Metalosate® Powered by Albion® Technology

Balchem® Plant Nutrition Research Paper

Bioavailability and Structure of Chelated Minerals

By Jennifer W. Hartle, M.S

Transition metals are necessary as cofactors in enzymatic reactions. Zinc, in particular, is involved in processes that affect transcription (1), cellular proliferation (2), oxidative stress (3), immunity (4; 5), and blood pH balance (6). In proteins, zinc is usually bound to either a nitrogen, sulfur or oxygen residue. In the case of zinc finger proteins, zinc is bound by a histidine nitrogen and cysteine sulfur forming a loop, or chelate (Figure 1) (7). The term chelate was initially coined by Morgan and Drew (8) when working with acetylacetones. They speculated organic molecules form bonds with metallic atoms akin to holding a metal at two points, like a lobster's claw. The word chelate was derived from the Greek word for the great claw, "chela". Martell defines a chelate as a substance combined with a metal containing two or more donor groups so that one or more rings are formed (9). The defining characteristic of a chelate is the heterocyclic ring structure.

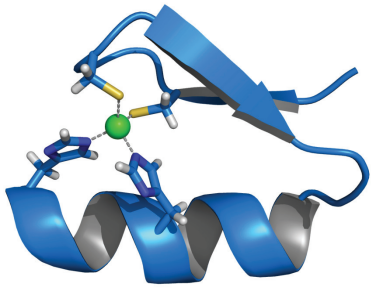


Figure 1. Zinc finger protein rendition. The zinc (green) is bound to two histidine residues (bottom bonds) through the amine moiety and two cysteine residues (top bonds) through the sulfur moiety (10).

Two abundant chelates in nature are hemoglobin and chlorophyll (Figure 2). Heme forms a chelate with ferrous iron, which is a portion of the hemoglobin molecule in blood. In plants, chlorophyll is a chelated form of magnesium (11). Chelating molecules are also important in medicine and industrial environments. One of the most widely used synthetic chelators is ethylenediamine tetra acetic acid (EDTA). The bonding affinity of EDTA with divalent cations such as calcium, magnesium, manganese and zinc is extremely high. This high affinity makes the molecule particularly useful as a treatment for victims of mercury or lead poisoning (12).

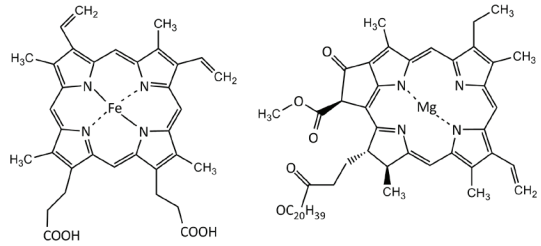


Figure 2. Examples of two chelates found in nature are heme (chelated Fe) and chlorophyll (chelated Mg).

A coordinate bond is one in which the atom on the ligand molecule acts as a Lewis base, donating a pair (or pairs) of electrons to the metal (13). This pair of electrons occupies the d orbital subshell(s), as opposed to either the s or p shells. Therefore the traditional or primary valence is unaffected. When ligands bind to metals, the number of attachments defines the chelate or complex bond. When there is only one point of attachment, a complex is formed with a unidentate bond, while more than one point of attachment in which a heterocyclic ring forms refers to bidentate, tridentate, and multidentate chelate bonds (Figure 3). This heterocyclic ring formation is a defining characteristic of a chelate. Since the valence of the outer shell is unaffected by coordinate bonds, there can be more than one type of bond in a chelate molecule; e.g., a sigma bond with the s orbital and a coordination bond with the d orbital(s) of the metal and possibly with the s or p orbital(s) of the ligand.

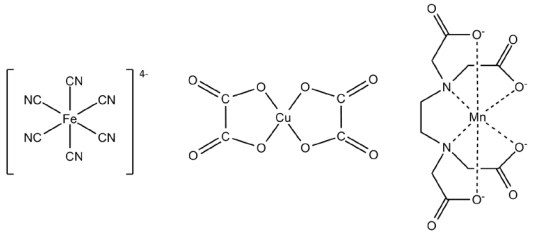


Figure 3. Examples of unidentate (ferrocyanide), bidentate (cupric bisoxalate) and multidentate (manganous EDTA) bonds.

The bonding affinity a chelating ligand has with a metal can have a tremendous effect on the bioavailability of the metal when using a chelated metal for supplementation or fortification. In a recent zinc study, it was found that diethylenetriamine

pentaacetic acid (DTPA) reduced the uptake of radiolabeled zinc in cells (14). Giroux and Prakash evaluated the effects of forced supplementation of a variety of zinc forms and zinc mixes in the sera of rats. They found that when rats were supplemented with zinc and a strong chelator such as phytic acid, edentate disodium or penicillamine, the concentration of zinc in the serum was greatly reduced. However, when the zinc was combined with natural chelators such as lysine, cysteine, glycine and histidine, higher sera values of zinc were observed in comparison to the strong chelators or with zinc sulfate alone (15). Therefore, the bond strength between the metal and the ligand plays an important role in the bioavailability of a chelated metal. The general stability of a metal to ligand bond can be evaluated by the formation or stability constant, K, which is the equilibrium constant for formation of the complex ion from the hydrated metal cation (16). The constant is expressed by Equation 1. In this and subsequent equations, M represents the metal and L represents the ligand.

$$ML_{N-1} + L = ML_N \qquad K_N = \frac{[ML_N]}{[ML_{N-1}][L]}$$

Equation 1. Equation for stability constant K (17).

The constant relates to the number of ligands with which the metal reacts. K1 is the equilibrium constant for a metal that reacts with one ligand, and so on. In this equation, there is only one ligand reacting with a complex; either the metal itself or the metal already complexed with a ligand(s). There are instances when the metal will react with more than one ligand. These constants are expressed as β, and are calculated by Equation 2.

$$M + L = ML_N \qquad \beta_N = \frac{[ML_N]}{[M][L]_N}$$

Equation 2. Equation for stability constant β (17).

Since there can only be N independent equilibria in a given system, the stability constants K and β are interrelated and can be mathematically correlated, as seen in Equation 3.

$$\textbf{If:} \quad \beta_3 = \frac{[ML_3]}{[M][L]^3} * \frac{[ML][ML_2]}{[ML][ML_2]}$$

$$\textbf{And:} \quad \beta_3 = \frac{[ML]}{[M][L]} * \frac{[ML_2]}{[ML][L]} * \frac{[ML_3]}{[ML_2][L]}$$

$$\textbf{Then:} \quad \beta_3 = K_1K_2K_3$$

Equation 3. Correlation of the constants K and β (17).

The constants K and β are called the stepwise formation constants and the overall formation constants, respectively (17).

The stability constants for several ligands with Zn+2 are listed in Table 1. The organic ligand stability constants were found in the National Institute of Standards and Technology (NIST) standard reference database for critically selected stability constants of metal complexes edited by Martell and Smith (18). (Of note is that the lysine stability constant listed is for MHL and MHL2 complexes. It was not referenced for the ML and ML2 complexes.) The inorganic stability constants were found in Sillen and Martell's book, Stability Constants of Metal-Ion Complexes (19). The higher the number, the more stable the ML or ML2 product. Penicillamine, EDTA, and DTPA all have stability constants over 16 with Zn+2, whereas amino acids stability constants range from 4 to 10. Phosphate, the portion of dietary phytate that binds to cations (Figure 4), also has a high stability constant with Zn+2. Dietary phytate, specifically the inositol hexaphosphates and pentaphosphates, have been linked to decreased mineral availability in the diet (20). This is due to the high binding constant of phosphate with minerals and has an observed effect on the bioavailability of supplemental transition elements.

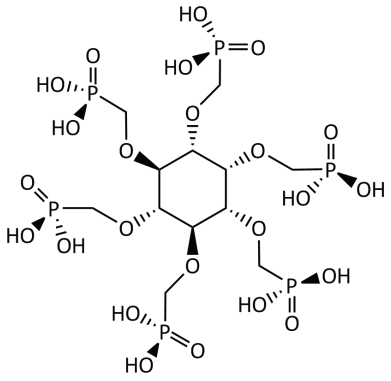


Figure 4. Inositol hexaphosphate is a constituent of dietary phytate which can bind cations through terminal phosphate groups.

Yoshikawa et al. evaluated the effect of stability constants on biological availability, specifically the effect zinc complexes with a coordination mode of Zn(N2O2) had on insulinomimetic activity in rat adipocytes. They found that zinc complexes including zinc complexed with aspartic acid, proline, glycine and others, with a β less than 10.5 had higher insulinomimetic activity than the two positive controls, zinc sulfate or vanadium (IV) oxysulfate. It

was also observed that zinc complexes, including zinc complexed with histidine, N,N'-ethylene-bis-glycine, N,N'-ethylene-bis-sarcosine, with a β greater than 11.0, showed essentially no insulinomimetic activity (21).

Ligand	Log K_1	Log K_2
EDTA	16.5	-
DTPA	18.2	4.48
Penicillamine	9.71	19.48
Aspartic Acid	5.87	10.16
Lysine	4.11	7.99
Glycine	4.96	9.19
Phosphate	12.44	6.71
Sulfate	2.28	-

Table 1. Stability constants for several ligands with Zn+2.

The bioavailability of the chemical form of a mineral plays an important role in the utilization of that mineral from diet, supplementation or agricultural application. Just as zinc complexed with different ligands injected into the body had different effects on insulinomimetic activity (21), the form that a mineral is ingested by the body or absorbed into the plant is also important. When a chelated molecule is formed, it exhibits different attributes than either the metal or the ligand alone (22). Therefore, the effect of a supplemental metal chelate, when given in a dry form such as a food additive, feed, or vitamin capsule, will be different than inorganic metals. In the literature some authors report no significant differences between an inorganic metal and a chelate (23; 24) while in others, a marked difference is noted (25; 26; 27). There are several reasons that could contribute to the differences observed including species, age of the animal, feed, dosage, matrix, delivery form, biomarkers analyzed and others. Hill noted that due to a limited number of quality studies in the published literature, few conclusions can be drawn when comparing organic and inorganic minerals in swine nutrition (28). However, one major factor that could contribute to the differences is the structure of the substances being evaluated. Utilizing a dry mixture of an inorganic metal and an amino acid, as opposed to a mineral reacted with an amino acid to form a chelate, could also have an effect on the outcome of biological trials.

In previous work done at Albion, a manufacturer of mineral glycinate chelates, a crystalline zinc bisglycinate chelate was synthesized from zinc oxide and glycine. The sample was analyzed by x-ray crystallography and yielded the structure seen in Figure 5 (29). The technique of x-ray crystallography analyzes the molecular density of atoms in a rigid crystalline structure. Based on the length of the space between the atoms, the bonds and molecular structure of a crystalline sample can be determined. The structure of a zinc bisglycinate chelate is a heterocyclic ring, with zinc bound to the carboxyl and the α-amine moieties of the glycine.

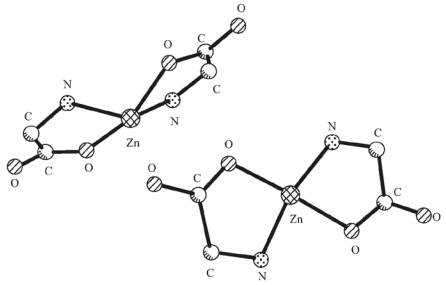


Figure 5. X-ray crystallography of zinc bisglycinate chelate.

The use of x-ray crystallography requires a crystalline sample for analysis. To evaluate bonding structure of a noncrystalline powdered sample, Fourier-Transforming Infrared Spectroscopy (FTIR) can be used. This type of spectroscopy analyzes the bonds in a sample based on the energy that is transmitted by a sample when subjected to infrared light. The energy transmissions detected correlate directly to the bonds and the energies of those bonds in a molecule. An FTIR was subsequently run on the zinc bisglycinate chelate crystal. Using this spectrum as a standard, the bonds relating to the chelate can be identified. The two major components of a chelate bond are the amine and carboxyl bonds with the metal. In midrange FTIR, which evaluates the wavelengths from 4000-400 cm-1, the metal to oxygen and metal to nitrogen bonds cannot be observed. However, there are specific molecular vibrations related to the amine and carboxyl functional groups that can be observed in a midrange FTIR spectrum. As glycine binds to the metal, some of those movements will be changed by the bond with the functional group to the metal and visualized as a peak shift in the spectrum, while other movements will be completely restricted upon bonding with the metal. Molecular vibrations of the alpha amine and carboxyl functional groups of amino acids observed in FTIR spectral analysis are presented in Table 2.

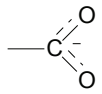
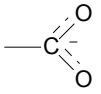
Group and Class	Peaks observed	Assignment
NH_2 in primary or aromatic amines	3520 - 3320 cm^{-1}	NH stretch
NH_3^+ in amino acids	3200 - 3000 cm^{-1}	NH_3^+ antisym stretch
NH_3^+ in amino acids	1530 - 1490 cm^{-1}	NH_3^+ deformation
O-C=O in carboxylic acids	700 - 590 cm^{-1}	O-C=O bending
 in amino acids	560 - 500 cm^{-1}	 rocking

Table 2. FTIR spectral analysis of amino acids.

Glycine is a zwitterionic molecule in solution between pH 3 and 9, meaning that it carries both a negative and a positive charge (Figure 6).

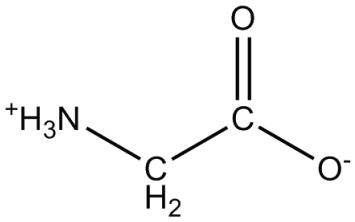


Figure 6. Glycine molecule in the zwitterionic state.

In order for a glycinate chelate to be formed, a change must occur with both the amine and carboxyl movements. The amine must lose a hydrogen atom to form a coordinate covalent bond with the metal. In glycine, there is an antisymmetric stretch of the NH3+ molecule observed at 3168 cm-1, as well as an NH3+ deformation observed at 1511 cm-1. When the amine moiety binds to the metal, the NH3+ loses a hydrogen atom to become NH2. In the chelate bond, this is observed as a shift from the NH3+ to NH stretching at 3453 cm-1. The NH3+ deformation disappears as that movement is no longer present. The carboxyl moiety in the glycine molecule has two prominent movements observable by FTIR. One is the rock, observed at 504 cm-1 and one is a bend observable at 694 cm-1. When the carboxyl moiety binds to the metal, its rocking motion is inhibited. Therefore, the peak at 504 cm-1 is not visible in the chelated glycine molecule. The bend changes when the carboxyl bonds to the metal, and shifts to 727 cm-1 (30). A summary of the spectral changes between an unbound glycine molecule and a glycine chelated to zinc is found in Table 3. The changes in the FTIR spectra can be seen in Figure 7.

In the FTIR spectra of amino acids, EDTA and related compounds bound to metals, the CH2 frequencies are of no interest in the metal chelate bond because they are not metal sensitive (31). Nakamoto evaluated the glycine chelate bonds in copper bisglycinate and found the strength and shift of the amine moiety indicated that the coordinate bond between the metal and the nitrogen would exhibit a covalent character. The changes in the carboxyl moiety indicate that the bond between the metal and the oxygen is more ionic in nature, though the oxygen in the chelate occupies a similar position as the nitrogen and therefore still has a covalent nature (32). Using the zinc bisglycinate crystal spectrum as a standard, the spectrum of noncrystalline powdered zinc bisglycinate products can be compared and evaluated for chelate bonds. Albion manufactures zinc bisglycinate chelate products. A sample of their chelated product was evaluated using FTIR. The amine shift as well as the

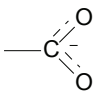
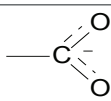
Glycine		*Zinc Bisglycinate Chelate*	
Assignment	**Peak Observed**	**Assignment**	**Peak Observed**
NH_3^+ antisym stretch	3168 cm^{-1}	NH stretch	3453 cm^{-1}
NH_3^+ deformation	1511 cm^{-1}	NH_3^+ deformation	Not observed
O-C=O bending	694 cm^{-1}	O-C=O bending	727 cm^{-1}
 Rocking	504 cm^{-1}	 rocking	Not observed

Table 3. FTIR spectral analysis of unbound and chelated glycine.

diminished carboxyl rock was observed, as seen in Figure 8. There are some minor differences between the product and the crystal spectra that are more than likely due to excipients used in manufacturing. However, the amine and carboxyl changes in the spectra used for identifying the chelate bonds are present.

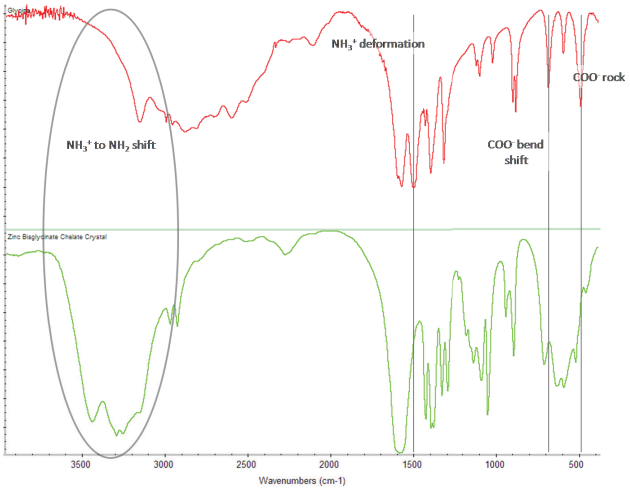


Figure 7. Spectral comparison of glycine (top) to a zinc bisglycinate chelate (bottom).

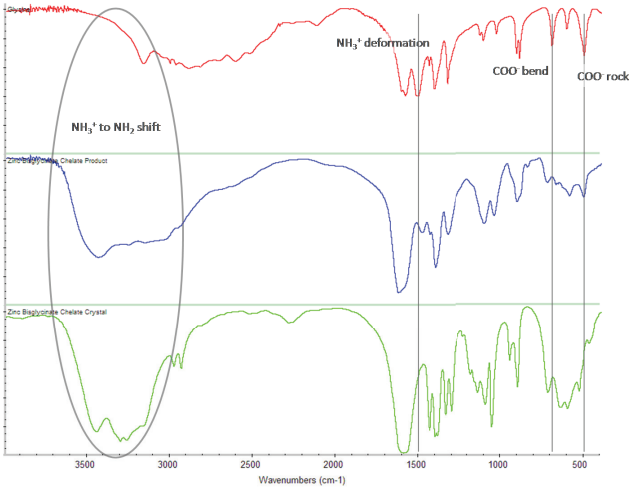


Figure 8. Spectral comparison of glycine (top), a zinc bisglycinate chelate product (middle), and a zinc bisglycinate chelate crystal (bottom).

Amino acid chelates have specific chemical structures that cannot be identified using analytical techniques which do not evaluate the actual bonds in a molecule. Albion produces amino acid chelates and can demonstrate using molecular vibrational spectroscopy that the chelated products produced, are in fact, chelates. These chelated products have been shown to have an increased bioavailability in both human and plant applications.

Bibliography

1. Deficiency of the zinc finger protein ZPR1 causes defects in transcription and cell cycle progression. **Gangwani, L.** *2006, The Journal of Biological Chemistry, Vol. 281, pp. 40330-40340.*

2. The zinc sensing receptor, a link between zinc and cell signaling. **Hershfinkel, M., Silverman, W. and Sekler, I.** *2007, Molecular Medicine, Vol. 13, pp. 331-336.*

3. The function of zinc metallothionein: a link between cellular zinc and redox state. **Maret, W.** *2000, The Journal of Nutrition, Vol. 130, pp. 1455S-1458S.*

4. Zinc and the immune system. **Rink, L. and Gabriel, P.** *2000, Proceedings of the Nutrition Society, Vol. 59, pp. 541-552.*

5. Zinc in human health: effect of zinc on immune cells. **Prasad, A.** *2008, Molecular Medicine, Vol. 14, pp. 353-357.*

6. Apo-human carbonic anhydrase II revisited: implications of the loss of a metal in protein structure, stability and solvent network. **Avvaru, B., et al.** *2009, Biochemistry, Vol. epub ahead of print.*

7. Recognition of DNA by Cys2, His2 zinc fingers. **Klevit, R.** *1991, Science, Vol. 253, pp. 1367-1393.*

8. CLXII.—Researches on residual affinity and co-ordination. Part II. Acetylacetones of selenium and tellurium. **Morgan, G. and Drew, H.** *1920, The Journal of the Chemical Society Transactions, pp. 1456-1465.*

9. **Martell, A. and Calvin, M.** *Chemistry of the Metal Chelate Compounds. New York : Prentice-Hall, Inc., 1952.*

10. **Splettstoesser, T.** *Zinc Finger DNA Complex. Published under the GNU Free Documentation License, s.l. : 2007.*

11. **Voet, D. and Voet, J.** *Biochemistry, third edition. Hoboken : John Wiley & Sons, Inc., 2004. p. 321.*

12. Heavy metal induced oxidative stress and its possible reversal by chelation therapy. **Flora, S., Mittal, M. and Mehta, A.** *2008, The Indian Journal of Medicinal Research, Vol. 128, pp. 510-523.*

13. Alfred Werner revisited: the coordination chemistry of anions. **Bowman-James, K.** *2005, Accounts of Chemical Research, Vol. 38, pp. 671-678.*

14. Zinc retention differs between primary and transformed cells in response to zinc deprivation. **Dutta, A., et al.** *2009, The Journal of Nutritional Biochemistry, pp. e-pub ahead of print.*

15. Influence of zinc-ligand mixtures on serum zinc levels in rats. **Giroux, E. and Prakash, N.** *1976, Journal of Pharmaceutical Sciences, Vol. 66, p. 391.*

16. **McMurray, J. and Fay, R.** *Chemistry. Englewood Cliffs : Prentice-Hall, Inc., 1995. pp. 647-650; 812.*

17. **Cotton, F., Wilkinson, G. and Gaus, P.** *Basic Inorganic Chemistry. New York : John Wiley & Sons, Inc., 1995. pp. 183-184; 503-516.*

18. **Martell, A. and Smith, R.** *NIST critically selected stability constants of metal complexes. College Station, Texas, USA : Texas A&M University, 2001. NIST Standard Reference Database 45 Version 6.0.*

19. **Sillen, L. and Martell, A.** *Stability Constants of Metal-Ion Complexes. London : The Chemical Society, 1964.*

20. Effect of dietary components upon zinc bioavailability. **O'Dell, B.** *1969, American Journal of Clinical Nutrition, Vol. 22, pp. 1315-1322.*

21. New insulinomimetic zinc(II) complexes of a-amino acids and their derivatives with Zn(N2O2) coordination mode. **Yoshikawa, Y., et al.** *2001, Chemical and Pharmaceutical Bulletin, Vol. 49, pp. 652-654.*

22. Synthetic chelating agents in clinical medicine. **Pullman, T., Lavender, A. and Forland, M.** *1963, Annual Reviews of Medicine, Vol. 14, pp. 175-194.*

23. Chemical characteristics and relative bioavailability of supplemental organic copper sources for poultry. **Guo, R., et al.** *2001, Journal of Animal Science, Vol. 79, pp. 1132-1141.*

24. Effects of organic forms of zinc on growth performance, tissue zinc distribution, and immune response in weanling pigs. **van Heugten, E., et al.** *2003, Journal of Animal Science, Vol. 81, pp. 2063-2071.*

25. Effects of metal amino acid chelates or inorganic minerals on three successive lactations in dairy cows. **Ashmead, D. and Samford, R.** *2004, The International Journal of Applied Research in Veterinary Medicine, Vol. 2, pp. 181-188.*

26. The effect of copper, zinc, and manganese amino acid chelates on dairy cow reproduction on eight farms: a field trial. **Bosseboeuf, Y., et al.** *2006, The International Journal of Applied Research in Veterinary Medicine, Vol. 4, pp. 313-319.*

27. Absorption and retention of zinc when administered as an amino-acid chelate in the dog. **Lowe, J., Wiseman, J. and Cole, D.** *1994, The Journal of Nutrition, Vol. 124, pp. 2572S-2574S.*

28. Organic and inorganic copper, zinc, iron and manganese. **Hill, G.** *Indianapolis : University of Illinois, 2005. Midwest Swine Nutrition Conference Proceedings. pp. 67-75.*

29. Characterization of metal amino acid chelates: zinc bisglycinate model. **Ashmead, S.** *Atlanta : AOAC 117th International Meeting, 2003.*

30. **Lambert, J., et al.** *Introduction to Organic Spectroscopy. New York : Macmillan Publishing Company, 1987. pp. 168-182.*

31. **Nakamoto, K.** *Infrared and Raman Spectra of Inorganic and Coordination Compounds. New York : John Wiley & Sons, Inc., 1986. pp. 233-239.*

32. Infrared spectra and normal coordinate analysis of metal glycino complexes. **Condrate, R. and Nakamoto, K.** *1965, The Journal of Chemical Physics, Vol. 42, pp. 2590-2598.*

balchem®
PLANT NUTRITION

balchem.com/plant-nutrition