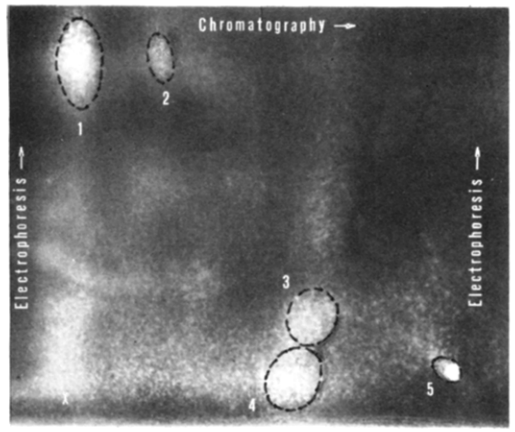

Figure 2. Peptide map of the plastein prepared with immobilized α-chymotrypsin from the low molecular weight peptide fraction of soybean protein hydrolysates.

work. The importance of this in preparation of plasteins for food use is not clear.

Peptides synthesized by using the immobilized enzyme were smaller than the smaller peptide prepared by using the soluble enzyme. The plasteins prepared with the immobilized enzyme (peptides 2 and 3) were found to be penta- and tetrapeptides, respectively. The average molecular weight was 1248 (10.4 residues) for the peptides from soluble α-chymotrypsin and 1084 (9.04 residues) for the peptides prepared from the immobilized enzyme. These results compared favorably with the results obtain previously (Pallavicini et al., 1980), which indicated an average molecular weight found for the soy plasteins in the current study is slightly higher than that obtained from soy hydrolysate and ^{14}C-labeled methionine ethyl ester substrate reported by Monti and Jost (1979) and lower than that of other plasteins prepared from soy by using soluble α-chymotrypsin (Yamashita et al., 1970b, 1974) and other free proteases (Yamashita et al., 1972, 1975; Tsai et al., 1974).

From the data presented above, we cannot completely exclude the possibility that small quantities of peptide material remain unresolved by the fingerprint technique. The immobilized enzyme system has great advantages in the preparation of plasteins in that the enzyme can be recovered and recycled, simplifying plastein purification. The plasteins prepared by using the immobilized enzyme are different than those from soluble enzyme.

ACKNOWLEDGMENT

We thank Gabriella Trentin for technical assistance.

Registry No. α-Chymotrypsin, 9004-07-3.

LITERATURE CITED

Eriksen, S.; Fagerson I. S. *J. Food Sci.* **1976**, *41*, 490.
Felix, A. M.; Jimenez M. H. *J. Chromatogr.* **1974**, *89*, 361.
Hofsten, B. v.; Lalasidis G. *J. Agric. Food Chem.* **1976**, *24*, 460.
Monti, J. C.; Jost, R. *J. Agric. Food Chem.* **1979**, *27*, 1281.
Noguchi, M.; Yamashita, M.; Arai, S.; Fujimaki, M. *J. Food Sci.* **1975**, *40*, 367.
Onoue, Y.; Riddle, V. M. *J. Fish Res. Board Can.* **1973**, *30*, 1745.
Pallavicini, C.; Finley, J. W.; Stanley, W. L.; Watters, G. G. *J. Sci. Food Agric.* **1980**, *31*, 273.
Savangikar, V. A.; Joshi, R. N. *J. Sci. Food Agric.* **1979**, *30*, 899.
Schiltz, E.; Schnackerz, K. D.; Gracy, R. W. *Anal. Biochem.* **1977**, *79*, 33.
Tsai, S.-J.; Yamashita, M.; Arai, S.; Fujimaki, M. *Agric. Biol. Chem.* **1974**, *38*, 641.
Yamashita, M.; Arai, S.; Aso, K.; Fujimaki, M. *Agric. Biol. Chem.* **1972**, *36*, 1353.
Yamashita, M.; Arai, S.; Gonda, M.; Kato, H.; Fujimaki, M. *Agric. Biol. Chem.* **1970a**, *34*, 1333.
Yamashita, M.; Arai, S.; Kokubo, S.; Aso, K.; Fujimaki, M. *J. Agric. Food Chem.* **1975**, *23*, 27.
Yamashita, M.; Arai, S.; Matsuyama, J.; Gonda, M.; Kato, H.; Fujimaki, M. *Agric. Biol. Chem.* **1970b**, *34*, 1484.
Yamashita, M.; Arai, S.; Tanimoto, S.-Y.; Fujimaki, M. *Biochim. Biophys. Acta* **1974**, *358*, 105.

Received for review July 1, 1982. Accepted March 2, 1983. This work was supported by the Consiglio Nazionale delle Ricerche, Roma, Italy.

On the Electrophoretical Differentiation and Classification of Proteins. 12. Comparative Investigation of Yeast Proteins of Different *Saccharomyces* Species and Various Strains Belonging to the Species *Saccharomyces cerevisiae* Hansen, by Means of Isoelectric Focusing in Polyacrylamide Gels

Friedrich Drawert* and Jaromír Bednář

Isoelectric focusing in cylindrical polyacrylamide gels was used to resolve the water-soluble proteins of *Saccharomyces cerevisiae*, *Saccharomyces uvarum*, *Saccharomyces bayanus*, *Saccharomyces rouxii*, *Saccharomyces aceti*, and seven strains of the species *Saccharomyces cerevisiae* Hansen. The cell disruption was carried out with freeze–pressing. About 30 protein bands were found with isoelectric points of 4.5–9.7. The trend in the pH gradient was determined by measuring the pH of the gel slices with a special one-rod electrode. The results were reproducible under constant working conditions. The protein patterns of the seven *S. cerevisiae* strains are found to be very similar, although those of the species are partly variable; hence, the question of correct systematic classification arises.

In recent years, attention has been focused on physicochemical methods for the identification of microorganisms which may provide alternatives to the conventional techniques. These new approaches include the gas chromatography (Drucker, 1981; Jantzen and Hofstad, 1981; Mitruka, 1975; Meuzelaar et al., 1975; Dees and Moss, 1978; Wasserfallen and Rinderknecht, 1978), mass spectrometry (Wieten et al., 1981; Kistemaker et al., 1975; Mitchell et al., 1978), impedance measurement (Ur and

Institut of Food Technology and Analytical Chemistry, Technical University of Munich, D-8050 Freising-Weihenstephan, West Germany.

0021-8561/83/1431-0848$01.50/0

Brown, 1975; Cady, 1975; Goldschmidt and Wheeler, 1975), microcalorimetry (Boling et al., 1973; Russell et al., 1975; Beezer et al., 1978), proton magnetic resonance (Gorin and Spencer, 1970), circular dichroism and absorption spectra (Torten and Schneider, 1973), and electrophoretical methods (Cato et al., 1982; Kersters and De Ley, 1975; Swings et al., 1976; Swings and De Ley, 1977; Wadström and Smyth, 1975; Yotis, 1977).

We have shown the dissimilarities of protein patterns between procaryotic cells, represented by bacteria *Pseudomonas ovalis* and *Achromobacter alcaligenes*, and eucaryotic cells, represented by two strains of yeast *Saccharomyces cerevisiae*, using thin layer isoelectric focusing in granular gels and isoelectric focusing in cylindrical polyacrylamide gels. Also, the protein patterns of eight different yeast genera resolved by means of isoelectric focusing in cylindrical polyacrylamide gels were dissimilar and hence useful in taxonomical identifications (Drawert and Bednář, 1979).

This paper deals with protein patterns of various species of the genus *Saccharomyces* and some strains of the species *S. cerevisiae* and presents a continuation of the previous work.

MATERIALS AND METHODS

Organisms. The strains of yeasts used in this study were obtained from Centraalbureau voor Schimmelcultures, Baarn, The Netherlands, as follows: *S. cerevisiae* Hansen 381, H. Naganishi; *S. cerevisiae* Hansen 439, fr. grapes, G. Krumbholz; *S. cerevisiae* Hansen 1171, T, fr. top yeast, van Wijk; *S. cerevisiae* Hansen 1395 (T, *Saccharomyces ellipsoideus*), Claussen; *S. cerevisiae* Hansen 1401, fr. wine; *S. cerevisiae* Hansen 1539; *S. cerevisiae* Hansen 2989, wine yeast, Steinberg 1917, Warmgärhefe Wiken; *Saccharomyces uvarum* Beijerinck 382 (T, *Saccharomyces logos*), fr. brewer's yeast, Lindner; *Saccharomyces bayanus* Saccardo 422, fr. beer, Schnegg; *Saccharomyces rouxii* Boutroux 732, T, fr. concentrated grape must, M. Sacchatti; *Saccharomyces aceti* Santa Maria 4054, fr. red wine, Santa Maria. All the strains have been described by Lodder (1971).

Culture Conditions. The cells were incubated in Difco YM Broth and the cultivation of the aerobic culture was carried out on a rotating roller drum apparatus at 25 °C and 200 rpm. The harvesting was done at the exponential growth phase.

Freeze–Pressing. The yeasts were washed in distilled water, centrifuged, then transferred into a Biox-X-Press disintegrator (LKB, Bromma, Sweden), and cooled at −25 °C. The disintegration of cells by the Biox-X technique has been described previously (Drawert and Bednář, 1974).

Isoelectric Focusing. The separation of proteins has been achieved in 7% (w/v) polyacrylamide gel with 5% (v/v) carrier ampholyte (Ampholine, LKB Bromma, Sweden) as described in our previous work (Drawert and Bednář, 1979).

Densitometry and pH Gradient. The colored gels were scanned with a Vitatron TLD 100 (Forschbach, West Germany), and parallel to this noncolored gels were sliced into pieces and incubated in distilled water, and the pH was measured directly.

RESULTS

With the exception of the strain *rouxii* and partially *aceti*, all other yeasts belonging to the genus *Saccharomyces* showed similarities. The protein fraction in the basic range with an isoelectric point at 8.4 was found in *S. cerevisiae* (1 in Figure 1), *S. bayanus* (3 in Figure 1) and *S. rouxii* (4 in Figure 1). Deviations were observed in *S. uvarum* and *S. aceti*; in *S. uvarum* this band occurred in a very pale intensity and in *S. aceti* it was absent. An additional protein band was found in *S. aceti* at pH 8.8. Relatively more deviation from the typical pattern was noticed in *S. rouxii* (Figure 1). In addition, the protein pattern had a distinctive band at pH 7.9, which did not occur in any other strain of this series. Also, a marked difference in the acid up to neutral range in the protein pattern of *S. rouxii* is noticed compared to that of other species of this genus. The absence of proteins around p*I* = 5.3 in the case of *S. rouxii* is striking, whereas for the other species this zone is heavily laid up. The general accumulation of proteins in the zone 4.8–6.0 is the case of *S. rouxii* shifted to the cathode, whereas the typical comparative accumulation of proteins for this species in the range p*I* = 6.6–7.2 is rather faint here.

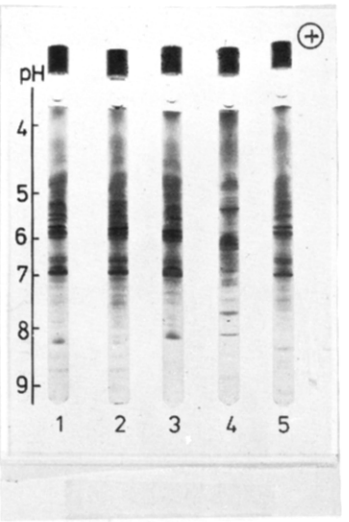

Figure 1. Isoelectric focusing of intracellular proteins from the following: 1, *S. cerevisiae*; 2, *S. uvarum*; 3, *S. bayanus*; 4, *S. rouxii*; 5, *S. aceti*.

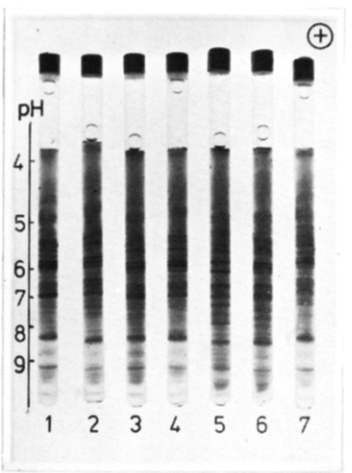

Figure 2. Isoelectric focusing of intracellular proteins from *S. cerevisiae*: 1, CBS 381; 2, CBS 439; 3, CBS 1171; 4, CBS 1395; 5, CBS 1401; 6, CBS 1539; 7, CBS 2989.

As expected, the seven strains of the species *S. cerevisiae* Hansen indicated a uniform distribution of proteins with the exception of the strain CBS 1401, which showed a slight deviation. In all strains this was the occurrence of protein bands at pH 8.4 as well as a band at pH 9. From the acid to neutral range most of the proteins appeared in two zones at pH 5.3–6.2 and 6.6–7.2. In the strain CBS 1401 there were slight deviations at pH 6.6 and 7.4 (Figure 2). In addition, the protein pattern had a band at pH 8.2, which was more intense compared to the other bands. A

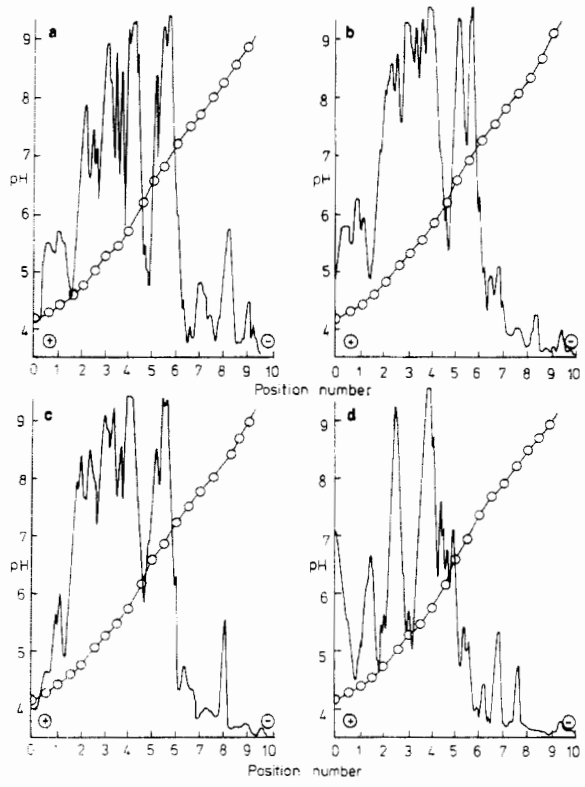

Figure 3. Densitometer tracing (—) and pH gradient (O) of the protein patterns after isoelectric focusing: (a) *S. cerevisiae*; (b) *S. uvarum*; (c) *S. bayanus*; (d) *S. rouxii*.

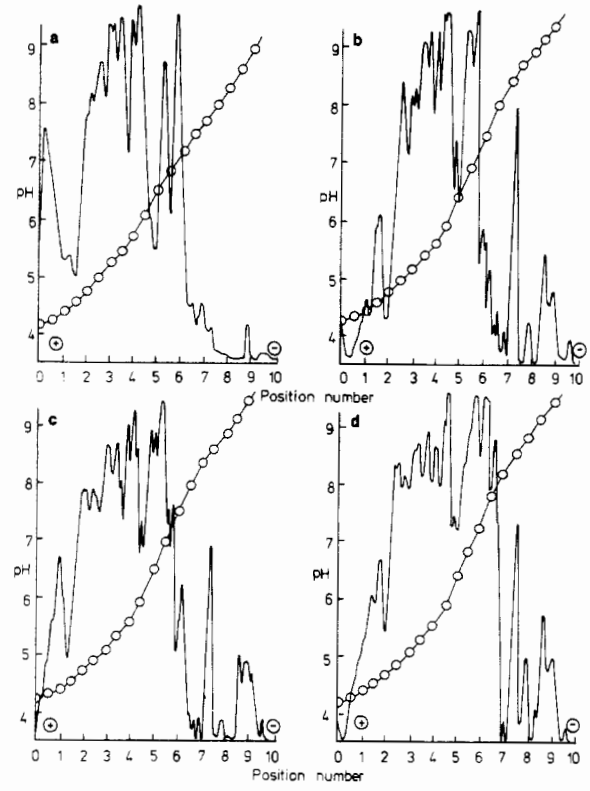

Figure 4. Densitometer tracing (—) and pH gradient (O) of the protein patterns after isoelectric focusing: (a) *S. aceti*; (b) *S. cerevisiae* CBS 381; (c) *S. cerevisiae* CBS 439; (d) *S. cerevisiae* CBS 1171.

similar deviation in this range also occurred in the strain CBS 1539.

Densitometric tracings according to the pH gradients were carried out for underlining the differences in protein patterns shown by the representative species (Figure 3 and Figure 4a) just like the similarity of themselves by the various strains of *S. cerevisiae* (Figure 4c,d and Figure 5c,d) with the exception of the strain CBS 1401 (Figure 5b). As focusing is being made in two series, the pH gradient course in the first series (Figure 3 and Figure 4a) differs from that of the second series slightly.

DISCUSSION

Among the physicochemical methods there has been an increase in the use of electrophoretic techniques for investigating the proteinic and enzymic heterogeneity of microbial cells for taxonomic purposes. Although there have been several publications describing the separation of cell proteins or enzymes using conventional agarose gel or acrylamide gel electrophoresis (Holdeman et al., 1982; Goullet, 1981; Tierno and Stotzky, 1978; Noble and Schell, 1978; Gross et al., 1978; Archer and Stevens, 1977; Brazil, 1978; Mytoon et al., 1978; Baptist et al., 1978), the adaptation of isoelectric focusing in acrylamide gels, based on the accumulation of the cell proteins at their isoelectric points, is found to be rare.

The isoelectric focusing brought good results in comparative investigations of various yeast genera (Drawert and Bednář, 1979). The protein patterns showed high-resolving power with good heterogeneity; thus, the differentiation of individual strains was possible without any difficulty. As expected, these investigations showed that the systematics of closely related strains poses similar protein patterns. The results indicate intimate relationships between some species (*S. cerevisiae*, *S. uvarum*, *S. bayanus*) as well as differences (*S. rouxii*, *S. aceti*). The high deviation of cell proteins of the strain *S. rouxii* from the standard type has also been serologically established

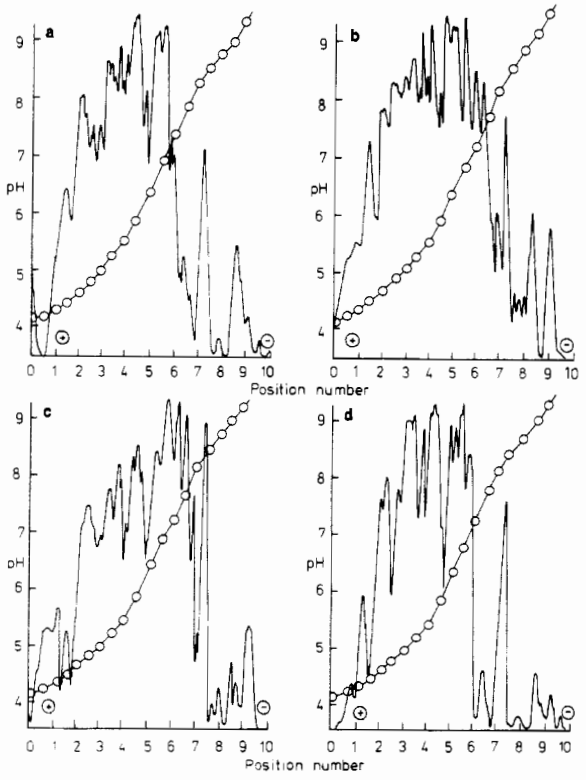

Figure 5. Densitomer tracing (—) and pH gradient (O) of the protein patterns after isoelectric focusing: (a) *S. cerevisiae* CBS 1395; (b) *S. cerevisiae* CBS 1401; (c) *S. cerevisiae* CBS 1539; (d) *S. cerevisiae* CBS 2989.

(Drawert et al., 1982). Now the question arises if *S. rouxii* and *S. aceti* are correctly classified at all.

Among the seven strains of the species *S. cerevisiae* Hansen there are no significant differences either in the

quality or in number of bands in the protein patterns. For the differentiation of yeasts at the species level, complementary and more sensitive methods, especially that of serological and enzymic, are necessary. A combination of isoelectric focusing with the enzymogram technique offers a relatively simple and efficient method in this connection. An application of the above technique on yeast enzymes will be published (Drawert and Bednář, 1982).

LITERATURE CITED

Archer, S. J.; Stevens, W. C. *Microbios Lett.* **1977**, *4*, 193.
Baptist, J. N.; Mandel, M.; Gherna, R. L. *Int. J. Syt. Bacteriol.* **1978**, *28*, 229.
Beezer, A. E.; Bettelheim, K. A.; Al-Salihi, S.; Shaw, E. J. *Sci. Tools* **1978**, *25*, 6.
Boling, E. A.; Blanchard, G. C.; Russell, W. J. *Nature (London)* **1973**, *241*, 412.
Brazil, R. P. *Ann. Trop. Med. Parasitol*, **1978**, *72*, 289.
Cady, P. In "New Approaches to the Identification of Microorganisms"; Hedén, C. G.; Illéni, T., Eds.; Wiley: New York, 1975; p 73.
Cato, E. P.; Holdeman, L. V.; Moore, W. E. C. *Int. J. Syst. Bacteriol.* **1982**, *32*, 77.
Dees, S. B.; Moss, C. W. *J. Clin. Microbiol.* **1978**, *8*, 61.
Drawert, F.; Bednář, J. *Chem., Mikrobiol., Technol. Lebensm.* **1974**, *3*, 69.
Drawert, F.; Bednář, J. *J. Agric. Food Chem.* **1979**, *27*, 3.
Drawert, F.; Bednář, J., Technical University of Munich, unpublished data, 1982.
Drawert, F.; Donhauser, S.; Bednář, J., Technical University of Munich, unpublished data, 1982.
Drucker, D. B. "Microbiological Applications of Gas Chromatography"; University Press: Cambridge, 1981.
Goldschmidt, M. C.; Wheeler, T. G. In "Microbiology-1975"; Schlessinger, D., Ed.; American Society of Microbiology: Washington, DC, 1975; p 6.
Gorin, P. A. J.; Spencer, J. F. T. In "Advances in Applied Microbiology"; Umbreit, W. W.; Perlman, D., Eds.; Academic Press: New York, 1970; p 25.
Goullet, Ph. *J. Gen. Microbiol.* **1981**, *127*, 161.
Gross, C. S.; Ferguson, D. A., Jr.; Cummins, C. S. *Appl. Environ. Microbiol.* **1978**, *35*, 1102.
Holdeman, L. V.; Moore, W. E. C.; Churn, P. J.; Johnson, J. L. *Int. J. Syst. Bacteriol.* **1982**, *32*, 125.
Jantzen, E.; Hofstad, T. *J. Gen. Microbiol.* **1981**, *123*, 163.
Kersters, K.; De Ley, J. *J. Gen. Microbiol.* **1975**, *87*, 333.
Kistemaker, P. G.; Meuzelaar, H. L. C.; Posthumus, M. A. In "New Approaches to the Identification of Microorganisms"; Hedén, C. G.; Illéni, T., Eds.; Wiley: New York, 1975; p 179.
Lodder, J. "The Yeasts, a Taxonomic Study", 2nd ed.; North-Holland Publishing Co.: Amsterdam, 1971.
Meuzelaar, H. L. C.; Kistemaker, P. G.; Tom, A. In "New Approaches to the Identification of Microorganisms"; Hedén, C. G.; Illéni, T., Eds.; Wiley: New York, 1975; p 165.
Mitchell, A.; Needleman, M.; Stratford, B. *Aust. J. Pharm. Sci.* **1978**, *7*, 25.
Mitruka, B. M. In "New Approaches to the Identification of Microorganisms"; Hedén, C. G.; Illéni, T., Eds.; Wiley: New York, 1975; p 123.
Mytton, L. R.; McAdam, N. J.; Portlock, P. *Soil Biol. Biochem.* **1978**, *10*, 79.
Noble, R. C.; Schell, S. C. *Infect. Immun.* **1978**, *19*, 178.
Russell, W. J.; Zettler, J. F.; Blanchard, G. C.; Boling, E. A. In "New Approaches to the Identification of Microorganisms"; Hedén, C. G.; Illéni, T., Eds.; Wiley: New York, 1975; p 101.
Swings, J.; De Ley, J. *Bacteriol. Rev.* **1977**, *41*, 1.
Swings, J.; Kersters, K.; De Ley, J. *J. Gen. Microbiol.* **1976**, *93*, 266.
Tierno, P. M., Jr.; Stotzky, G. *Ann. Clin. Lab. Sci.* **1978**, *8*, 42.
Torten, M.; Schneider, A. S. *J. Infect. Dis.* **1973**, *127*, 319.
Ur, A.; Brown, D. F. J. In "New Approaches to the Identification of Microorganisms"; Hedén, C. G.; Illéni, T., Eds.; Wiley: New York, 1975; p 61.
Wadström, T.; Smyth, C. J. In "Isoelectric Focusing"; Arbuthnott, J. P.; Beeley, J. A., Eds.; Butterworths: London, 1975; p 152.
Wasserfallen, K.; Rinderknecht, F. *Chromatographia* **1978**, *11*, 128.
Wieten, G.; Haverkamp, J.; Meuzelaar, H. L. C.; Engel, H. W. B.; Berwald, L. G. *J. Gen. Microbiol.* **1981**, *122*, 109.
Yotis, W. W. In "Biological and Biomedical Applications of Isoelectric Focusing"; Catsimpoolas, N.; Drysdale, J., Eds.; Plenum Press: New York, 1977; p 265.

Received for review October 19, 1982. Accepted February 22, 1983.

Calcium Binding to Phytic Acid

Ernst Graf[1]

Phytic acid (*myo*-inositol hexaphosphate), a substance present in large amounts in most plant seeds, may, through chelation, suppress the absorption of important polycationic nutrilites such as Ca^{2+}, Zn^{2+}, and Fe^{3+}. I have, therefore, investigated the binding of Ca^{2+} to phytic acid and its dependence on temperature, pH, and ionic strength by Ca^{2+}-selective potentiometry. Scatchard plots showed downward curvature, indicating the existence of intrinsically different binding sites. Their affinities for Ca^{2+} increased sharply with pH, and between 5 and 40 °C the interactions displayed positive entropy and enthalpy changes. The study also showed the presence of two soluble Ca^{2+}–phytate species, Ca_1–phytate and Ca_2–phytate, whereas all other Ca^{2+}–phytate complexes precipitated even at low pH. The important nutritional consequences of this phenomenon are discussed.

The occurrence of phytic acid [*myo*-inositol 1,2,3,4,5,6-hexakis(dihydrogen phosphate)] as a mixed Ca^{2+}–Mg^{2+} salt in aleurone grains was first reported in 1872 (Pfeffer, 1872). It constitutes 1–6% by weight of most cereals, legumes, and oilseeds. It has been shown to interfere with the nutritional bioavailability of minerals by forming insoluble complexes with di- and trivalent cations (Maddaiah et al., 1964; Vohra et al., 1965). With a continually expanding global need for food protein, increasing concern arises over the presence of antinutritional agents such as phytic acid in plant-derived foods. Several excellent recent review articles discuss the occurrence, chemistry, and nutritional aspects of phytic acid (Erdman, 1979; O'Dell, 1979; Cheryan, 1980; Maga, 1982).

Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61604.

[1]Present address: Department of Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, MN 55455.