AMINO ACIDS OF TWO INSECT VIRUSES*

by

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INTRODUCTION

KNIGHT[1] found that different strains of tobacco mosaic virus varied in amino acid composition and suggested that this was an underlying reason for the differences in their biological properties. In view of KNIGHT's findings it was of interest to determine whether analogous chemical variations occur in the highly specific insect viruses.

In the present paper, the investigation is reported of the polyhedral virus of the silkworm, *Bombyx mori* (L.), and the capsule virus of *Cacoecia murinana* (Hb.). In both cases the infectious agents are rod-shaped particles; these are enclosed in non-infectious polyhedral bodies and capsules respectively. Each polyhedron contains many virus rods whereas each capsule contains either one or two rods.

Little work has been done on the amino acid composition of insect viruses. Quantitative analyses have been made on the silkworm polyhedral protein (DESNUELLE AND CHANG[2,3] and DESNUELLE, CHANG AND FROMAGEOT[4]), which at that time was thought to be the active virus (BERGOLD[5]). Polyhedral protein is available in sufficient amounts for quantitative analysis, but quantitative work on purified active virus particles is limited by the minute amounts available. Hence, for the time being, qualitative analyses were made by paper chromatography (CONSDEN, GORDON AND MARTIN[6]).

MATERIALS AND METHODS

The viruses** were separated from their characteristic protein by dissolving in alkali and centrifuging (BERGOLD[7,8]). Six milligrams of *Bombyx mori* virus and 3 mg of *Cacoecia murinana* virus were obtained from 105 mg of polyhedra and capsules respectively.

For satisfactory chromatography of the dissolved polyhedral and capsular proteins it was necessary to dialyse the alkali from these proteins before hydrolysis. The dialysis was carried out in a revolving cellophane bag for one week at 1° C with 10 gallons of distilled water passing through the dialysing vessel.

Individual hydrolyses were done by refluxing the samples in an automatically controlled glycerine bath at 120° C with 200 times the sample weight of 6 N HCl twice distilled from glass (STEIN AND MOORE[9]). For each hydrolysis 3–5 mg of material was used.

Samples of 0.02 to 0.08 ml hydrolysate containing 100 to 400 μg hydrolysed material were applied to Whatman paper No. 1 in μl amounts, the paper being allowed to dry after each application. Hydrogen peroxide was added to oxidize any methionine, cysteine, or cystine present, thus giving better separation and identification of these amino acids. The papers were run by capillary ascent

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(WILLIAMS AND KIRBY[10]). A mixture of ethanol (60 parts), tertiary butanol (20 parts), ammonia (5 parts), and water (15 parts) was used for the first run; water-saturated phenol containing 0.002% 8-hydroxyquinoline in an atmosphere of ammonia was used for the second run. This combination resolved all the amino acids except leucine, isoleucine, and phenylalanine. These three were separated by a one-dimensional run in water-saturated benzyl alcohol (CONSDEN, GORDON AND MARTIN[6]).

Tryptophan was determined separately in unhydrolysed samples (GORDON AND MITCHELL[11]).

RESULTS AND DISCUSSION

Amino acid composition of acid hydrolysates of the two purified insect viruses and their enclosing proteins proved to be qualitatively similar (Table I). The following amino acids have been found in the acid hydrolysates of the two insect viruses and their corresponding polyhedral and capsular protein: cysteic acid, aspartic acid, glutamic acid, serine, threonine, alanine, tyrosine, methionine sulfone, histidine, lysine, arginine, proline, valine, leucine, isoleucine, phenylalanine, and glycine.

TABLE I

AMINO ACIDS FOUND IN VIRUS AND ENCLOSING PROTEINS FROM *Bombyx mori* (L.) AND FROM *Cacoecia murinana* (Hb.)

Amino acids	*B. mori* (L.)				*C. murinana* (Hb.)		
	Total polyhedra	Purified virus	Purified polyhedral protein	DESNUELLE *et al.*[2,3]	Total capsules	Purified virus	Purified capsular protein
No. of chromatograms	10	5	16		10	4	8
Alanine	×	×	×	4.4%	×	×	×
Arginine	×	×	×	5.6%	×	×	×
Aspartic Acid	×	×	×	—	×	×	×
Cyst(e)ine	×	×	×	0.5%	×	×	×
Glutamic acid	×	×	×	—	×	×	×
Glycine	×	×	×	—	×	×	×
Histidine	×	×	×	2.5%	×	×	×
Hydroxyproline	o	o	o	o	o	o	o
Isoleucine	×	×	×	—	×	×	×
Leucine	×	×	×	—	×	×	×
Lysine	×	×	×	10.6%	×	×	×
Methionine	×	×	×	3.3%	×	×	×
Phenylalanine	×	×	×	6.7%	×	×	×
Proline	×	×	×	—	×	×	×
Serine	×	×	×	—	×	×	×
Threonine	×	×	×	—	×	×	×
Tyrosine	×	×	×	9.6%	×	×	×
Valine	×	×	×	—	×	×	×
Tryptophan	×	?	3.5%	3.3%	×	?	3.0%

× present; o absent; — not reported; ? doubtful

Fig. 1 shows a representative amino acid chromatogram of the acid hydrolysate of purified silkworm polyhedral virus. Determinations of tryptophan in the unhydrolysed samples indicated one difference between the purified viruses and their inclusion proteins. The polyhedral and capsular proteins had 3.5% of this amino acid, whereas the viruses

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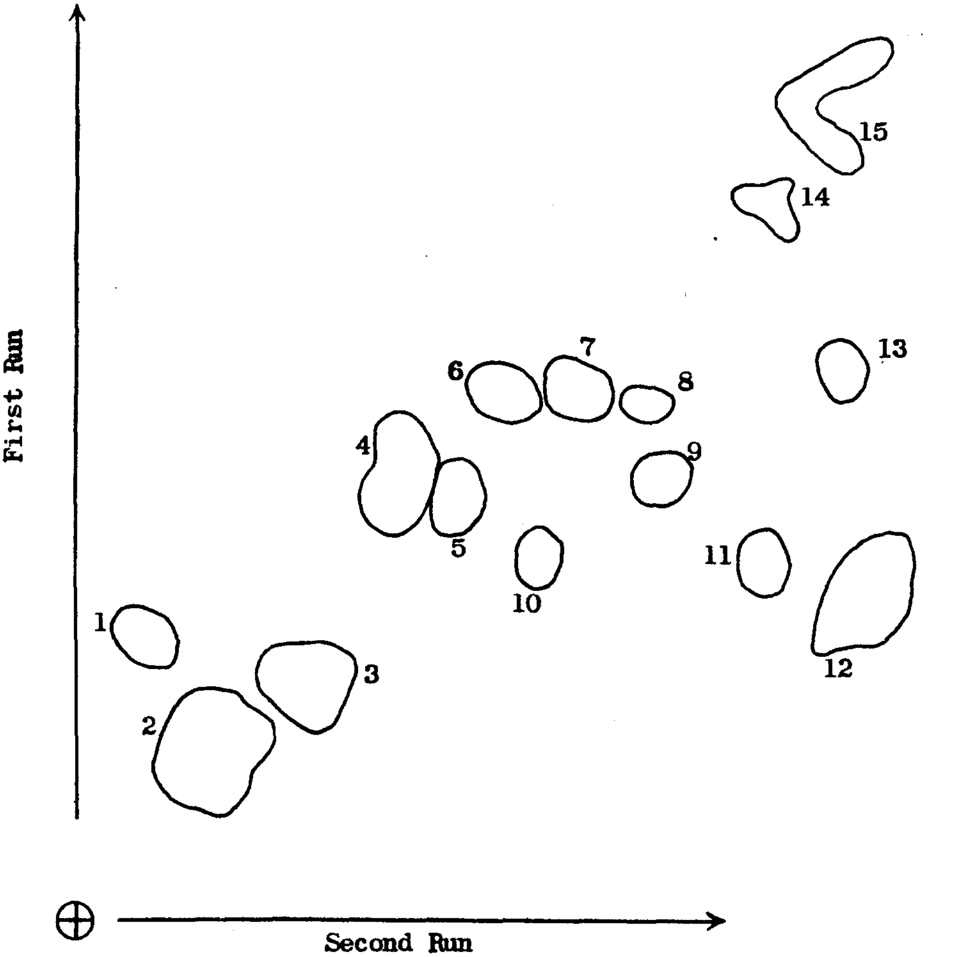

Fig. 1a. Two-dimensional chromatogram of the *Bombyx mori* virus hydrolysate. The hydrolysate was applied at the circle. The paper was run first with mixed alcohols and ammonia for 24 hours, then in water-saturated phenol with 0.002% 8-hydroxyquinoline in an atmosphere of ammonia for 24 hours. The following amino acids are visible: 1. cysteic acid, 2. aspartic acid, 3. glutamic acid, 4. serine, 5. glycine, 6. threonine, 7. alanine, 8. methionine sulfone, 9. histidine, 10. tyrosine, 11. lysine, 12. arginine, 13. proline, 14. valine, 15. leucine, isoleucine, and phenylalanine.

did not give the characteristic green fluorescence by this method, perhaps because of some interfering substance in the virus.

Table I includes the results of DESNUELLE *et al.*[2,3,4] with *Bombyx mori* "virus". DESNUELLE's material has since been shown to be chiefly the non-infectious polyhedral protein (BERGOLD[7]). The present results are in agreement with DESNUELLE *et al.* for those amino acids which they determined. In addition, aspartic acid, glutamic acid, glycine, proline, serine, threonine, valine, leucine, and isoleucine have been identified.

It should not be overlooked that the glycine in the viruses and their surrounding proteins may have been derived, wholly or in part, from the hydrolysis of the purine fraction of the nucleic acids rather than from the proteins themselves (MARKHAM AND SMITH[12]). This applies especially to the virus, since the nucleic acid content is about 14%

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Fig. 1b. Photograph of the original chromatogram showing the distribution of the amino acids illustrated diagramatically in Fig. 1a

(BERGOLD[7]). Quantitative determinations of both amino acids and nucleic acids in progress may clarify this point.

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SUMMARY

Acid hydrolysates of purified polyhedral virus of *Bombyx mori* and of purified capsule virus of *Cacoecia murinana* and their respective inclusion proteins were analysed by paper chromatography. The analyses showed qualitative similarity of amino acid composition of these two purified viruses and their polyhedral and capsular proteins. The following amino acids were found: cysteic acid, aspartic acid, glutamic acid, serine, threonine, alanine, tyrosine, methionine sulfone, histidine, lysine, arginine, proline, valine, leucine, isoleucine, phenylalanine, and glycine.

Separate quantitative determination of tryptophan showed that capsular and polyhedral proteins contained 3 and 3.5% respectively of this amino acid. The viruses, however, did not give the greenish fluorescence characteristic of tryptophan.

RÉSUMÉ

Les hydrolysates acides du virus purifié polyédral du *Bombyx mori* et du virus purifié capsulaire du *Cacoecia murinana* et leurs respectives protéines enfermées étaient analysées par l'emploi de la methode de la chromatographie sur papier. Les analyses se montraient similaires qualitatives de la composition des acides aminés de ces deux virus purifiés et leurs protéines polyédrales et capsulaires. Les suivants acides aminés s'étaient trouvés: acide cystéique, acide aspartique, acide glutamique, sérine, thréonine, alanine, tyrosine, méthionine sulfone, histidine, lysine, arginine, proline, valine, leucine, isoleucine, phénylalanine, et glycine.

La détermination quantitative separée du tryptophane montrait que les protéines capsulaires et polyédrales contenaient respectivement 3 et 3.5% de cet acide aminé. Les virus, cependant, ne donnaient pas le verdâtre de la fluorescence caractéristique du tryptophane.

ZUSAMMENFASSUNG

Säurehydrolysate von gereinigtem Polyedervirus von *Bombyx mori* und von gereinigtem Kapselvirus von *Cacoecia murinana* und deren zugehörige Einschlusskörper-Proteine wurden mittels Papierchromatographie analysiert. Die Analysen ergaben qualitative Ähnlichkeit der Aminosäuresusammensetzung der zwei gereinigten Viren und deren Polyeder- und Kapselproteine. Die folgenden Aminosäuren wurden gefunden: Cysteinsäure, Asparaginsäure, Glutaminsäure, Serin, Threonin, Alanin, Tyrosine, Methioninsulfon, Histidin, Lysin, Arginin, Prolin, Valin, Leucin, Isoleucin, Phenylalanin und Glycin.

Getrennte quantitative Bestimmüng von Tryptophan ergab, dass das Kapsel-und Polyederprotein 3 und 3.5% dieser Aminosäure enthält. Die Viren zeigten jedoch nicht die grüne Fluoreszenz, die für Tryptophan charakteristisch ist.

REFERENCES

[1] C. A. KNIGHT, *J. Biol. Chem.*, 171 (1947) 297.
[2] P. DESNUELLE AND C. T. CHANG, *Ann. Inst. Pasteur*, 69 (1943) 248.
[3] P. DESNUELLE AND C. T. CHANG, *Ann. Inst. Pasteur*, 71 (1945) 264.
[4] P. DESNUELLE, C. T. CHANG AND C. FROMAGEOT, *Ann. Inst. Pasteur*, 69 (1943) 75.
[5] G. H. BERGOLD AND G. SCHRAMM, *Biol. Zentr.*, 62 (1942) 105.
[6] R. CONSDEN, A. H. GORDON AND A. J. P. MARTIN, *Biochem. J.*, 38 (1944) 224.
[7] G. H. BERGOLD, *Z. Naturforsch.*, 2b (1947) 122.
[8] G. H. BERGOLD, *Z. Naturforsch.*, 3b (1948) 338.
[9] W. H. STEIN AND S. MOORE, *J. Biol. Chem.*, 176 (1948) 337.
[10] R. J. WILLIAMS AND HELEN KIRBY, *Science*, 107 (1948) 481.
[11] M. GORDON AND H. K. MITCHELL, *J. Biol. Chem.*, 180 (1949) 1065.
[12] R. MARKHAM AND J. D. SMITH, *Nature*, 164 (1949) 1052.

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