecules [13,57]. The temperature dependence of the volume of this space and its corresponding contribution to compressibility may be one of the reasons for the unusual temperature dependence of the partial molar compressibility of nonpolar solutes and atomic groups [13,57]. In addition to the relaxation compressibility, β_r, the 'instant' compressibility, β_∞, (see section 3.1) of water in the hydration shell of nonpolar groups and the temperature dependence of β_∞ may be altered compared with bulk water. At present, the individual contributions of each of the above mentioned factors (relaxation, 'instant' compressibility, void volume) to the total partial compressibility of the nonpolar groups are unknown.

Previous studies have shown that heat capacity and entropy changes upon solvation of nonpolar molecules in water are proportional to the surface area of the solute molecules (or to the number of water molecules within the first coordination shell) [98,102,107]. By extension, it is reasonable to assume that other thermodynamic characteristics of nonpolar solutes which describe hydrophobic hydration phenomenon (e.g., the partial molar compressibility) also will be proportional to the surface area of the nonpolar moiety [13]. The contribution of the nonpolar moiety of a complex molecule to the partial molar compressibility can be found by multiplying the compressibility contribution of the $-CH_2-$ group by the ratio of the total nonpolar surface area of the solute molecule to the surface area of a single $-CH_2-$ group.

5. Hydration of biopolymers

The experimental data base derived from studies on low molecular weight compounds such as those described above, can, in principle, be used to interpret the partial compressibility of biopolymers in terms of hydration from the knowledge of the surface accessible atomic groups. Such an analysis of biopolymer compressibility data, in general, requires the following common assumptions and/or considerations: (i) hydration of a biopolymer predominantly is determined by the accessible surface atomic groups, which influence the neighboring water molecules in the same way as model low molecular weight compounds; (ii) in contrast to low molecular weight compounds, the intrinsic compressibility, K_M, of biopolymers cannot always be neglected due to the significant size

of the volume that is inaccessible to water; (iii) for biopolymers, one must consider potential relaxation contributions, K_r, to the partial compressibility, which result from the redistribution of biopolymer conformational substates due to pressure and temperature variations in the field of the ultrasonic waves; (iv) for biopolymers, regular repeating structures may cooperatively influence their hydration properties [25], particularly, when the periodicity of the regular structures correlates with the second (4.5 Å) and third peaks (7 Å) in the radial distribution function of water molecules [108,109]. This latter feature currently is only a hypothesis, with no direct experimental evidence that confirms or refutes its veracity.

In the sections that follow, we consider two classes of biologically important polymers, proteins and DNA duplexes, for which partial compressibility data have been reported. We will discuss how such data can be interpreted in terms of biopolymer hydration.

5.1. Proteins

In comparing proteins of very different molecular weights, M, it is convenient to focus on the partial or apparent specific adiabatic compressibilities ($\varphi K_S = \phi K_S/M$ or $\bar{k}_S = \bar{K}_S/M$) rather than the partial or apparent molar adiabatic compressibilities (ϕK_S or $\bar{K}_S$) [16]. The partial specific adiabatic compressibility, $\bar{k}_S$, of a protein can be represented as a sum of three terms [110]:

$$\bar{k}_S = k_{SM} + \Delta k_h + k_r$$
$$= k_{SM} + n_h(\bar{K}_{Sh} - \bar{K}_{S0})/M + k_r, \qquad (10)$$

where k_{SM} is the positively contributing intrinsic compressibility of a protein molecule (which is mostly determined by the compressibility of void volumes within the molecule due to the imperfect packing [21,55]); Δk_h is the negative contribution due to hydration; and k_r is the relaxation component, which originates from the ability of the solvated flexible macromolecule under the influence of pressure and temperature changes in an ultrasonic wave field to shift its equilibrium between its subconformations. Assuming that the hydration shell mainly involves the first layer of water molecules around a protein molecule, the hydration number, n_h, in Eq. (10) can be found as a ratio of the accessible surface area, S_M, of the protein molecule to the effective cross section of a water molecule, S_W; that is, $n_h = S_M/S_W$ (see section 4.2), where S_W can be taken to be equal to 9 Å^2.

At neutral pH, the term, k_r, in Eq. (10) is only a few per cent of the total value of the partial specific adiabatic compressibility, $\bar{k}_S$, for globular proteins, and almost equal to zero for unfolded polypeptide chains [110]. Consequently, the relaxation contribution, k_r, in Eq. (10) usually is neglected when considering proteins at neutral pH.

A characteristic feature of proteins is that the partial specific adiabatic compressibilities, $\bar{k}_S$, of globular proteins are mostly positive [21,54,55], while they are negative for the unfolded polypeptide chains [16,17,110,111]. Specifically, at 25°C, the partial specific adiabatic compressibilities, $\bar{k}_S$, of globular proteins range from -1×10^{-6} to 10×10^{-6} cm^3 g^{-1} bar^{-1} [16,21,54,55,112,113].

For globular proteins with molecular weights, M, larger than 10 kDa, the positive contribution of the intrinsic compressibility, k_{SM}, in Eq. (10) prevails over the negative contribution of the hydration term, Δk_h. By contrast, for unfolded polypeptide chains, the hydration contribution dominates. Because the 'hydration number', n_h, is proportional to S_M, the accessible surface area of a solute molecule, it can be seen from Eq. (10) that the absolute value of Δk_h, the hydration contribution to the protein partial specific compressibility, will be proportional to the ratio S_M/M. Among macromolecules, compact globular proteins have the lowest surface/volume ratios. Thus, the ratio S_M/M also will be small since the intrinsic volume of a globular protein is proportional to its molecular weight [114]. As a result, the positive intrinsic term, k_{SM}, in Eq. (10) prevails over the negative hydration term, Δk_h. The accessible surface area, S_M, of an 'average' globular protein molecule has been estimated to be proportional to $M^{2/3}$; specifically, $S_M = 11.12\, M^{2/3}$ (Å^2) [114]. Thus, for an 'average' globular protein, the hydration contribution, Δk_h, in Eq. (10) will be proportional to $M^{-1/3}$ (i.e. S_M/M). Taking into account that $n_h = S_M/S_W$,

one can obtain for an 'average' globular protein the following relationship for Δk_h, the hydration contribution to $\bar{k}_S$:

$$\Delta k_h = 1.24\, M^{-1/3}\left(\bar{K}_{Sh} - \bar{K}_{S0}\right). \quad (11)$$

Water in the hydration shell of a protein molecule has a heterogeneous character and is influenced by the different charged, polar, and nonpolar surface atomic groups. Consequently, the partial molar adiabatic compressibility, $\bar{K}_{Sh}$, of water in the hydration shell of a protein molecule used in Eq. (10) is an integral value reflecting the 'average' hydration of a protein. The difference between the values of $\bar{K}_{Sh}$ corresponding to different proteins is determined by the difference in the distribution of the surface atomic groups. For example, the specific adiabatic compressibility, $\bar{k}_S$, of globular proteins has been shown to correlate positively with their hydrophobicity [21,55]. Furthermore, for three globular proteins (lysozyme, myoglobin, and ribonuclease) the hydration term, Δk_h, has been calculated [16] using a simple additivity approach based on knowledge of the surface atomic groups [115] and their individual compressibility contributions. In this approach, all surface accessible atomic groups within a protein are classified as polar, nonpolar, and charged [16]. The hydration term, Δk_h, in Eq. (10) then is calculated as a sum [16]:

$$\Delta k_h = M^{-1} \sum n_i \bar{K}_{Shi}, \quad (12)$$

where subscript i denotes polar, nonpolar, or charged atomic groups; n_i is the number of the solvent accessible groups of each class; $\bar{K}_{Shi}$ is the partial molar adiabatic compressibility contribution of an atomic group of each class; M is the molecular weight of a protein. In this additive approach [16], the compressibility contribution of polar groups is taken to be equal to that of 'closely situated' polar groups (see section 4.4). The number of nonpolar groups used in Eq. (12) is determined as the ratio of the total nonpolar accessible surface area of a protein to the accessible surface area of a single methylene group, while the partial molar adiabatic compressibility contribution of the latter (see section 4.5) is used in Eq. (12) as the compressibility contribution of nonpolar groups [16].

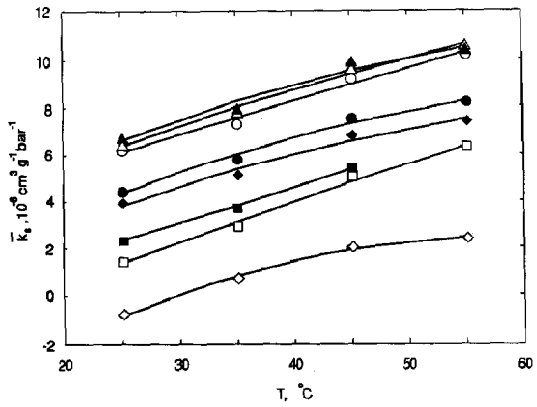

Fig. 5. The temperature dependences of the partial specific adiabatic compressibilities of the globular proteins: (○) bovine serum albumin, ovalbumin (●), cytochrome C (□), chymotrypsin (■), trypsin (◇), lysozyme (◆), myoglobin (△), and hemoglobin (▲) [T.V. Chalikian, A.P. Sarvazyan, and K.J. Breslauer, unpublished data]. Measurements were performed in pure water.

The impressive success of such additive calculations [16] supports the assumption that the components of globular proteins are solvated in a manner similar to that exhibited by small model compounds. Thus, the conclusions and observations derived from studies on low molecular weight model compounds are applicable to biopolymer hydration studies. Based on this approach, the coefficient of adiabatic compressibility, β_{Sh}, of water solvating globular proteins was estimated to be $35 \pm 4 \times 10^{-6}$ bar^{-1} at 25°C [16], while the coefficient of adiabatic compressibility, β_{SM}, of the interior of native globular proteins was estimated to be $13 \pm 3 \times 10^{-6}$ bar^{-1} at 25°C [15–17,55]. In this connection it is interesting to note that a recent study on equine cytochrome C [116] reveals the intrinsic compressibility of the 'molten globule' state of this protein to be slightly higher than that of the native form.

As reflected in the trends shown in Fig. 5, globular proteins in their native states manifest qualitatively similar temperature dependences of $\bar{k}_S$, their partial specific adiabatic compressibilities. At 25°C, the temperature slope, $\Delta\bar{k}_S/\Delta T$, is essentially the same for the globular proteins shown in Fig. 5 and, on average, equals 1.5 ± 0.2

$\times 10^{-7}$ cm^3 g^{-1} bar^{-1} K^{-1}. An increase in the partial specific adiabatic compressibility, $\bar{k}_S$, of native globular proteins with temperature can be ascribed to two processes [112,113]: (i) a decrease in the absolute value of the hydration term Δk_h; and (ii) an increase in the intrinsic compressibility, k_{SM} (see Fig. 5). To estimate the contribution from each process to the total increase in $\bar{k}_S$ with increasing temperature, we have used Eq. (12) in conjunction with our data on the temperature dependences of the partial specific adiabatic compressibilities, $\bar{k}_S$, of myoglobin and lysozyme shown in Fig. 5. Our estimates reveal that for these two proteins in their native globular states the contribution, on average, of the first process to the total increase in $\bar{k}_S$ with increasing temperature is dominant (more than 70%), while the contribution of the second process is less than 30% [unpublished data from our laboratory]. We believe, that these estimates are, also, held for other small globular proteins. However, additional studies are required to assess the generality of our results on myoglobin and lysozyme.

A review of the literature reveals that relatively few studies have used changes in the partial specific adiabatic compressibility of globular proteins to define hydration changes accompanying processes, such as the oxidation of cytochrome C [117–119], ligand binding to lysozyme [120,121], antigen–antibody interactions [122], and the temperature and pH induced denaturation of globular proteins [116,123,124]. Nevertheless, these studies illustrate the sensitivity of compressibility measurements to hydration changes in biomolecular processes. For example, using compressibility data Kharakoz and Krshchikov [123] have shown that at low ionic strength, the acidic denaturation of myoglobin is accompanied by two transitions: the first occurs at pH 4, is cooperative, and leads to a compact denatured protein state (perhaps, similar to the 'molten globule' state [125,126]); while the second transition at pH 2.5, results in more complete unfolding of the protein, with exposure of the buried atomic groups and an accompanying increase in hydration.

In the aggregate, published studies that report compressibility data on proteins and their components [15,16,54,55,110–113,116–124] already illustrate the value of such measurements for characterizing protein hydration properties.

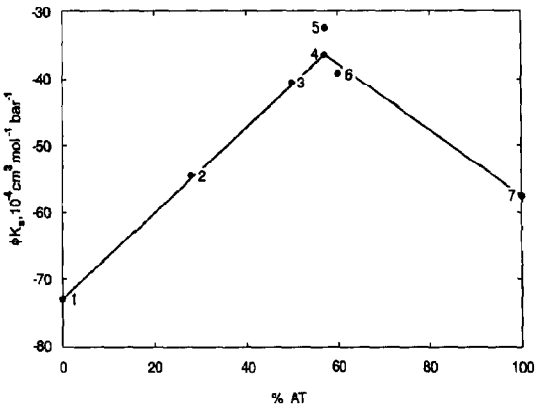

Fig. 6. Dependence of the apparent molar adiabatic compressibility of DNA duplexes on the AT content at 25°C [25]: 1 - poly(dGdC)poly(dGdC), 2 - Micrcoccus Lysodeikticus, 3-E. Coli, 4 - Salmon Testes, 5 - Herring Testes, 6 - Human Placenta, 7 - poly(dA)poly(dT) and poly(dAdT)poly(dAdT). Measurements were performed in a pH 6.7 buffer consisting of 10 mM cacodylic acid-sodium cacodylate, 0.1 mM Na_2EDTA, and 8 mM NaCl.

5.2. *Nucleic acids*

Compressibility studies on nucleic acids are even more rare than those on protein systems. Nevertheless, based on the few reports in the literature, it is clear that DNA double helices exhibit highly negative apparent molar adiabatic compressibility values, ϕK_S [25,56,127]. In fact, in the most comprehensive study to date, we recently showed that at 25°C the apparent molar adiabatic compressibilities of 5 natural and 3 synthetic DNA double helices fall between -73×10^{-4} and -32×10^{-4} cm^3 mol^{-1} bar^{-1} [25] (see Fig. 6). Furthermore, we estimated the coefficient of adiabatic compressibility of water in the DNA hydration shell to be about 20–35% lower than the compressibility of bulk water [25]. We interpreted this reduced compressibility of the solvating water as reflecting the fact that the hydration shell of DNA double helices is dictated predominantly by electrostatic solute–solvent interactions, a conclusion which coincides with the conventional wisdom.

As noted above, we found that water in the hydration shell of B-form DNA duplexes exhibits a decreased compressibility relative to bulk water. More quantitatively, we found the following linear relationship between the density, ρ_h, and the coefficient of adiabatic compressibility, β_{Sh}, of water in the hydration shell of B-form duplexes:

$$\beta_{Sh}(\mathrm{bar}^{-1}) = 1.15 \times 10^{-4} - 0.85 \times 10^{-4} \rho_h\,(\mathrm{gcm}^{-3}). \quad (13)$$

An important general conclusion implicit in this quantitative relationship is that solute–solvent interactions increase the density while decreasing the coefficient of adiabatic compressibility of water in the hydration shells of B-form DNA duplexes. More specifically, our data allowed us to estimate the 'intrinsic' density of water in the hydration shells of B-form DNA duplexes to be in the range of 1.17 to 1.26 g cm^{-3}, with the corresponding coefficient of adiabatic compressibility, β_{Sh}, falling between 30×10^{-6} and 35×10^{-6} bar^{-1}. Significantly, the latter values of β_{Sh} are only 65 to 80% of those of bulk water and reflect the strong influence that the DNA duplex exerts on the compressibility properties of the solvating water molecules, a feature that underscores the usefulness of the compressibility parameter as a measure of hydration.

In general, we found that duplexes which exhibit reduced apparent molar adiabatic compressibilities relative to bulk solvent can be described as 'more hydrated'. In connection with this generalization, we emphasized that the phenomenon of DNA hydration depends not just on the 'quantity' of hydration (the number of water molecules in the hydration shell, n_h), but also the 'quality' of hydration (the strength of the solute–solvent interactions as reflected by the density and compressibility data). In fact, we showed that the 'quantity' of hydration, n_h, was about the same for all the B-form duplexes studies, while the 'quality' of this water often differed from one duplex to another (as reflected in the density and compressibility data), thereby yielding differences in the overall hydration properties.

We also investigated the influence of base composition and sequence on the hydration properties. As reflected by the data shown in Fig. 6, we found that base composition significantly influences the hydration properties of DNA duplexes, with the influence of base sequence being relatively minor. Specifically, our studies revealed that alternating all-GC duplex poly(dGdC)poly(dGdC) exhibits an apparent molar adiabatic compressibility, ϕK_S, of $-74.0 \pm 2.0 \times 10^{-4}$ cm^3 mol^{-1} bar^{-1}, which is significantly lower than either the corresponding alternating all-AT duplex poly(dAdT)poly(dAdT) or the homopolymeric all-AT duplex poly(dA)poly(dT) ($-57.5 \pm 2.0 \times 10^{-4}$ cm^3 mol^{-1} bar^{-1}). Since, the accessible surface areas, S_M, of AT and GC base pairs within a B-DNA are essentially the same [128], the hydration numbers, n_h, (equal to the ratio of S_M to the effective cross section of a water molecule, S_W) also should be approximately the same for the all-GC and all-AT DNA duplexes. If one recalls that the intrinsic compressibility, K_{SM}, of DNA duplexes is small and essentially the same for all DNA duplexes [127], then according to Eq. (7), the lower value of ϕK_S for poly(dGdC)poly(dGdC) compared with the all-AT duplexes can be interpreted as reflecting a lower compressibility for the water solvating the all-GC duplex. We have interpreted these data as suggesting that solute–solvent interactions in the proximity of GC base pairs are stronger than those around AT base pair. This result may reflect solvent interactions with two functional groups: (i) the polar N-2 amino group of guanine in the minor groove of the GC base pair, which enhances hydration by forming additional hydrogen bonds with water molecules, and (ii) the hydrophobic influence of the methyl group of thymine on water molecules in the major groove of AT base pairs, which not only reduces hydration by disrupting some of the strong electrostatic DNA–water interactions in the groove, but also partly shields the polar O4 atom of thymine, thereby decreasing its accessibility to solvent.

Further inspection of the trends plotted in Fig. 6 reveals that DNA duplexes with base compositions of 55–60% AT base pairs exhibit the least negative values of the apparent molar adiabatic compressibility values. This intriguing result can be interpreted as suggesting that duplexes with

such base compositions are the mostly 'weakly' hydrated, while increases and/or decreases in AT content from this range of values lead to enhanced DNA hydration. Thus, the hydration dependent characteristics of a DNA duplex cannot be estimated as a simple weighted sum of contributions from AT and GC base pairs. Perhaps the regularity of some sequences produce periodic structural scaffolds that promote resonance extension of hydration networks, thereby causing duplex hydration properties to be influenced by more than global base composition or local sequence effects. In other words, long-range solute–solvent interactions may be facilitated by a regular matrix of solvent binding sites produced by a periodic DNA structure. For this reason, DNA duplexes with patterned, repeating sequences (e.g., 100% AT or GC base pairs) rather than random sequences, may have hydration shells which include more solvent molecules, particularly, involving waters from the second and even third layers of the surrounding solvent. In fact, the periodicity of regular DNA structures may correlate with the second and/or third peaks in the radial distribution function of bulk water, thus causing some additional coordination of water at distances which exceed that associated with local hydration near isolated atomic groups. Clearly, further studies are required to understand the origin of the intriguing maxima in Fig. 6.

Interestingly, at 25°C and low ionic strength, we found no difference between the apparent molar compressibilities, ϕK_S, of the poly(dA)-poly(dT) and poly(dAdT)poly(dAdT) duplexes [25]. If one assumes that the two duplexes have the same n_h values, this similarity in ϕK_S may be interpreted as reflecting equivalent 'qualities' of hydration for these two all-AT polymeric duplexes. Alternatively, if under the conditions of our study the poly(dA)poly(dT) duplex assumed a non canonical conformation with a narrow minor groove and a distorted helical axis (a reasonable assumption based on previous studies [129]), this might cause the number of water molecules in its hydration shell, n_h, to be lower than that in the B-form poly(dAdT)poly(dAdT) duplex. Such a reduction in n_h would result in a lower compressibility for water in the hydration shell of the poly(dA)poly(dT) duplex compared with the poly(dAdT)poly(dAdT) duplex. Thus, the conventional picture that the homopolymer is more hydrated may reflect stronger DNA–solvent interactions (the 'quality' of hydration) rather than more waters of hydration, n_h (the 'quantity' of hydration).

Very recently, we also have demonstrated the value of compressibility data for elucidating the role of hydration in drug–DNA binding studies (K.J. Breslauer, G.E. Plum, and T.V. Chalikian, manuscript in preparation). Specifically, we have used a combination of acoustic and densimetric measurements to investigate the binding of netropsin to two all-AT duplexes, poly(dA)poly-(dT) (the homopolymer), and poly(dAdT)poly-(dAdT) (the alternating copolymer), as well as an all-AT triplex, poly(dT)poly(dA)poly(dT). With regard to the two DNA duplexes, we interpreted our compressibility data (in the context of several assumptions) as suggesting that netropsin binding to the homopolymeric duplex causes release of 40 water molecules, which is 15 to 20 more waters released than when netropsin binds to the alternating copolymeric duplex. This result appears to agree conceptually with previously published enthalpy, entropy, and volume data, however these data have been interpreted in terms of greater hydration of the drug-free poly(dA)poly(dT) duplex initial state [130–132]. By contrast, our compressibility data suggest that the larger release of water associated with netropsin binding to the homopolymeric duplex reflects a lower degree of hydration for the netropsin-poly(dA)poly(dT) complex (compared with the netropsin-poly-(dAdT)poly(dAdT) complex) rather than exclusively a greater degree of hydration for the drug-free poly(dA)poly(dT) initial state. Our compressibility data also suggest that netropsin binding to the poly(dT)poly(dA)poly(dT) triplex is accompanied by the release of 10 to 15 more water molecules than netropsin binding to the corresponding poly(dA)poly(dT) duplex. Additional studies are needed to characterize the microscopic basis for this intriguing observation.

Independent of the veracity of the molecular interpretations discussed above, compressibility data provide important new characterizations of

DNA structures that ultimately are required to understand the central role that hydration plays in modulating DNA properties.

6. Concluding remarks

Despite the limited number of compressibility measurements that have been conducted on proteins, nucleic acids, and their low molecular weight analogs, it already is clear that this macroscopic parameter is exquisitely sensitive to hydration properties. This sensitivity is particularly important considering the recent emphasis on the role of hydration in stabilizing biopolymer structure and in modulating biopolymer recognition events. We believe that compressibility measurements represent a relatively untapped yet powerful means of probing and characterizing solute–solvent interactions, particularly, as they relate to biologically relevant questions.

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Discussion to the paper by T.V. Chalikian, A.P. Sarvazyan and K.J. Breslauer

Comments

By A. Rashin

(1) Entropies of hydration represent some measure of water structure around solutes. We found (see the paper by Rashin and Bukatin in this Issue) that for solutes with van der Waals radii of 2 Å hydration entropies are similar for non-polar solutes, ionic singly charged solutes and partially charged solutes. They remain similar for larger non-polar and positively (singly or partially) charged solutes. Negatively charged solutes may behave somewhat differently for at least some interval of sizes above 2 Å (see the same paper in this Issue). Did or can your methods detect such disappearance of differences in characteristics of water around solutes of different types with changes in solute radii? In a more simplified form one can ask at what size ions become really hydrophobic.

(2) Your results show certain temperature dependencies of compressibilities for different types of groups and suggest some interpretations of these dependencies. Can you correlate or combine your findings with calorimetrically determined characteristics (and/or their temperature dependencies) for hydration of different types of groups?

Responses by T.V. Chalikian et al. to Comments

To A. Rashin

(1) As we have discussed in our paper in this Issue, the waters solvating charged, polar, or hydrophobic groups differ significantly from each other with respect to both the absolute value of the compressibility and of its temperature dependence. Therefore, compressibility data, definitely, will be sensitive to changes in the character of solvating water with increasing the charged solute radius, as your theoretical calculations predict. One can find examples supporting your conclusions among a wealth of existing data on partial compressibility of various electrolytes, particularly in the data on the partial compressibility of alkali halides as a function of the size of con-

stituent ions. At 25°C, the partial molar adiabatic compressibility contribution of halide ions changes greatly going from fluorine ion (with the radius ≈ 1.3 Å), -40.8×10^{-4} cm^3 mol^{-1} bar^{-1}, to chlorine ion (with the radius ≈ 1.8 Å), -17.0×10^{-4} cm^3 mol^{-1} bar^{-1}, to bromine ion (with the radius ≈ 2.0 Å), -9.5×10^{-4} cm^3 mol^{-1} bar^{-1}, and to iodine ion (with the radius ≈ 2.2 Å), $+1.8 \times 10^{-4}$ cm^3 mol^{-1} bar^{-1} [J.G. Mathieson and B.E. Conway, J. Solution Chem. 3 (1974) 455], which qualitatively agrees with your conclusions, considering that the average contribution of the methylene group is -1.8×10^{-4} cm^3 mol^{-1} bar^{-1} (see our paper in this Issue). More general and quantitative comparison of the compressibility data with your entropy based predictions requires additional systematic studies of the temperature dependencies of partial molar compressibilities of large variety of substances.

Exploration of this subject is one of our long term objectives in studies of hydration of biopolymers, because the charge density is a very important factor determining the influence of the charged groups on surrounding water molecules.

(2) Just a correlation between results of two methods usually conveys only limited information and may lead to misinterpretations if its basis is poorly understood. A combination of independent measurements is more attractive as it allows to ascribe more characteristics to a phenomenon. An example of the new insight derived from such a combined experimental approach is provided by our recent studies of hydration changes accompanying drug–DNA binding. Specifically, we have used acoustic, densimetric, and calorimetric techniques to characterize the hydration changes accompanying netropsin binding to two DNA duplexes (poly(dA)poly(dT) and poly(dAdT)poly(dAdT)). Based on the volume and compressibility data, we have calculated that netropsin binding to the poly(dA)poly(dT) duplex causes release of about 18 more water molecules than netropsin binding to the poly(dAdT)poly(dAdT) duplex. Based on calorimetric measurements under the same experimental conditions, we determined the difference in netropsin binding entropy, $\Delta\Delta S^0$, for the two duplexes to be 28 cal K^{-1} mol^{-1}. Dividing $\Delta\Delta S^0$ by 18, the calculated difference in water molecules released, we obtain 1.6 cal K^{-1} mol^{-1}. Thus, each water of hydration released to bulk solvent contributes 1.6 cal K^{-1} mol^{-1} to the observed netropsin binding entropy. With the reasonable assumption that the difference in entropy changes predominantly reflects differences in hydration, this value corresponds to the average difference between the partial molar entropies of water in the bulk state and in the hydration shell of the two all-AT DNA duplexes. This evaluation is possible because the values of the number of waters released and $\Delta\Delta S^0$ were determined independently by unrelated methods, thereby avoiding trivial coupling of their values. In summary, by conducting parallel compressibility and calorimetric measurements it is possible to derive an energetic characterization of waters in the hydration shell of duplex DNA.

In general, macroscopic observables, such as compressibility and heat capacity, 'sense' the entire population of solute-perturbed water molecules, often referred to as hydration shell. Macroscopic characterization of hydration shells are distinct from their microscopic counterparts (e.g., X-ray crystallography) in that they are sensitive not only to the 'quantity' of solvating waters (the number of solute-perturbed solvent molecules), but also to the 'quality' of the perturbed solvent (the extent to which the solute-induced perturbation alter a spectrum of solvent physicochemical properties). Insight derived from a specific characterization of the 'quality' of hydrated water vary with the nature of the observable. Consequently, macroscopic characterization of hydration derived from a combination of experimental methods is desirable since it will define a range of physicochemical properties (the 'quality') exhibited by the same population of waters. This information rich, multifaceted nature of 'quality' is lost if one only designates the 'quantity' of water as expressed in the so-called 'hydration number'. For this reason, a multiparametric experimental approach is required to obtain a complete characterization of the hydration phenomenon and to define how it influences solute properties. Since very few studies have used multiple macroscopic methods to characterize hydration of a common

system, the opportunity to analyze the physics of hydration is limited. Such an analysis requires not only ΔG^0, ΔH^0, and ΔS^0, but also their pressure (P) and temperature (T) derivatives over a wide range of P–T space.

Traditionally, the principal thermodynamic potentials, used to describe a system (ΔG^0, ΔH^0, and S^0), have been obtained indirectly by measuring the temperature dependence of some equilibrium property. A more direct approach for obtaining the relevant thermodynamic data, including ΔC_p, is based on calorimetric techniques. Significantly these thermodynamic parameters also can be derived by measuring the speed of sound, U, over a range of pressures and temperatures, since U is a simple function of the derivative of density, ρ, over pressure and, consequently, also the second derivative of free energy over pressure. By measuring $U(P, T)$, one can obtain $\rho(P, T)$, $V(P, T)$, which allows calculation of any thermodynamic function.

Table 1 shown below illustrates the complementary nature of the calorimetric and acoustic derivation of thermodynamic data. Note that the application of calorimetry is based on the use of two conjugate intensive and extensive variables: C_p and T. By contrast, the acoustic approach uses the conjugate variables V and P.

Table 1

Measured	Calorimetry	Acoustics
	$C_p(T)$	$U(P, T) = (\partial\rho/\partial P)_s^{-1/2}$ $\Rightarrow V(P, T)$
evaluated	$\Delta H^0(t)$ $= \int C_p(T)\,dT$	$\Delta G^0(P, T)$ $= \int V\,dP$
	$\Delta S^0(T)$ $= \int C_p(T)\,dT/T$	$\Delta S^0(P, T)$ $= -\int(\partial V/\partial P)_p\,dP$
	$\Delta G^0(T)$ $= \Delta H^0(T) - T\Delta S^0(T)$	$\Delta H^0(P, T)$ $= \int[V - T(\partial V/\partial T)_p]\,dP$

In recognition of the need for a multiparametric characterization of hydration, our laboratory at Rutgers, in conjunction with Dr. Theodor Funck and Dr. Leo deMaeyer at the Max-Planck-Institute of Biophysical Chemistry (Göttingen, Germany), is developing an acoustic method for determining the equation of state for aqueous solutions of biologically relevant solutes. The example presented above concerning the entropy contribution associated with the release of one water of hydration, as well as some of the new material in our paper, provide illustrations of the early stages of this program. Future work will involve expanding these measurements in P–T space to obtain $V(P, T)$, $G^0(P, T)$, $H^0(P, T)$ and $S^0(P, T)$, thereby providing a comprehensive characterization of hydration phenomena.