ELSEVIER

Biophysical Chemistry 112 (2004) 245–252

Biophysical Chemistry

www.elsevier.com/locate/bpc

Anionic regulation of biological systems: the special role of chloride in the coagulation cascade

Enrico Di Stasio*

Istituto di Biochimica e Biochimica Clinica, Universitû Cattolica del Sacro Cuore, Largo F. Vito no. 1, 00168 Rome, Italy

Received 11 March 2004; accepted 1 July 2004

The author wish to respectfully dedicate this article to the memory of professor John D. Ferry: Animula vagula blandula; Hospes comesque corporis; Quae nunc abibis; In loca pallidula rigida nudula; Nec ut soles dabis iocos. Publius Aelius Hadrianus, Imperator Romanus (76–138 AD).

Abstract

The discovery that previously unidentified allosteric properties of several proteins, such as fibrinogen and myoglobin, can be triggered by anions binding, has suggested the possibility to design a new ãactiveã role of chloride in the modulation of a broad range of biological systems. The molecular bases of the anions binding to proteins depends by their charge density in turn regulating the ability to bind water molecules and interact with basic groups on proteins. This review reports the role of the physiologically relevant chloride, and of other anions, in the regulation of several proteins, with special attention to the coagulation cascade. Moreover, possible mechanisms of modification of plasma, intra- or extracellular chloride concentration are listed.

Keywords: Anions binding to proteins; Coagulation cascade; Cryoglobulins; Cl^-–protein interaction

1. The charge density of ions: theoretical background

Recent progress in the characterization of ion–protein interactions for several proteins, such as serine proteases, fibrinogen, immunoglobulins, and DNA binding proteins has suggested the existence of a broad range of previously unidentified ion-dependent allosteric systems [1–7]. Individual ions may be systematically classified as chaotropes or kosmotropes by the sign of the Jones-Dole viscosity B coefficient that correlates with charge density and the strength of interaction with water molecules [8]. Collins et al. [8–12] have shown that many of the properties of aqueous ionic solutions are a function of the charge density of the ions. Small ions of high-charge density (kosmotropes) bind water molecules strongly, whereas large monovalent ions of low-charge density (chaotropes) bind water molecules weakly relative to the strength of water–water interaction in bulk solution (which acts as a critical reference energy level) [8,11,12]. Large ions paired with small ions give highly soluble salts, whereas salts containing just one of the two species are only moderately soluble [11]. Moreover, anions are more strongly hydrated than cations for a given charge density, since the anions begin to bind strongly the immediately adjacent water molecules at a lower charge density [8]. This phenomenon can be ascribed to: (i) quantum-mechanical calculations indicate that the anions, interacting with the hydrogen atom of water, allow intrashell hydrogen bonding of the solvating waters, differently from cations, which interact with the oxygen atom of water [13]; (ii) charge transfer to solvent characterizes strong hydration; because of high electronegativity of the water oxygen atom, it is easier to accept negative charge from anions than the positive from cations [11].

Small ions are strongly hydrated because their point charge is close to that of opposite charge on the water molecule, whereas large ions are weakly hydrated since

Abbreviations: NaCl, sodium chloride; Na_2SO_4, sodium sulphate; $NaNO_3$, sodium nitrate; NaSCN, sodium thiocyanate; $NaClO_4$, sodium perchlorate; NaF, sodium fluoride.

* Tel.: +39 6 30154215; fax: +39 6 3053598.

E-mail address: e.distasio@uniserv.ccr.rm.cnr.it.

0301-4622/$ - see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.bpc.2004.07.026

their point charge is distant from that of opposite sign on the water molecule. Ions in solution have most often been treated with the model of continuum electrostatics, assuming that chaotropes do not exist [8,14]. The model proposed by Collins [8] offers an alternative description of how ions behave in aqueous solution treating charge density as a central determinant of the structure and function of biological systems. In particular, they hypothesize that the principal properties of biological systems arise from the fact that kosmotropic ions bind water molecules tightly taking up a greater size of their anhydrous volume. This property makes them scarcely reactive with proteins. On the other hand, chaotropic ions bind water molecules weakly allowing an easy removal of them from the complex with a limited steric impairment. Among the anions of physiological relevance, Cl^- is the only chaotrope and tends to interact with basic groups of proteins with significant affinity. Detailed discussion on the thermodynamic of ion interaction can be found in Collins [8], Robinson and Stokes [15] and Krestov [16].

2. Ion interaction with proteins

The effect of salts on proteins and amino acids can be divided roughly into specific, and nonspecific (i.e. ionic strength/electric screening effect) [17,18]. The first is due to a direct and differential binding of ions in different sites on the macromolecule, the second to a change in the dielectric of the solution causing long-range electric field effects on the solutes [19]; however, Kiriukhin and Collins [10] have recently suggested that ionic strength effects may largely arise from local effects related to the hydrated volume of the ion, again suggesting a difference in the role of each specific ion.

In a theoretical paper, Record et al. [20] have listed five potential origins for salts interactions with protein: (1) differential cation binding, (2) differential anion binding, (3) differential hydration (at high electrolyte concentration), (4) differential screening (Debyeã Huckel) effects of electrolyte on the macroion charges, reflected in a variation of the macromolecular activity coefficient, and (5) effects of electrolyte on the activity coefficient of the ligand.

Both specific and nonspecific effects play a fundamental role in the stability, folding and activity of proteins in biological systems. However, especially in human physiopathology, ionic strength effects are of limited interest, since that only slight variation can occur. Major change in the I.S. could be of ravaging consequences due to indiscriminate effects on all molecules and to cellular osmotic lyses. On the other hand, specific binding of different ion could play a key role in the modulation of protein activity as a consequence of a driven exchange of ions between intracellular and extracellular compartment either plasma or other biological fluids. Therefore, the amount of protein bound or not by an ion can be regulated by local change in the concentration of the ion, in turn causing a modulation in the activity of the complex.

The molecular nature of the specific interactions can be described as follow.

Up to a concentration of about 0.1–0.3 M (the pseudo-physiological range), the effects are due mostly to the neutralization of charges on the protein [21]. These effects can lead to:

(1) a conformational change reflecting an allosteric nature of the protein with different activity triggered by the ion binding
(2) salting in (increased solubility) when intramolecular ion pairs (ion pairs within or between protein subunits) are replaced by protein–small ion pairs [22–24]
(3) salting out (decreased solubility) when the formation of protein–small ions pairs abolishes net charge on the protein [23,24].

Above 0.1–0.3 M salt, the interaction are largely on the neutral parts of the protein, producing a salting out effect because of a salt-induced increase in surface tension when kosmotropic ions are used (e.g. SO_4^{-2}) [21] or a salting in effect when chaotropic ions are used (e.g. guanidinium) because of direct interactions with the protein [25]. Moreover, kosmotropes at high concentration stabilize proteins, but chaotropes at the same concentration destabilize them. Chaotropic anions are known to adsorb to nonpolar surfaces [12] and thus are also expected to adsorb to the nonpolar portions of arginine, histidine and lysine side chains; the chaotropic anions may therefore not be well matches in charge density to the protein cations being neutralized.

3. Intracellular and extracellular anions: the special role of chloride

All of the major intracellular anions (phosphates, sulfates and carboxylates) are kosmotropes, whereas the major intracellular monovalent cations (K^+, arginine, histidine and lysine said chains) are chaotropes; together they form highly soluble, solvent separated ion pairs that keep the contents of the cell in solution. Hofmeister interactions, consisting in changes of protein solubility, denaturation and enzyme activity triggered by neutral electrolytes cosolutes, are dominated by the anions [11] and an important effect of the kosmotropic polyphosphates in the cell is to produce an environment highly stabilizing for native protein structure; these polyphosphates appear to exist as soluble K^+ salts in vivo [26]. The concentrations of anions in different biological fluid are reported in Table 1.

The negatively charged Cl^-, which is in low intracellular concentration (3 mM), with respect to the extracellular (~100 mM) [27], is marginally a chaotrope. Strong chaotropic anions are NO_3^-, ClO_4^-, and SCN^-, all not

Table 1
Anions concentration in biological fluids

Anion	Plasma	Intracellular	Sweat	Gastric juice	Bile
Cl^-	98–106	1–3	<40	150–200	75–95
HCO_3^-	21–28	8–12	–	–	40–70
Phosphates	1–1.4	85–100	1–2	10	12
Sulfates	1	17–22	<1	1	1

Anion	Pancreatic juice	Interstitial fluid	Cerebrospinal fluid	Saliva	Urine, mEq/die
Cl^-	60–75	113	119–131	25–50	125–300
HCO_3^-	70–80	27	20–25	10–20	3
Phosphates	1	2	0.4–0.7	1–10	16–32
Sulfates	<1	1	1	1	0.16–0.34

Values were obtained from Refs. [8,27,32–34], and expressed as mM.
Plasma total concentration, bound and not-bound to protein, of other anions are as follow: Bromide 40–60 μM, Fluoride 4–6 μM, Iodine 2–4 M, Thiocyanate 20–30 μM, Nitrate 35–40 μM.

physiological anions, and I^-, present only at low level (nanomolarã micromolar range) in the organism.

Because Cl^- is the only chaotropic anion present in physiological fluids, it appears to have a particularly intimate relationship with the chaotropic cation groups on proteins. Thus, the weakly hydrated Cl^- should readily form inner sphere ion pairs with nitrogen based protein cationic side chains. When the pH is lowered below 7 and more positively charged imidazolium groups are created on proteins, Cl^- binding increases stabilizing the protein [28]. Lysozyme, with its net positive charge, crystallizes most readily from solutions containing Cl^- or other chaotropic anions [29], and acid denaturated proteins are more compact when Cl^- or other chaotropic anions are present [30]. Cl^- has a much bigger salting in effect than does sulphate, and lysine and arginine residues have been implicated in the Cl^- channel of the cystic fibrosis transmembrane conductance regulator [31].

A specific interaction of Cl^- with different proteins is been found in numerous biological systems. However, Cl^- is generally considered an "inert" anion, following passively the movement of sodium and in exchange with bicarbonate anions from intracellular department to extracellular fluids, plasma or secretions to neutralize the charge differences and to maintain the homeostasis of pH and electrolytes [32]. A specific role of chloride is determined in the interaction with hemoglobin in the "chloride shift" effect (see above) [33].

The mean serum concentration of chloride is ranging from 98 to 110 mM [32]. Generally, only slight modification in the serum concentration of chloride occurs (±10 mM) [32]. However, substantial alteration in the plasma chloride level can be found in many pathological conditions. An increasing in chloride concentration (140–170 mM) can be associated to renal insufficiency, renal tubule acidosis, hyperparathyroidism, ureterosigmoid anastomosis, dehydration and overtreatment with saline solution [32].

Decreased level can be associated to gastrointestinal diseases with loss of gastric and intestinal fluids (vomiting, diarrhea, and gastrointestinal suction), renal insufficiency, overtreatment with diuretics, chronic respiratory acidosis, diabetic acidosis, adrenal insufficiency, and extremely low level (45–70 mM) can be found in the ipochloremic alkalosis syndrome.

Moreover, the role of chloride in the modulation of different proteins activity should be considered also in terms of local fluctuation in anion concentration, linked to the intra-extracellular movement across the cellular membrane.

4. Chloride ions and the coagulation cascade

Humans have evolved an intricate haemostatic system designed to maintain blood in a fluid state in physiological conditions but primed to react towards vascular injury in an explosive manner to stem blood loss by sealing the defect in the vessel wall. The normal vascular endothelium maintains blood fluidity inhibiting blood coagulation and platelet aggregation and promoting fibrinolysis by providing a protective barrier that separates blood cells and plasma factors from highly reactive elements in the deeper layers of the vessel wall, such as collagen, von Willebrand factor (vWF) and tissue factor, respectively, promoting platelet adhesion and triggering blood coagulation. Platelets, stimulated by subendothelial collagen, expose membrane glycoproteins IIb and IIIa which are capable to bind fibrinogen and vWF cofactors for platelet recruitment and aggregation. Protein cofactors, such as Factor V, secreted by platelets or derived from plasma, serve as nidus for assembling enzyme-cofactor complexes on the platelets surface, thereby accelerating Factor X and prothrombin activation, leading to thrombin formation and successive conversion of fibrinogen, circulating in the plasma as a dimer of three chains and in fibrin monomers. The monomers aggregate to form protofibrils that, after fibrinopeptide B release, associate laterally forming ticker fibers. The process leads to formation of fibrin clot that anchors platelets to the site of injury and initiates processes that stop the bleeding and promote wound repair and healing [34]. Ferry and Morrison [35] demonstrated, for the first time, that fibrin

clots formed at different ionic strength are dramatically different with fibers becoming apparently thinner at high pH and salt concentration. This seminal observation has been reproduced by numerous other investigators, but these studies have not accessed the role of specific ion interaction in fibrin polymerization. When the effect of different salts on clot structure was examined [3–36], it was found that the concentration of Cl^- in solution is the most important variable that controls the size of fibrin fibers.

Data on fibrin clot grown in the presence of different anions, show that the fibers thickness is dependent by the anion and its concentration, and the strength of the binding interaction follows the properties of the anion in the Jones–Dole scale. F^-, cacodylate, acetate and organic phosphates behave as inert anions. On the other hand, almost complete inhibition of lateral aggregation of fibrin is found in the presence of Cl^- and Br^-. An even larger effect is seen in the presence of iodide and perchlorate ions. The observed salt-specific effect is also observed under physiological conditions of pH and temperature and the chloride ion acts around its physiological concentration, proving unequivocally that concentration of the physiological anion Cl^- plays a key role in determining the thickness of fibrin fibers. This conclusion is directly supported by scanning electron microscopy of clots made in the presence of Cl^- or F^- [3].

Although identification of the ionizable groups remains elusive, specific domains of the fibrin monomer can be targeted with anionic ligands to mimic the physiological effect of Cl$^{\tilde{a}}$. This information can be exploited to search for ligands that specifically inhibit fibrin polymerization by binding to the domain responsible for Cl^- interaction. Moreover, results on the role of Cl^- also provide new insights into the role of fibrinopeptide B (FPB) release in fibrin polymerization. In presence of Cl^-, the release of FPB tends to increase the mass–length ratio of fibrin fibers. Conversely, in absence of Cl$^{\tilde{a}}$ the effect of FPB cleavage is actually reversed, such that fibrin fibers grow thicker when only fibrinopeptide A is released, demonstrating that there is no direct relationship between FPB cleavage and lateral aggregation. Under physiological condition, in the presence of Cl^-, fiber thickness can be regulated by FPB cleavage promoting lateral aggregation of protofibrils. Thus, Cl^- allows the production of fibers of appropriate thickness for different conditions to guarantee the optimal mechanical properties of the fibrin scaffold.

Among fibrinolytic factors chloride anion exerts its action on the activation in serum of human [Glu^1]plasminogen by tissue plasminogen activator (TPA) [37]. The plasminogen activation is inhibited by the normal serum levels of Cl^- and enhanced by physiological levels of fibrinogen in the presence or absence of chloride. The anion induces a conformation in [Glu^1]plasminogen less favorable for its activation. The enhancing effect of fibrinogen surpassed the inhibitory effect of chloride over a wide range of recombinant TPA concentrations in physiological serum. Data suggest that anion and fibrinogen effects both need consideration when TPA is used as a thrombolytic agent; the influence of these effects on the degradation of circulating fibrinogen and the thrombus-specificity of TPA therapy must be weighed.

The role of circulating Cl^- and fibrinogen, as modulators of the fibrinolytic process might also be considered under the more physiological conditions associated with endogenously provoked thrombosis and hemorrhage.

Gaffney et al. [37] have shown that in the physiological milieu of human serum, fibrinogen promotes activation of [Glu^1]plasminogen by TPA, whereas Cl^- inhibits that reaction. One explanation may lie in the ability of Cl^- to conform the substrate [Glu^1]plasminogen unfavorably for activation. However, this effect is drastically decreased during lyses of fibrin clot, suggesting that this chloride property relates more to molecular interactions in solution than to such interactions on the insoluble fibrin surface and pointing out a role of chloride, and circulating fibrinogen, in controlling the plasma level of activated [Glu^1]plasminogen more than an inhibition of the lyses of already formed clots.

There is a specific binding interaction of Cl^- to fibrin polymer that affects the sizes of the fibers and eventually determines the fine or coarse nature of the clot. The two most abundant ions in the blood participate in crucial aspects of its coagulation. Na^+ increases the fraction of thrombin in the procoagulant fast form, while Cl^- reduces the thickness of the fibrin fibers in the developing clot so that decrease the rate of plasmin formation. Both effects are large and physiologically important, certainly playing a key role in the pathogenesis of thrombosis and fibrinolysis. Clots formed in high concentration of NaCl develop fast and involve thin fibers that should be degraded slower by the inhibited plasmin formation. Clots formed in low concentration of NaCl develop slowly leading to thick fibers and to a higher rate of plasmin formation. The equilibrium established between these two conditions is probably responsible of the fine regulation of coagulation system suggesting a possible new therapeutical approach for coagulation or fibrinolytic diseases. Interestingly, specific binding of Cl^- to thrombin has been suggested to occur at the fibrinogen recognition site, although no evidence of absorbed anions at this site can be found crystallographically [2].

The resistance of fibrin clot grown at different concentration of Cl^- has to be clarified. The mechanical resistance of clot formed in absence of chloride seems to be higher than in its presence. However, the possibility of such clots to be degraded faster or slower than those grown in absence of this anion, has still to be accessed.

It is ascertained that Cl^- ions, in physiological concentration, have to be considered an important factor of coagulation cascade and thrombolytic events. This observation, together with the well-known role of calcium as

activator of the coagulation cascade and of sodium as modulator of pro- or anti-coagulant activity of thrombin [2,34], suggests an intriguing ionic regulative mechanism of haemostatic and thrombolytic events.

5. Anions and proteins

A specific interaction of anions with different proteins is been found in numerous biological systems. Some examples of protein strongly modulated by anion binding are reported below.

5.1. Respiratory proteins

The heme groups of hemoglobin reversibly combine with gaseous ligands (e.g. O_2, CO and NO) having different affinities and cooperativities; these compounds are called "heme ligands". The binding of heme ligands is influenced by reversible interaction of other types of ligand to specific sites on the globin moiety. These effectors, named "nonheme ligands", are principally H^+, CO_2 and various anions, such as 2,3-diphosphoglycerate, adenosine triphosphate, inositol hexaphosphate, and Cl^-. Chloride ions pass across the erythrocyte membrane in exchange with bicarbonate anions and its successive interaction with a cluster of positive residues on hemoglobin leads directly to O_2 release.

Myoglobin is a monomeric heme protein, which represents a paradigmatic case for protein molecules, which do not display any significant functional modulation either by homotropic or heterotropic effectors [33]. De Rosa et al. [4] have studied the effect of increasing concentrations of several anions on the azide binding properties of ferric myoglobins. Surprisingly, a number of anions may act as heterotropic effectors, decreasing the affinity of myoglobins for azide mirroring the increase in their charge density. Only a slight effect can be observed in the presence of chloride, whereas the binding of perchlorate and iodine to myoglobins ($K_d \approx 200$ mM at 20 °C and pH 6.5) produces a ≈4–8 fold increase of the dissociation constant for azide.

5.2. Enzymatic systems

Chloride ions were found to regulate the biosynthetic activity of glucose-6-phosphatase (Glc-P-ase) [38]. Certain amino acids stimulate glycogenesis from glucose (Glc) and the regulatory volume decrease mechanism explaining this effect involves amino acid-induced swelling of hepatocytes in turn resulting in loss of chloride ions, leading to disinhibition of glycogen synthase phosphatase. This results in an enhanced conversion of the inactive to active form of glycogen synthase and thus in an increased glycogen synthesis. With undisrupted microsomes, chloride ion competitively inhibits carbamyl phosphate: glucose phosphotransferase more extensively than Glc-6-P phosphohydrolase. Reduced concentration of Cl^- will disinhibit the biosynthetic activity of Glc-6-Pase, while Glc-6-P hydrolysis is still inhibited, leading to an increased cellular concentration of Glc-6-P (an important glycogenic intermediate as well as allosteric activator of glycogen synthase). This phenomenon increases the active form of glycogen synthase by disinhibiting glycogen synthase phosphatase both through the previously defined mechanism and via Glc-6-P-enhanced conversion of glycogen synthase from its inactive to active form. Thus, the biosynthetic activity of Glc-6-Pase may act in concert with glycogen synthase during amino acid-induced glycogenesis from glucose [38].

The physiological ligands for Na,K-ATPase (the Na,K-pump) are ions, and electrostatic forces that could be revealed by their ionic strength dependence are therefore expected to be important for their reaction with the enzyme [39]. Norby and Esmann [39] found that the affinities for ADP^{3-}, $eosine^{2-}$, *p*-nitrophenylphosphate, and *V*(max) for Na,K-ATPase and K^+-activated *p*-nitrophenylphosphatase activity, were all decreased by increasing salt concentration and by specific anions. The apparent affinity for ADP decreased up to 30 times. At equal ionic strength, I.S., the ranking of the salt effect was $NaCl \sim Na_2SO_4 \sim$Na-acetate$< NaNO_3 < NaSCN < NaClO_4$, where the influence of NaCl and Na_2SO_4 on K_d for the enzyme and ADP was considered as a "pure" ionic strength effect.

The NO_3^- effect was compatible with competitive binding of NO_3^- and ADP in addition to the general I.S. effect. Results reported indicate that the reversible interactions between ions and Na,K-ATPase can be grouped according to either simple Debye–Huckel behavior or to specific anion interactions with the enzyme.

5.3. DNA–protein interactions

Ha et al. [1] have shown that binding of the lac repressor to DNA in vitro is 40- to 300-fold stronger when Cl^- is replaced by glutamate (glu^-, the physiologically important anion in *E. coli*) and similar finding have been reported for at least six other DNA-binding proteins. Only the kosmotrope F^- has an effect comparable to that of glu^- [1]. The authors concluded that glu^- is an inert anion, whereas Cl^- competes with DNA phosphate groups in binding the lac repressor. It occurs presumably because Cl^- competes with DNA for cationic binding sites on proteins that the intracellular Cl^- concentration is kept low compared to the extracellular concentration.

5.4. Prions

Prion diseases are associated with the conversion of cellular prion protein, PrPC, into a misfolded oligomeric form, PrPSc. Chemical unfolding studies show that at low concentrations (below approximately 50 mm), salts

($NaSO_4$, NaF, Na-acetate, and NaCl), significantly reduced the thermodynamic stability of the protein promoting conformational conversion of the recombinant prion protein into a PrPSc-like form [40]. This highly unusual response to salts was observed for the full-length prion protein as well as the N-truncated fragments huPrP90-231 and huPrP122-231. At higher salt concentrations, the destabilizing effect was gradually reversed, and salts behaved according to their ranking in the Hofmeister series. These data indicate that electrostatic interactions play an unusually important role in the stability of the prion protein by an ion-induced destabilization of salt bridges (Asp144–Arg148 and/or Asp147–Arg151) in the extremely hydrophilic helix 1, suggesting that anionic species present in the cellular environment may control the PrPC to PrPSc conversion by modulating the thermodynamic stability of the native PrPC isoform [40].

5.5. The particular case of cryoglobulins

Cryoglobulins are immunoglobulins exhibiting temperature-dependent insolubility at low temperature associated with a number of infectious, autoimmune and neoplastic disorders. The formation of cryoaggregates induced by exposure to cold is the key pathogenetic mechanism. The subsequent intravascular precipitation can account for some clinical signs of the peripheral vasculitis, but fails to explain the precipitation of cryo-immunoglobulins in districts where no significant temperature changes take place. Middaugh and Litman [41] were the first to demonstrate that cryoglobulin precipitation at different ionic strength changes dramatically. Di Stasio et al. [6] have assessed the role of possible specific ion binding interactions affecting cryoglobulin aggregation and it was found that the concentration of Cl^- present in solution controls the size and the rate of formation of cryoglobulin aggregates both during cold-aggregation and at physiological temperature. The authors suggest that chloride anion could be involved in the pathogenesis of events on the basis of disease manifestation in organs, such as kidneys, where no temperature changes occur but the local Cl^- concentration changes according to blood electrolytic homeostasis and acid–basic equilibrium. This study also showed that the effect of chloride is specific, it takes place at physiological concentration range and it is specific for various cryoglobulins. This recent observation may have a profound impact on our mechanistic understanding of cryoglobulin aggregation and the factors that regulate it. If specific binding interactions of Cl^- determine the dimension and the formation kinetic of aggregates, the nonspecific electrostatic components invoked in previous analyses become of marginal importance, and identification of a specific structural domain responsible for Cl^- binding may provide new targets for drugs selectively designed to interfere with cryoglobulin aggregation [6].

Moreover, the inhibition of fibrin clot formation by anions [3] could help in the reperfusion of vessels already obstructed by vasculitis-associated associated thrombus and recover of nephron functionality.

5.6. Other protein

Although no mechanism has been established, chaotropic anions have also been shown to shift the insulin hexamer from the T to R state [42]; and the chaotropic anion ClO_4^- at 10 mM potentiates excitation–contraction coupling in mammalian skeletal muscles [43].

Cl^- arginine interactions have been identified in thermolysin [44] and shown to be the basis of Cl^- transport by halorhodopsin [45]. Cl channels are generally permeable to many small chaotropic anions such as Br^-, I^-, NO_3^- and SCN^-, as might be expected for a channel containing a chaotropic cationic binding sites for Cl^- [45].

6. Concluding remarks

From a psychopathological point of view, Cl^- is considered an ãinertã ion, following the movement of sodium and bicarbonate anion from intracellular department to extracellular fluids, plasma or secretions. The role of chloride was thought to neutralize charge difference between the intracellular and extracellular compartment of the cellular membrane due to the movement of sodium and potassium cations, driven by the Na^+/K^+ATPase for cellular depolarization preceding cellular activity and contraction, or in exchange with bicarbonate anion to maintain the homeostasis of pH and electrolytes.

We should evaluate the possibility of an active role of chloride in modulating the function of different protein and of the importance of local fluctuation of their concentration linked to the movement of the anion from the inside to outside of cellular compartment and the gradient that can be established in blood flow in particular districts, such as kidney and every tissue where ions movement occurs. Moreover, especially for the coagulation system, should be considered the role of the modification of chloride concentration during vascular lesions due to disruption of cellular membrane, which can release its low Cl^- concentration contents in the fluid, thus modifying the local amount of chloride that could interact with fibrinogen in growing the fibrin network on the basis of coagula formation. Attempts should be undertaken to establish the possible role of Cl^- in the physiopathology of diseases associated to its concentration modifications and the eventually compensatory mechanism involved in its homeostasis.

Finally, the evidence that the binding of anions to different proteins is site-specific encourages attempts to identify the possible binding site(s). Their identification

could in turn open the way to engineer molecules able to site-specifically bind proteins and to modulate their activity.

Acknowledgements

The author would like to acknowledge, with gratitude, Prof. Bruno Giardina and Prof. Barbara Tavazzi for their scientific support and helpful suggestions during the preparation of this manuscript.

References

[1] J.H. Ha, M.W. Capp, M.D. Hohenwalter, M. Baskerville, M.T. Record Jr., Thermodynamic stoichiometries of participation of water, cations and anions in specific and non-specific binding of lac repressor to DNA. Possible thermodynamic origins of the "glutamate effect" on protein–DNA interaction, J. Mol. Biol. 228 (1992) 252–264.
[2] E. Di Cera, Q.D. Dang, Y.M. Ayala, Molecular mechanism of thrombin function, Cell. Mol. Life Sci. 53 (1997) 701–730.
[3] E. Di Stasio, C. Nagaswami, J.W. Weisel, E. Di Cera, Cl^- regulates the structure of the fibrin clot, Biophys. J. 75 (1998) 1973–1979.
[4] M.C. De Rosa, C. Bertonati, B. Giardina, E. Di Stasio, A. Brancaccio, The effect of anions on azide binding to myoglobin: an unusual functional modulation, Biochim. Biophys. Acta 1592 (2002) 341–352.
[5] E. Di Stasio, P. Bizzarri, M. Bove, M. Casato, B. Giardina, M. Fiorilli, A. Galtieri, L.P. Pucillo, Analysis of the dynamics of cryoaggregation by light-scattering spectrometry, Clin. Chem. Lab. Med. 41 (2003) 152–158.
[6] E. Di Stasio, P. Bizzarri, M. Casato, A. Galtieri, M. Fiorilli, L.P. Pucillo, Cl^- regulates cryoglobulin aggregation: a new hypothesis for physiopathological mechanism of cryoglobulin associated glomerulonephritis, Clin. Chem. Lab. Med. 42 (2004) 614–620.
[7] N. Griffon, E. Di Stasio, Thermodynamics of Na+ binding to coagulation serine proteases, Biophys. Chemist. 90 (2001) 89–96.
[8] K.D. Collins, Charge density-dependent strength of hydration and biological structure, Biophys. J. 72 (1997) 65–76.
[9] K.D. Collins, Sticky ions in biological systems, Proc. Natl. Acad. Sci. 92 (1995) 5553–5557.
[10] M.Y. Kiriukhin, K.D. Collins, Dynamic hydration numbers for biologically important ions, Biophys. Chemist. 99 (2002) 155–168.
[11] K.D. Collins, M.W. Washabaugh, The Hofmeister effect and the behaviour of water at interfaces, Q. Rev. Biophys. 18 (1985) 323–422.
[12] M.W. Washabaugh, K.D. Collins, The systematic characterization by aqueous column chromatography of solutes which affect protein stability, J. Biol. Chem. 261 (1986) 12477–12485.
[13] J.E. Combariza, N.R. Kestner, J. Jortner, Energyã structure relationship for microscopic solvation of anions in water clusters, J. Chem. Phys. 100 (1994) 2851–2864.
[14] J. Perkyns, B.M. Pettitt, Integral equation approaches to structure and thermodynamics of aqueous salt solutions, Biophys. Chemist. 51 (1994) 129–142.
[15] R.A. Robinson, R.H. Stokes, Electrolyte Solutions, Butterworths Scientific Publications, London, 1959.
[16] G.A. Krestov, Thermodynamics of Solvation: Solution and Dissolution, Ion and Solvents, Structure and Energetics, Horwood, New York, 1991.
[17] C. Tanford, Physical Chemistry of Macromolecules, John Wiley and Sons, New York, NY, 1966.
[18] I. Tinoco, et al., Physical Chemistry, Prentice-Hall, Englewood Cliffs, NJ, 1985.
[19] R. Leberman, The Hofmeister series and ionic strength, FEBS Lett. 284 (1991) 293–294.
[20] M.T. Record Jr., C.F. Anderson, T.M. Lohman, Thermodynamics analysis of ion effects on the binding and conformational equilibria of proteins and nucleic acids: the roles of ion association or release, screening and ion effects on water activity, Q. Rev. Biophys. 11 (1978) 103–178.
[21] W. Melander, C. Horvath, Salt effect on hydrophobic interactions in precipitation and chromatography of proteins: an interpretation of the lyotropic series, Arch. Biochem. Biophys. 183 (1977) 200–221.
[22] S. Fox, J.S. Foster, Introduction to Protein Chemistry, Wiley, New York, 1957.
[23] M.M. Ries-Kautt, A.F. Ducruix, Relative effectiveness of various ions on the solubility and crystal growth of lysozyme, J. Biol. Chem. 264 (1989) 745–748.
[24] M.M. Ries-Kautt, A.F. Ducruix, Crystallization of basic proteins by ion pairing, J. Cryst. Growth 110 (1991) 20–25.
[25] T.Y. Lin, S.N. Timasheff, On the role of surface tension in the stabilization of globular proteins, Protein Sci. 5 (1996) 372–381.
[26] H.J. Guttman, S. Cayley, M. Li, C.F. Anderson, M.T. Record Jr., K(+)-ribosome interactions determine the large enhancements of 39K NMR transverse relaxation rates in the cytoplasm of *Escherichia coli* K-12, Biochemistry 34 (1995) 1393–1404.
[27] J.B. West, Physiological Basis of Medical Practice, Williams and Wilkins, Baltimore, 1990.
[28] C.R. Johnson, P.E. Morin, C.H. Arrowsmith, E. Freire, Thermodynamic analysis of the structural stability of the tetrameric oligomerization domain of p53 tumor suppressor, Biochemistry 34 (1995) 5309–5316.
[29] M.M. Ries-Kautt, A.F. Ducruix, Relative effectiveness of various ions on the solubility and crystal growth of lysozyme, J. Biol. Chem. 264 (1989) 745–748.
[30] A.L. Fink, Compact intermediate states in protein folding, Annu. Rev. Biophys. Biomol. Struct. 24 (1995) 495–522.
[31] D.C. Gadsby, G. Nagel, T.C. Hwang, The CFTR chloride channel of mammalian heart, Annu. Rev. Physiol. 57 (1995) 387–416.
[32] R.K. Murray, D.K. Granner, P.A. Mayes, V.W. Rodwell, Harper's Biochemistry, Prentice-Hall International, NJ, 2000.
[33] E. Antonini, M. Brunori, Hemoglobin and Myoglobin in their reactions with ligands, North-Holland Publishing, Amsterdam, 1971.
[34] R.W. Colman, J. Hirsh, V.J. Marder, E.W. Salzman, Hemostasis and Thrombosis: Basic Principle and Clinical Practice, III ed., J.B. Lippincott, Philadelphia, 1994.
[35] J.D. Ferry, P.R. Morrison, Preparation and properties of serum and plasma proteins: VIII. The conversion of human fibrinogen to fibrin under various conditions, J. Am. Chem. Soc. 69 (1947) 388–400.
[36] A. Vindigni, E. Di Cera, Release of fibrinopeptides by the slow and fast forms of thrombin, Biochemistry 35 (1996) 4417–4426.
[37] P.J. Gaffney, T. Urano, V.S. de Serrano, M. Mahmoud-Alexandroni, A.R. Metzger, F.J. Castellino, Roles for chloride ion and fibrinogen in the activation of [Glu^1]plasminogen in human plasma, Proc. Natl. Acad. Sci. U. S. A. 85 (1988) 3595–3598.
[38] B.A. Pederson, M.A. Nordlie, J.D. Foster, R.C. Nordlie, Effects of ionic strength and chloride ion on activities of the glucose-6-phosphatase system: regulation of the biosynthetic activity of glucose-6-phosphatase by chloride ion inhibition/disinhibition, Arch. Biochem. Biophys. 353 (1998) 141–151.
[39] J.G. Norby, M. Esmann, The effect of ionic strength and specific anions on substrate binding and hydrolytic activities of Na, K-ATPase, J. Gen. Physiol. 109 (1997) 555–570.
[40] A.C. Apetri, W.K. Surewicz, Atypical effect of salts on the thermodynamic stability of human prion protein, J. Biol. Chem. 278 (2003) 22187–22192.
[41] C.R. Middaugh, G.W. Litman, Effect of solutes on the cold-induced insolubility of monoclonal cryoimmunoglobulins, J. Biol. Chem. 252 (1977) 8002–8006.

[42] P.S. Brzovic, W.E. Choi, D. Borchardt, N.C. Kaarsholm, M.F. Dunn, Structural asymmetry and half-site reactivity in the T to R allosteric transition of the insulin hexamer, Biochemistry 33 (1994) 13057–13069.
[43] E.M. Gallant, N.S. Taus, T.F. Fletcher, L.R. Lentz, C.F. Louis, J.R. Mickelson, Perchlorate potentiation of excitationã contraction coupling in mammalian skeletal muscles, Am. J. Physiol. 264 (1993) C559–C567.
[44] J.J. Yang, D.R. Artis, H.E. Van Wart, Differential effect of halide anions on the hydrolysis of different dansyl substrates by thermolysin, Biochemistry 33 (1994) 6516–6523.
[45] M.S. Braimann, T.J. Walter, D.M. Briercheck, Infrared spectroscopic detection of light-induced change in chloride–arginine interaction in halorhodopsin, Biochemistry 33 (1994) 1629–1635.