Table 1. Capacity of different plaque-purified Shiga-like toxin–converting phages to form plaques on various *E. coli* hosts. Phage stocks were diluted 10^{-2} and plated in a soft agar overlay. The designations C600(H-19J), C600(H-19A), C600(933J), C600(H-19B), and C600(933W) indicate *E. coli* K-12 substrain C600 lysogenized with the phage H-19J, H-19A, 933J, H-19B, or 933W, respectively. Symbols: (+) formation of a large number (>10^4) of plaques on the host lawn after overnight incubation, (−) no plaques, and (*) less than 50 plaques.

Host strain of *E. coli*	Phage				
	H-19J	H-19A	933J	H-19B	933W
C600	+	+	+	+	+
C600(H-19J)	−	−	−	−	+
C600(H-19A)	−	−	−	−	+
C600(933J)	−	−	−	−	+
C600(H-19B)	+	+	+	−	+
C600(933W)	+	+	*	+	−

19B are converting phages that determine production of high titers of Shiga-like toxin (*2*).

2) *Escherichia coli* 933 also contains two different Shiga-like toxin–converting phages. Phage 933J is closely related to phage H-19A/J, as judged by similarities in morphology, virion proteins, restriction endonuclease fragments of genomic DNA, DNA-DNA hybridization in Southern blots, immunity specificity, and heat stability. Nevertheless, the restriction of plaquing of phage 933J but not H-19A/J on lawns of C600(933W) demonstrates that they are not identical.

3) A family of Shiga-like toxin–converting phages exists in nature. The distribution of members of this family and the extent of relatedness among them is not fully established and requires further study. We recently observed that ^{32}P-labeled DNA from phage H-19J hybridizes in Southern blots with specific restriction fragments of genomic DNA from selected strains of *E. coli* 0145, 0111, and 026 associated with diarrheal disease in humans. This finding suggests that phages belonging to the family of Shiga-like toxin–converting phages are also present in these strains of *E. coli*.

How the converting phages from *E. coli* 933 and H-19 control production of Shiga-like toxin has not yet been established. Because *E. coli* K-12 makes low levels of Shiga-like toxin (*6, 19*), it appears that the genome of *E. coli* K-12 contains the toxin structural gene or genes. However, lysogenization of *E. coli* K-12 with a toxin-converting phage results in a dramatic increase (up to 10,000-fold) in the amount of Shiga-like toxin produced. Converting phages could contain either the toxin structural genes or regulatory elements that act on toxin structural genes already present in the host bacterium. To determine which of these possibilities is correct, it will be necessary to isolate and characterize the toxin-converting genes from phage DNA by recombinant DNA techniques. The potential medical relevance of Shiga-like toxin–converting phages is suggested by the strong correlation between production of high levels of Shiga-like toxin and the ability of *E. coli* to produce bloody diarrhea or hemorrhagic colitis in humans (*19*), the presence of converting phages in such clinical isolates of *E. coli*, and the ability of the converting phages to determine high levels of toxin production in *E. coli* K-12 (*21*).

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21. After our manuscript was submitted for publication, H. R. Smith, N. P. Day, S. M. Scotland, J. R. Gross, and B. Rowe [*Lancet* **1984-II**, 242 (1984)] also reported the existence of Shiga-like toxin–converting phages in *E. coli* strains of serogroup 0157.
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Molecular Weight Determinations of Proteins by Californium Plasma Desorption Mass Spectrometry

Abstract. *The plasma desorption mass spectrometry method is used to determine the molecular weights of larger molecules than before, to determine the molecular weights of proteins and peptides in mixtures, and to monitor protein modification reactions. Proteins up to molecular weight 25,000 can now be studied with a mass spectrometric technique. Protein-peptide mixtures that could not be resolved with conventional techniques were successfully analyzed by this technique. The precision of the method is good enough to permit one to follow the different steps in the conversion of porcine insulin to human insulin.*

Molecular weight (MW) determination is normally one of the first steps in characterizing unknown protein molecules. The present commonly used techniques, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and gel chromatography, allow a precision of 10 to 20 percent, and the MW determination may be influenced by other properties of the molecule such as conformational state and hydrophobicity. For smaller molecules mass spectrometry (MS) is the method of choice. In the last few years methods based on

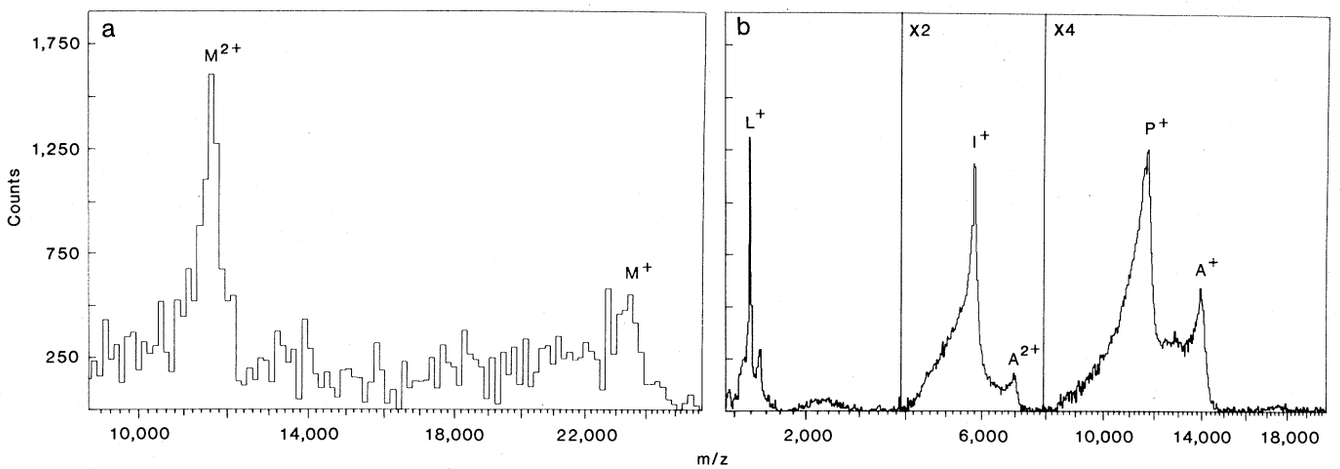

Fig. 1. (a) Positive-ion mass spectrum of porcine trypsin (m/z, mass-to-charge ratio). The molecular weight determined from the centroids of the M^+ and M^{2+} peaks is 23,406 ± 140 (calculated MW is 23,463). (b) Positive-ion mass spectrum of a mixture of LHRH (L), porcine insulin (I), pancreatic spasmolytic peptide (P), and phospholipase A2 (A).

particle bombardment of solid and liquid surfaces have allowed analysis of considerably larger molecules. Thus ^{252}Cf plasma desorption mass spectrometry (PDMS) (*1*) has been used to study peptides and proteins in the MW range 1,000 to 14,000 (*2–5*). Also fast atom bombardment (*6*) has been applied to protein molecules such as bovine insulin (*7*) and bovine proinsulin (*8*).

The PDMS technique originally suggested by Macfarlane and his co-workers (*1*) and used in this study is based on ultrafast ion impact on solid samples. The primary ions are fission fragments from a ^{252}Cf source. The features of the PDMS spectra are described in detail by Sundqvist *et al.* (*5*).

We have demonstrated that the technique can be applied to molecules up to MW 14,000 (*5*). In Fig. 1a the positive ion spectrum of porcine trypsin (MW 23,463) is shown. This is, to our knowledge, the largest monomeric protein ion observed in the gas phase thus far. The peak corresponding to doubly charged ions is about three times as intense as that of singly charged ions. The abundance of doubly charged ions increases with the increasing size of the protein studied; by extrapolation from earlier results (*5*), a protein of this dimension is expected to show this behavior. When comparing the intensities, one should remember that the doubly charged ions have been accelerated to twice the energy of the singly charged ions and therefore are detected with higher efficiency.

We have tested the possibility of determining the MW of proteins and peptides in mixtures on several samples. In Fig. 1b the positive-ion mass spectrum of a 1:1:1:1 mixture (on a molar basis) of luteinizing hormone releasing hormone (LHRH) (MW 1,182), porcine insulin (MW 5,778), pancreatic spasmolytic peptide (PSP) (MW 11,711), and phospholipase A2 (MW 13,980) is shown. The four components of the mixture can be easily identified, and the MW's were determined to 1,183 ± 0.5, 5,778 ± 2, 11,705 ± 15, and 13,963 ± 20, respectively. Multiply charged ions of the components are seen in the spectra, whereas mixed clusters, which would be a complication for the interpretation of the spectra, are not observed. The same mixture has been analyzed with SDS-PAGE and high-performance gel-permeation chromatography. The PSP and phospholipase A2 could not be separated with the use of either of these techniques, and LHRH was lost in the SDS-PAGE procedure.

In order to follow MW changes in protein modification reactions, we examined the preparation of semisynthetic human insulin based on porcine insulin (Fig. 2). The C-terminal alanine residue of the B-chain of porcine insulin (Fig. 2a) is cleaved off, resulting in des-ala insulin. The success of this operation is verified by the PDMS spectrum in Fig. 2b. In the next step a threonine residue is coupled to the C-terminal of the B-chain of des-ala insulin, resulting in a semisynthetic human insulin (Fig. 2c). The synthesis

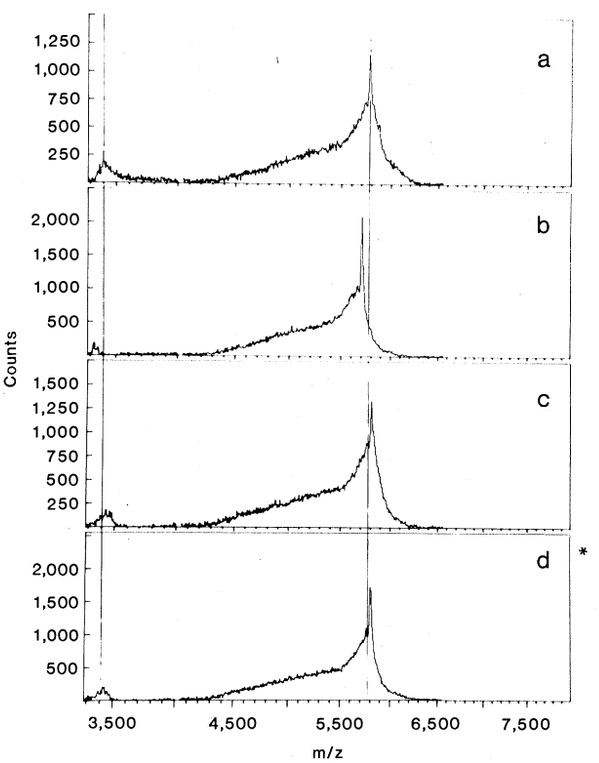

Fig. 2. The high mass region of porcine insulin (a), B des-ala insulin (b), semisynthetic human insulin (c), and human insulin (d), showing the molecular-ion and B-chain peaks. The mass differences observed for the molecular-ion peaks relative to that of porcine insulin are −71.5 ± 3.0, +31.9 ± 3.0, and +27.4 ± 3.0. The corresponding calculated values are −71, +30, and +30, respectively.

can be checked by comparison with the spectrum of authentic human insulin (Fig. 2d). The spectra show both the B-chain and the molecular-ion region of the insulins, and the masses determined give the mass differences expected for the reactions on the assumption that the molecule is in its protonated form.

The sample amount used in the present study was ~10 μg of each protein. However, in the case of insulin, tests have indicated a distinct molecular-ion peak with as small an amount as 10 ng, that is, 2 pmol. The precision of the method is to some extent a function of the purity of the sample because an intense quasi-molecular ion peak gives a more accurate centroid determination. A high content of low-MW components seems to give a low signal-to-noise ratio. At present, the precision in the MW determination of components in the region up to MW 15,000 is better than 0.2 percent. This is approximately two orders of magnitude better than the precision of SDS-PAGE or high-performance gel permeation chromatography.

Our results demonstrate that PDMS is a viable method for MW determination up to 25,000. The precision obtained is sufficient to monitor even minor protein modifications. Therefore, possible applications are control of products from genetic engineering experiments and protein modification reactions in vivo and in vitro. We have also shown that the MW of components in a mixture can be determined and this for mixtures which are difficult or impossible to analyze with conventional techniques. The precision of the MW determination also makes PDMS useful for verifying sequence analysis.

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Human Ornithine Transcarbamylase Locus Mapped to Band Xp21.1 Near the Duchenne Muscular Dystrophy Locus

Abstract. *The gene for the mitochondrial enzyme ornithine transcarbamylase was mapped to the short arm of the X chromosome by in situ hybridization experiments, with DNA complementary to the human ornithine transcarbamylase gene used as a probe. A series of cell lines with X chromosome abnormalities was used to localize the gene to band Xp21.1. Because the gene maps near the Duchenne muscular dystrophy locus, the ornithine transcarbamylase probe may be useful in carrier detection and prenatal diagnosis of Duchenne muscular dystrophy as well as of ornithine transcarbamylase deficiency.*

Ornithine transcarbamylase (OTC), a trimeric enzyme composed of identical subunits, catalyzes the second step of the urea cycle. Although active in the mitochondrion, OTC is encoded by nuclear DNA and is X-linked in humans (*1*) and the mouse (*2*). Male infants, hemizygous for OTC deficiency, suffer from ammonia intoxication and protein intolerance; they often do not survive the

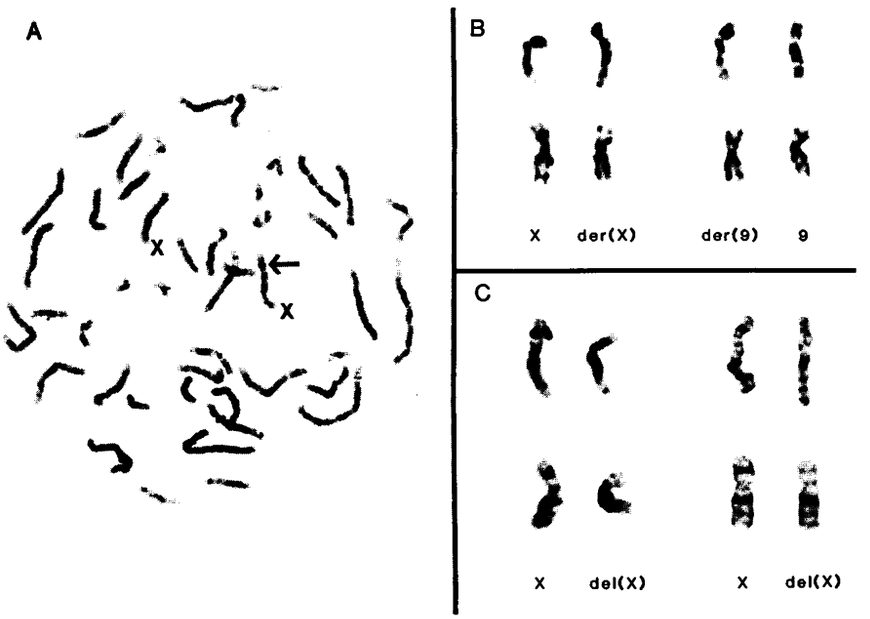

Fig. 1. Wright-stained chromosomes after in situ hybridization with plasmid pHO-731. Hybridizations followed the method of Harper and Saunders (*20*). The 1.5-kb Pst I fragment of OTC complementary DNA cloned into pBR332 was labeled with the tritiated triphosphates of deoxyadenosine, deoxycytosine, and thymidine, by nick translation to a specific activity 1.6×10^7 cpm/μg. The probe was hybridized to chromosome spreads overnight at a concentration of 25 ng/ml at 37°C. Photographic emulsion was applied to the slides, which were then exposed for 9 to 13 days. Chromosomes were stained with quinacrine mustard dihydrochloride (*21*) and photographed with a fluorescence microscope. The slides were then treated with Wright stain and, under bright light, a second photograph was taken of the cells previously chosen for analysis. (A) Representative normal human metaphase spread (46,XX) with a silver grain over the short arm of an X chromosome in the region of band p21 (arrow). (B) Partial karyotypes of two cells from lymphoblastoid cell line GM 6007 [46,X,t(X;9)(p21)(p22)], illustrating typical labeling of the der(X) and the X chromosomes in the region of band Xp21. (C) On the left, X chromosomes from two GM 7773 [46.X, del(X)(p21.1p21.3)] cells, showing labeling of the normal X short arm but not of the deleted X short arm. On the right, for comparison, are the X chromosomes from two GTG-banded cells that were not hybridized with the probe.