2 A. Streitwieser, Jr., C. J. Chang and A. T. Young, *J. Am. Chem. Soc., 94,* 4888 (1972).

The Composite Physical and Chemical Approach to the Solution Spatial Structure of Polypeptide Neurotoxins

V. F. BYSTROV

Shemyakin. Institute of Bioorganic Chemistry, U.S.S.R. Academy of Sciences, Moscow, U.S.S.R.

The most fruitful approach to the solution structure of peptides and proteins is a dovetailing of physical (experimental and theoretical) and chemical (selective modification) methods so as to best attain the common purpose of assessing the intra- and intermolecular interactions of the given substance. Of the physical methods the most informative in such study is high resolution NMR spectroscopy.

We have made extensive use of this technique within the framework of the above approach in an analysis of the spatial structures of some polypeptide neurotoxin components of bee and snake venoms.

For signal assignment in the ^{1}H and ^{13}C NMR spectra of the bee venom component apamin (18 amino acid residues, two disulfide bonds) a multifrequency homo- and heteronuclear decoupling procedure was developed, aimed at identification of the spin systems of the particular amino acid residues and determination of the residue position in the amino sequence. The apamin spatial structure has been elucidated by selection of the optimal calculated conformation on the basis of the NMR parameters of the native toxin and its selectively modified analogs.

In a study of snake neurotoxins, mainly neurotoxin II (61 amino acid residues, four disulfide bonds) isolated from the venom of the Central Asian cobra *Naja naja oxiana,* use was made of the dependence of the ^{1}H NMR parameters on the conditions of the aqueous medium, and of selective chemical modification, in particular, insertion of spin labels. Additional information was obtained from comparison of the spectra with those of homologous toxins from other snakes. The contacts revealed between the amino acid side chains provided a general picture of the folding of the backbone and detailed information on the antiparallel β-structure of its central segment. A comparison is made with the known X-ray structure of hydrophidae snake venom erabutoxins, as well as with NMR data for *Naja naja oxiana* cytotoxins I and II.

An EPR study of the binding of selectively spin labeled neurotoxin II derivatives to the *Torpedo marmorata* acetylcholine receptor revealed the role played by lysine residues in this specific interaction.

Synthetic Molecular Membranes and Their Functions

TOYOKI KUNITAKE* and YOSHIO OKAHATA

Department of Organic Synthesis, Faculty of Engineering, Kyushu University, Fukuoka 812, Japan

A variety of dialkyl amphiphiles with alkyl chain lengths of C_{10} to C_{20} and with cationic, anionic, nonionic and zwitterionic head groups form stable bilayer membranes spontaneously when dispersed in water. Further assemblage of the bilayer produces vesicles and lamellae, as examined by electron microscopy. These aqueous membranes undergo the crystal-liquid crystal transition in a way similar to the biolipid membranes.

The bilayer and monolayer membranes are similarly formed from one-headed and two-headed single-chain amphiphiles which contain the rigid segment. Their morphologies are drastically changed by the chemical structure of the rigid segment.

These synthetic molecular membranes provide unique microenvironments for reaction and several examples were found in which the membrane fluidity affected the reaction kinetics.

Intramolecular Hydrophobic and Aromatic-ring Stacking Interactions in Ternary Complexes in Solution

HELMUT SIGEL

Institute of Inorganic Chemistry, University of Basle, Spitalstrasse 51, CH-4056 Basle, Switzerland

One possibility of influencing the stability and structure of mixed ligand complexes [1] is through the formation of intramolecular ligand–ligand bonds. *Covalent bond formation* is well-known, *e.g.,* coordinated pyruvate and glycinate form a Schiff base within the coordination sphere of a metal ion [2]. *Ionic bonds* may be formed between oppositely charged side chains: several amino acids are predestinated for such electrostatic interactions [3, 4]. *Hydrogen bond formation* also seems possible [5]

and is known to exist in solid mixed ligand complexes [6].

Aromatic-ring stacking is another very subtle possibility for an influence and equilibrium (1) may even be shifted to its right side, *i.e.* for the logarithm of the corresponding equilibrium constant, $\Delta \log K_M$

$$M(A) + M(B) \rightleftharpoons M(A)(B) + M \quad (1)$$

$$\Delta \log K_M = \log K^{M(A)}_{M(A)(B)} - \log K^{M}_{M(B)} = \log K^{M(B)}_{M(B)(A)} - \log K^{M}_{M(A)} \quad (2)$$

(eqn. 2) [1], positive values are observed. For example, for the Mg^{2+} or Ca^{2+}/1,10-phenanthroline (Phen)/adenosine 5'-triphosphate (ATP^{4-}) system $\Delta \log K_M \simeq 0.5$ due to the intramolecular stacking between the purine moiety of ATP^{4-} and the aromatic-ring system of Phen (studied by potentiometric pH-titrations, UV-difference spectrophotometry and ^{1}H-NMR shift measurements) [7]. This leads to an intramolecular equilibrium (3) between an 'open' and 'closed' isomer of the ternary com-

$$M(A)(B)_{open} \rightleftharpoons M(A)(B)_{closed} \quad (3)$$

plex, M(A)(B). About 90% of Ca(Phen)$(ATP)^{2-}$ exist in the 'closed' form [4], while for M(tryptophanate)$(ATP)^{3-}$ where M^{2+} = Mn^{2+}, Cu^{2+} or Zn^{2+} the corresponding percentages are 55, 41 and 76 [4].

Hydrophobic interactions are observed between the isopropyl residue of leucinate (Leu) and Phen; *e.g.* of Zn(Phen)$(Leu)^+$ exist about 30% in the 'closed' form [8]. Estimations for M(phenylalaninate)(norvalinate) complexes, where M^{2+} = Co^{2+}, Ni^{2+} or Cu^{2+}, give 21, 5 and 11%, respectively, for the isomer with an intramolecular hydrophobic interaction [4, 8].

(Research supported by the Swiss National Science Foundation.)

References

1 H. Sigel, *Angew. Chem. Int. Ed., 14,* 394 (1975); H. Sigel, B. E. Fischer, and B. Prijs, *J. Am. Chem. Soc., 99,* 4489 (1977).
2 D. L. Leussing, *Met. Ions Biol. Syst., 5,* 1 (1976).
3 G. Brookes and L. D. Pettit, *J. Chem. Soc. Dalton Trans.,* 1918 (1977); T. Sakurai, O. Yamauchi, and A. Nakahara, *Bull. Chem. Soc. Jpn., 51,* 3203 (1978).
4 H. Sigel, to be published in the *Conference Volume of the XXth Internat. Conf. Coord. Chem.* (Dec. 1979, Calcutta) by I.U.P.A.C. through Pergamon Press, Oxford.
5 K. H. Scheller, T. H. J. Abel, P. E. Polanyi, P, K. Wenk, B. E. Fischer and H. Sigel, submitted for publication.
6 L. G. Marzilli and T. J. Kistenmacher, *Accounts Chem. Res., 10,* 146 (1977).
7 P. R. Mitchell and H. Sigel, *J. Am. Chem. Soc., 100,* 1564 (1978).
8 B. E. Fischer and H. Sigel, submitted for publication.

Protonation and Complexation Equilibria of Macromolecular Bioligands in Aqueous and Mixed Solvent Solutions. The Solvent Effect

KÁLMÁN BURGER* and BÉLA NOSZÁL

L. Eötvös University, Department of Inorganic and Analytical Chemistry, H-1443 Budapest, P.O. Box 123, Hungary

Coordination chemical equilibria of macromolecular polyfunctional ligands in solution are extremely sensitive to changes in the composition of the solvent. The conformation of such molecules is usually solvent dependent and their conformational changes may result in the alteration of the H-bridge formation reactions in the system. These are reflected by the protonation and complexation equilibria [1, 2].

The characterization of the functional groups taking part in such processes by equilibrium constants is made, however, difficult by the overlap of several analogous (*e.g.* protonation) equilibria in the system, resulting *e.g.* in the formation of protonation isomers (species of equal ligand: bound proton ratios but of different structure).

For the characterization of the single functional groups in overlapping protonation or complexation equilibria a new evaluation method [3] was elaborated leading to the determination of 'group constants'.

Protonation equilibria of several macromolecular polypeptides (*e.g.* corticotropin fragments, angiotenzine analogs and the basic tripsin inhibitor Kunitz Base) were studied in aqueous and mixed solvent solutions and the new evaluation method was used for the determination of the equilibrium constants. The silver and zinc complex formation of these peptides has been also studied.

The effect of the solvent on the equilibria in solution will be discussed in the lecture.

References

1 K. Burger, 'Metal Ions in Biological Systems', M. Dekker, New York, Vol. 9, p. 213 - 249 (1979).
2 K. Burger, F. Gaizer, B. Noszál, M. Pékli and G. Takácsi-Nagy, *Bioinorg. Chem., 7,* 335 (1977).
3 B. Noszál and K. Burger, *Acta Chim. Acad. Sci. Hung., 100,* 275 (1979).