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# Cytokinin-binding protein in grape berries

by

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## Ein Cytokinin-bindendes Protein in Traubenbeeren

Zusammenfassung. — Aus Traubenbeeren der Sorte Delaware (*Vitis labruscana*) wurde mit Hilfe der Affinitätschromatographie an Benzyladenin- (BA-)gekoppelter Sepharose 4B ein Cytokinin-bindendes Protein isoliert. Das adsorbierte Protein wurde aus der BA-Sepharose-Säule mit 50 mM NaOH eluiert. Es besteht aus mindestens zwei Glykoproteinkomponenten, die gegenüber Cytokinin und Concanavalin A eine hohe bzw. niedrige Affinität aufweisen.

## Introduction

In grapevine, many investigations suggested that cytokinins play an important role in the regulation of flower initiation (SRINIVASAN and MULLINS 1979), sex expression (NEGI and OLMO 1966) and berry development (WEAVER *et al.* 1966, POOL 1975). These actions of cytokinins seem to be closely related to RNA and protein synthesis, but precise mechanisms of their activity are still unknown. Since MATTHYSSE and ABRAMS (1977) described a cytokinin receptor protein from pea bud chromatin that enhanced RNA synthesis in an in vitro system, further evidence indicating the presence of receptors for cytokinins has been reported. Recently, FOX and ERION (1975) described a cytokinin-binding protein and its biochemical functions using equilibrium dialysis, and TAKEGAMI and YOSHIDA (1975, 1977) isolated a binding protein from tobacco leaves by affinity chromatography on which benzyladenine (BA) has been introduced as an affinity ligand. This paper reports the presence of a cytokinin-binding protein in grape berries by affinity chromatography and describes some properties of this binding protein.

## Materials and methods

### 1. Plant materials

Mature grapevines, *Vitis labruscana* cv. Delaware, growing in the field at Yamagata University were used. Berry samples were collected within one week after anthesis.

### 2. Preparation of crude extract

Immediately after collecting about 10 g of berries (which included seeds) were homogenized for 3 min with 100 ml of 0.2 M phosphate buffer (pH 7.2) containing insoluble PVP (0.2 g/g berries) previously swelled, 2 mM EDTA, 5 mM L-cystein monohydorate, 2 mM DTT and 0.5 % ascorbate sodium salt. The homogenate was

strained through four layers of cheese cloth and then centrifuged for 20 min at 15000 *g*. The supernatant was used as crude extract. All operations were carried out at below 5 °C.

### 3. Immobilization of BA on Sepharose 4B

The BA-Sepharose column was prepared by the method of TAKEGAMI and YOSHIDA (1975, 1977). Sepharose 4B activated with cyanogen bromide was washed with $10^{-4}$ M BA solution for 18 h at 37 °C to link BA covalently to Sepharose 4B. The BA-Sepharose obtained was packed into column (15 × 0.9 cm), washed with 0.1 M NaOH, 5 % TCA and 50 mM ethanolamine and extensively with 0.2 M phosphate buffer (pH 7.2).

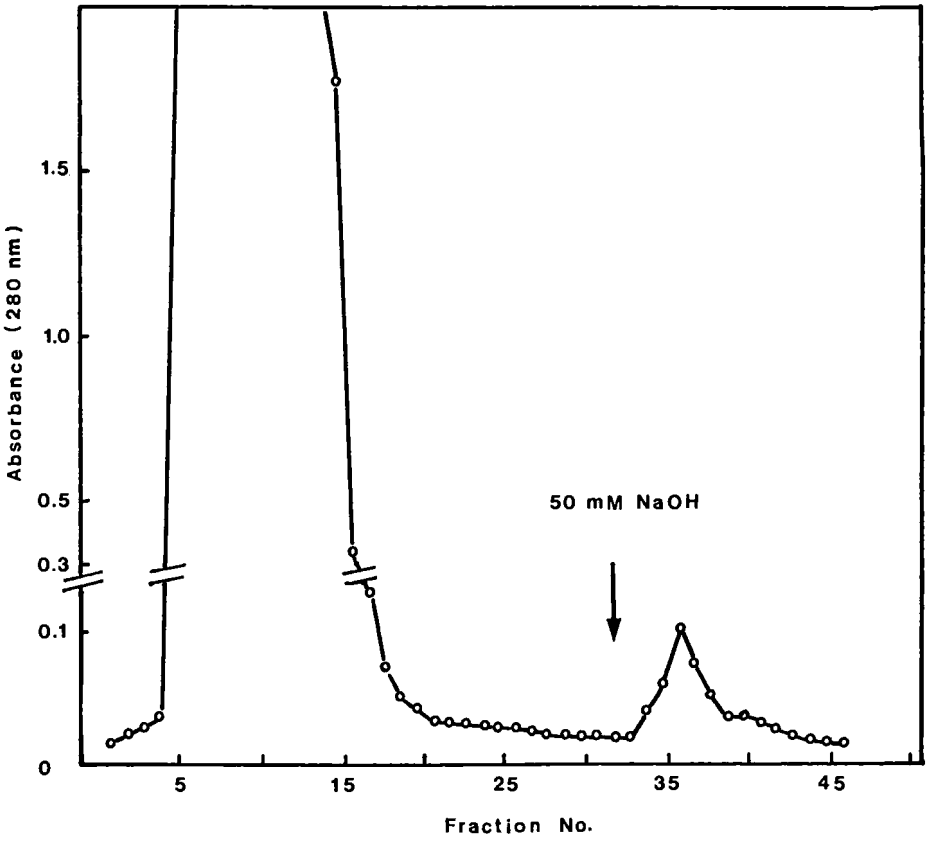


Fig. 1: Isolation of cytokinin-binding protein by BA-Sepharose affinity chromatography. The scale of the vertical axis is changed between 0.1 and 0.3. The volume of the fractions is always 4 ml/tube.

Isolierung des Cytokinin-bindenden Proteins mit Hilfe der Affinitätschromatographie an BA-Sepharose. Auf der senkrechten Achse ändert sich der Einteilungsmaßstab zwischen 0,1 und 0,3. Das Volumen der Fraktionen beträgt stets 4 ml/Probenglas.

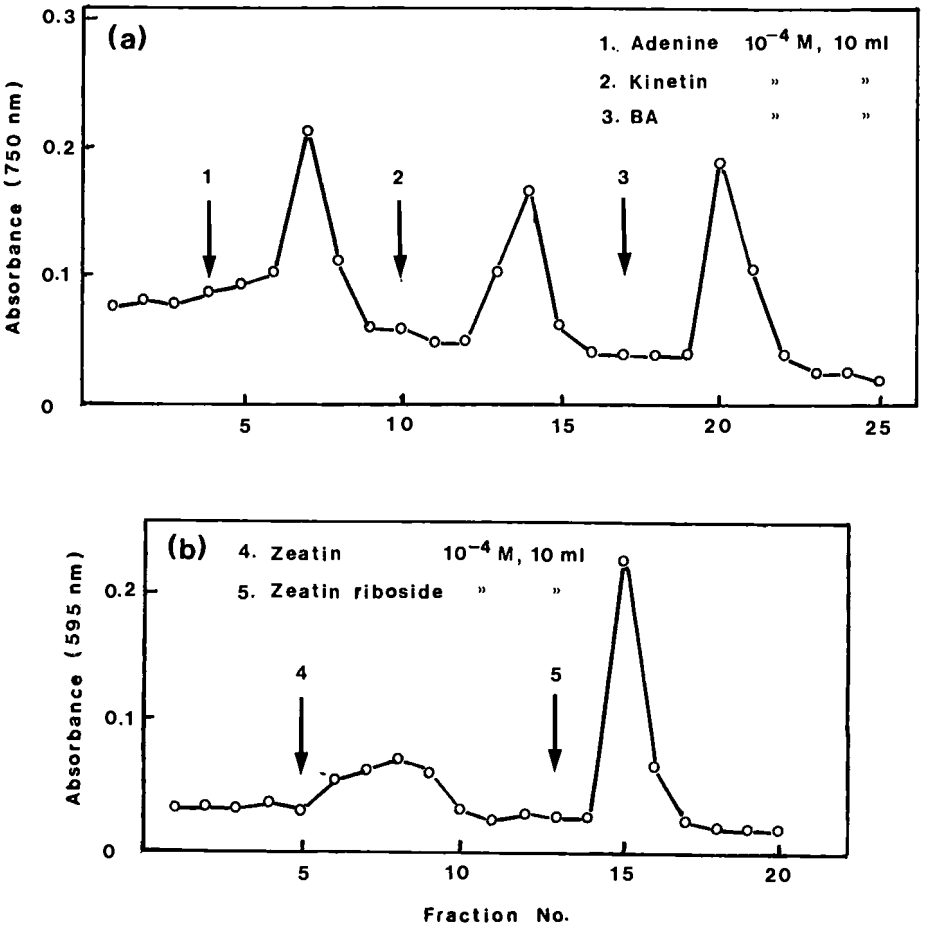


Fig. 2: Elution of the adsorbable proteins from BA-Sepharose column. Protein determination was carried out by the method of LOWRY *et al.* (1951) (a) and BRADFORD (1976) (b).

Elution der adsorbierten Porteine aus der BA-Sepharose-Säule. Die Proteinbestimmung erfolgte nach LOWRY *et al.* (1951) (a) und BRADFORD (1976) (b).

## 4. Immobilization of binding protein on Sepharose 4B

Affinity adsorbants were prepared by the general method of CUATRECASAS and ANFINSEN (1970).

## 5. Affinity chromatography

About 50 ml of crude extract of grape berries were layered onto the BA-Sepharose column. After passage of the extract, the column was washed with starting buffer containing 2 mM DTT to remove non-adsorbable protein and suscessively was eluted with 10 ml of 50 mM NaOH or $10^{-4}$ M cytokinin solution.

### 6. ConA-Sepharose affinity chromatography

Cytokinin-binding protein solution or crude extract of grape berries was layered onto the Concanavalin A (ConA)-Sepharose 4B column (0.5 × 20 cm). The column was washed with 0.2 M phosphate buffer pH 7.2 and adsorbable protein was eluted by addition of 0.1 M α-methyl-D-glycoside.

### 8. Cytokinin bioassay

The radish cotyledon bioassay was used to determine cytokinin activity (LETHAM 1971).

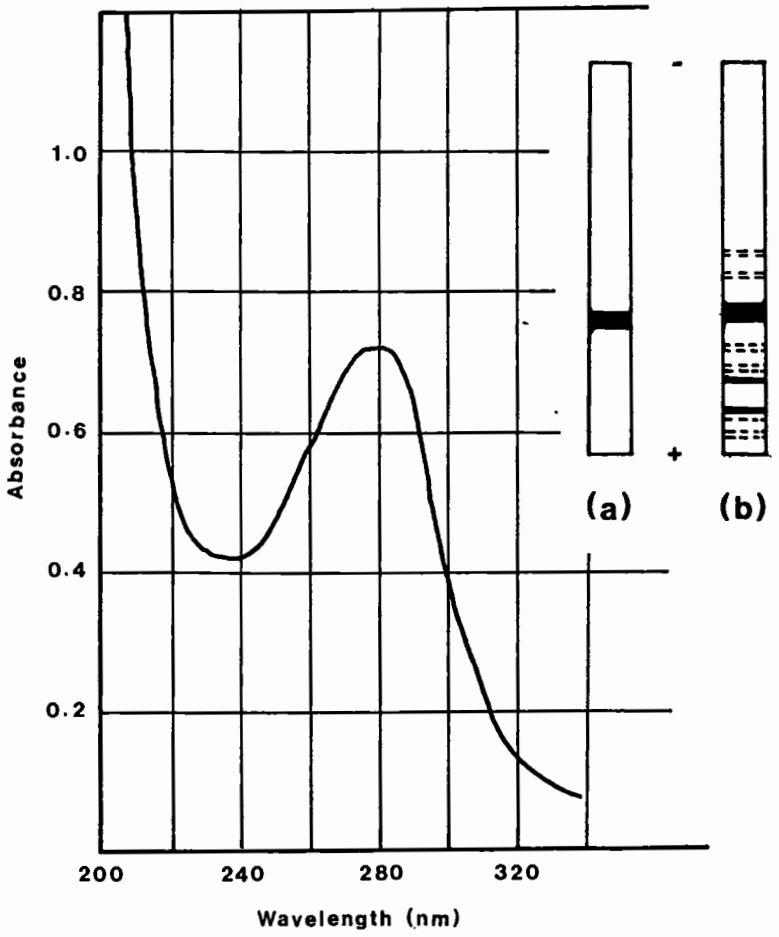


Fig. 3: UV absorption spectrum and diagram of gel isoelectric profile of the adsorbable fraction. a) Adsorbable fraction. b) Crude extract. Seperation by isoelectric focusing in a pH 3.5—10. Protein staining with Coomassie Blue G.

UV-Absorptionsspektren und Profile der adsorbierten Fraktion nach isoelektrischer Fokussierung. a) Adsorbierte Fraktion. b) Rohextrakt. Trennung durch isoelektrische Fokussierung im pH-Gradienten 3.5—10. Proteinfärbung mit Coomassie-Blau G.

## Results and discussion

The result of affinity chromatography is presented in Fig. 1. Crude extract was applied to the BA-Sepharose 4B column and was thoroughly washed with starting buffer and eluted with 50 mM NaOH. Small quantities of protein which seemed to contain the cytokinin-binding protein were eluted. The adsorbable fraction could be partially eluted from the column with synthetic cytokinins (BA, Kinetin), natural cytokinins (zeatin, zeatin riboside) and adenine. Zeatin was an inferior elutant for BA-Sepharose column, while BA, kinetin and zeatin riboside were highly efficient (Fig. 2). The adsorbable fraction was not eluted with 1 M NaCl and acetic acid. Protein components were responsible for the binding judging from the UV absorption curve and the electrophoresis profile obtained for the adsorbable fractions. (Fig. 3). The experiment illustrated in Fig. 4 was designed to confirm the binding activity of this eluted protein to BA. The adsorbable protein-linked Sepharose was prepared by the general method of CUATRECASAS and ANFINSEN (1970) and BA ($10^{-4}$ M) in 10 ml of 0.2 M phosphate buffer pH 7.2 was applied to the adsorbable protein-Sepharose column. After the non-adsorbable fraction was eluted with phosphate buffer, the adsorbable fraction was eluted with 50 mM NaOH. The cytokinin bioassay with

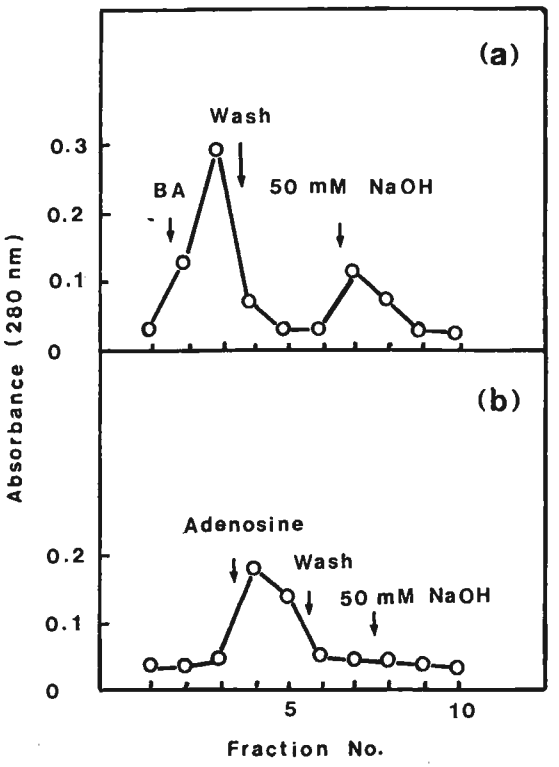


Fig. 4: Determination of the binding capacity of immobilized protein for BA (a) and adenosine (b).

Bestimmung der Bindungskapazität des immobilisierten Proteins für BA (a) und Adenosin (b).

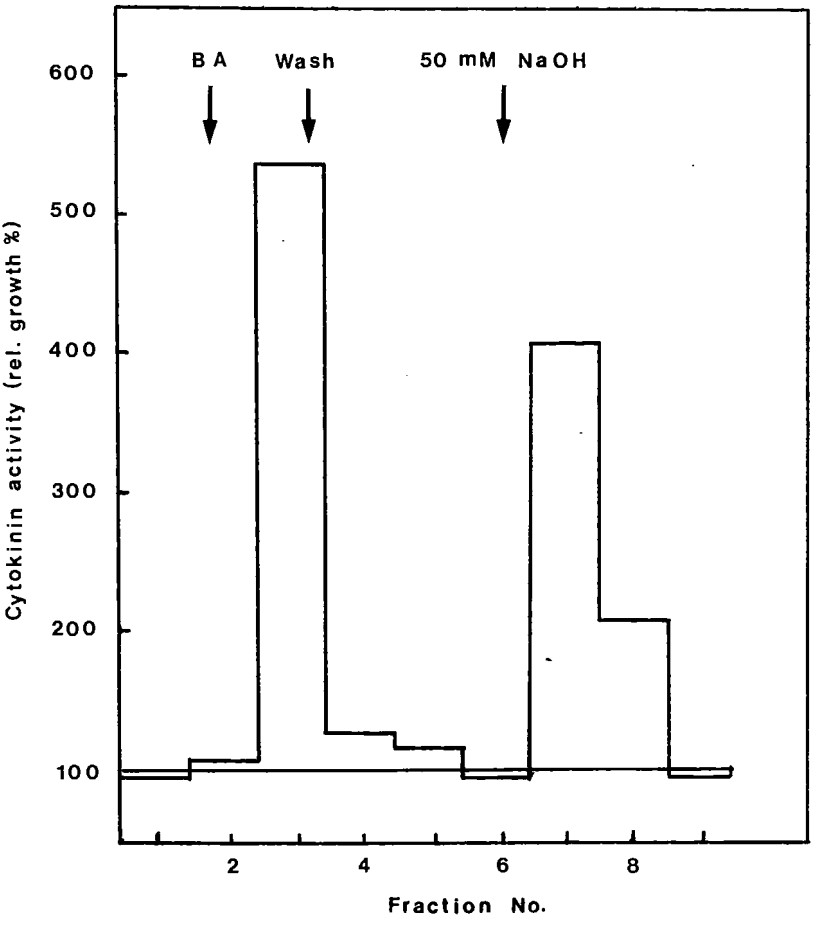


Fig. 5: Bioassay after chromatography on adsorbable protein-Sepharose.

Biologische Bestimmung nach Chromatographie an dem Komplex adsorbierbares Protein-Sepharose.

the UV absorption curve obtained for this adsorbable fraction indicated that BA combined to the affinity column. On the other hand, adenosine (no cytokinin activity)) was not retained by the column (Fig. 5). These results indicate the presence of the cytokinin-binding protein in grape berries.

LeJohn and Cameron (1973) and LeJohn (1975) reported that a calcium-binding glycoprotein isolated from fungi not only bound calcium but also cytokinin and auxin. In order to determine if the cytokinin-binding protein in grape berries is a glycoprotein, the binding activity of this protein to ConA was tested. ConA interacts specifically with $\alpha$-D-mannopryranosyl, $\alpha$-D-glycopyranosyl and $\alpha$-D-N-acetylglucosamine residues located at the terminal, non-reducing ends of the polysaccharide chain. The cytokinin-binding protein could be resolved into at least two fractions by affinity chromatography on ConA-Sepharose column. One of these fractions was eluted with $\alpha$-methyl-D-glycoside (Fig. 6). The glycoproteins were then isolated from grape berries with the ConA-Sepharose column, and the binding activity

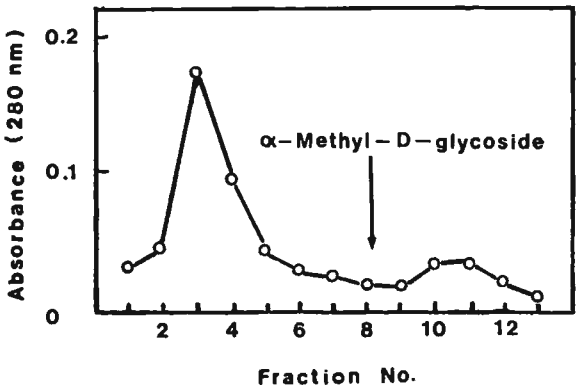


Fig. 6: ConA-Sepharose chromatography of the cytokinin-binding proteins.

ConA-Sepharose-Chromatographie der Cytokinin-bindenden Proteine.

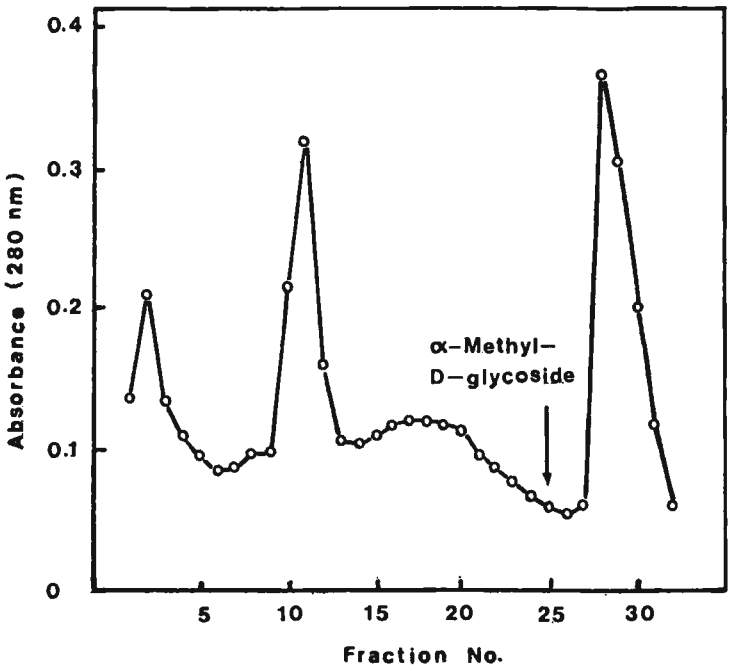


Fig. 7: Isolation of glycoproteins from grape berries by ConA-Sepharose chromatography (rechromatogram of glycoprotein fractions is shown).

Isolierung von Glykoproteinen aus Traubenbeeren mit Hilfe der ConA-Sepharose-Chromatographie (dargestellt ist das Rechromatogramm der Glykoproteinfraktionen).

between each glycoprotein and BA was examined. From grape berry extract, at least three kinds of glycoprotein were isolated (Fig. 7). The fraction which eluted with starting buffer was named fraction 1, and that which eluted with α-methyl-D-glycoside was named fraction 2. When each fraction was applied to the BA-Sepharose column, the bulk of the proteins were adsorbed to each column (Fig. 8). To confirm the binding activity of each fraction to BA the fraction-1 and the fraction-2-linked Sepharose were packed into columns (0.5 × 10 cm), and BA ($10^{-4}$ M) was layered onto each column. The UV absorption curve obtained from each eluted fraction coincided with that of the BA solution. As can be seen in Fig. 9, both columns retained BA, which indicates that fraction 1 and fraction 2 had binding activity to BA. The fraction 1 retained more BA than fraction 2. It is clear from the results that the cytokinin-binding protein in grape berries consists of at least three protein components, and two of these are glycoproteins which can bind to ConA. Although the mechanism of action of the cytokinin-binding protein is still unknown, Berridge *et al.* (1970) investigated the interaction of cytokinin with ribosomes isolated from Chinese cabbage leaves, and detected reversible binding of cytokinin to 83S ribosomes. Recently, Fox and Erion (1975) confirmed the result of Berridge *et al.* (1970) by showing BA bound of ribosomes and crude ribosome proteins of wheat germ and tobacco callus. Takegami and Yoshida (1977) isolated the cytokinin-binding protein from tobacco leaves by affinity chromatography and described that the cytokinin-binding protein bound to 40S ribosome subunits and its binding was stimu-

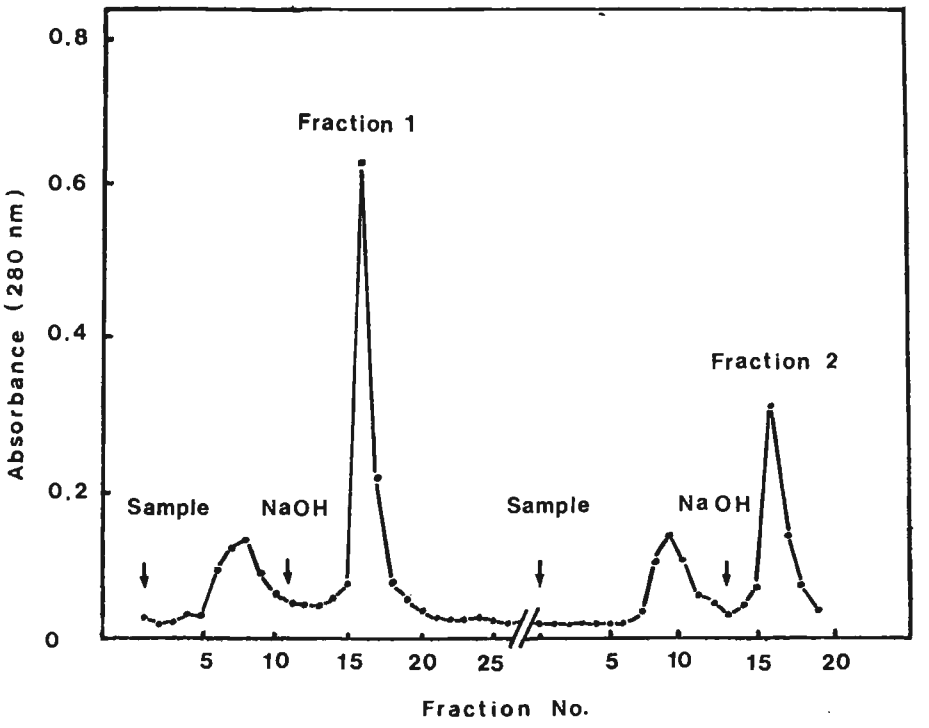


Fig. 8: BA-Sepharose chromatography of fraction 1 and fraction 2.
BA-Sepharose-Chromatographie von Fraktion 1 und Fraktion 2.

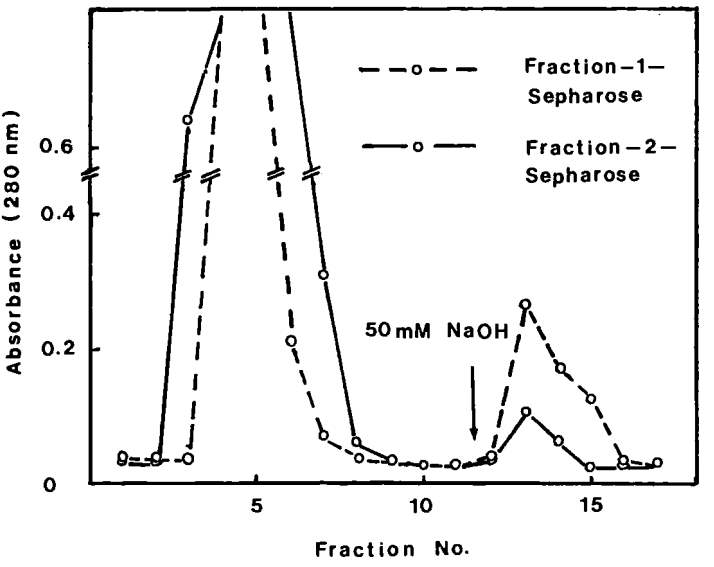


Fig. 9: Determination of the binding capacity of the fraction-1-Sepharose and the fraction-2-Sepharose for BA.

Bestimmung der Bindungskapazität von Fraktion-1-Sepharose und Fraktion-2-Sepharose für BA.

lated by BA. Accordingly, it is assumed that cytokinins, like animal hormones, react with some protein factor or cellular organelles to enhance RNA and protein synthesis. The cytokinin-binding protein in grape berries includes one more class of glycoprotein components and these glycoproteins bind to phytolectins like ConA. However, little information is available on the subcellular location of lectins in plants, lectin activity has been detected in various membrane fractions isolated from mung bean hypocotyls (BOWLES and KAUSS 1975). It is therefore possible that lectins play a role in the binding of the cytokinin-binding protein to ribosomes.

## Summary

A cytokinin-binding protein was isolated from grape berries (*Vitis labruscana* cv. Delaware) by affinity chromatography on benzyladenine-linked Sepharose 4B. The adsorbable protein was eluted with 50 mM NaOH from the BA-Sepharose column. This protein consisted of at least two glycoprotein components which exhibited high or low affinity to cytokinin and Concanavalin A, respectively.

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