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HLA membrane antigens: Sequencing by intrinsic radioactivity*

(HLA antigens A1,B8/detergent-solubilized HLA antigens/antiserum to beta$_2$-microglobulin/immunoadsorption/amino acid sequence)

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ABSTRACT **Radiochemical sequence data are presented for the amino termini of mixed HLA antigens A1 and B8 isolated from a human lymphoblastoid cell line. Cells were labeled intrinsically in small-scale tissue culture with ^{14}C-labeled amino acids and [^{14}C]pyruvate. The specific activities obtained were sufficiently high and uniform to permit direct radiochemical sequencing of the antigens. HLA antigens were isolated by adsorption to a solid-phase anti-β_2-microglobulin immunoadsorbent followed by electrophoresis. The method should be generally applicable and useful for sequencing all parts of the molecule.**

Many laboratories are directing their efforts towards determining the primary structures of membrane proteins and cell surface antigens. The desired proteins usually constitute only a small fraction of the total cellular protein, making isolation of sufficient material difficult. Three general approaches to the problem are being exploited: Terhorst *et al.* (1) have successfully isolated and sequenced human HLA antigens using tissue cultures of the order of several hundreds of liters and conventional biochemical techniques. Another approach is to increase sequencer sensitivity. Bridgen and coworkers (2) have used phenyliso[^{35}S]thiocyanate (3) to sequence human HLA antigens purified by lectin affinity chromatography and immunoadsorption. Cultures of the order of tens of liters were required. A third approach to the problem employs radioisotopes biosynthetically incorporated throughout the whole of the molecules of interest. The procedure has the advantages that only small tissue cultures (20–100 ml) are needed, and that the results obtained are insensitive to the presence of unlabeled contaminants such as extrinsic antibody; the method is applicable to all parts of the molecule.

Intrinsic labeling of proteins for radioisotope sequencing purposes was successfully used by Jacobs *et al.* (4), who labeled their protein with single amino acids. Silver and Hood (5), Henning *et al.* (6), Vitetta *et al.* (7), and Ewenstein *et al.* (8) have used this approach for the study of H-2 antigens purified by precipitation with alloantisera. The single amino acid method requires that the labeling be repeated many times with different radioactive precursors. It also requires a subsequent analysis when a given precursor labels more than one amino acid. Our procedure of intrinsic radioisotope sequencing (9), in which all amino acids in the protein are labeled, requires in principle only a single radiolabeling. However, it necessitates a quantitative analytical step to identify the labeled amino acid derivatives obtained during the sequencing.

For the present study, we isolated mixed HLA antigens with an anti-β_2-microglobulin (β_2m) immunoadsorbent, then separated the HLA antigenic polypeptide chain from β_2m by analytical-scale polyacrylamide gel electrophoresis. The procedure has yielded useful sequence data on HLA antigens from tissue cultures as small as 20 ml with an acceptable expenditure of radioisotopes (less than 500 μCi) and of labor. The method appears to be generally applicable to any protein that can be biosynthetically labeled in culture and for which a specific purification method (not necessarily an immunoadsorbent) is available.

MATERIALS AND METHODS

Cells. The source of histocompatibility antigens was human lymphoblastoid line TLCL-19 (HLA-A1,B8) developed by W. Leibold, and given to us by R. Gatti. Cultures were maintained in stationary bottles under an atmosphere of 10% CO_2 in air at density of 1 to 5 $\times$ 10^5/ml. The medium was RPMI 1640 supplemented with 15% fetal calf serum.

Labeling. The medium used for uniform labeling was essentially RPMI 1640 supplemented with 5% fetal calf serum (undialyzed) and 0.1% pluronic acid; the concentrations of unlabeled and ^{14}C-labeled amino acids and of [^{14}C]pyruvate were adjusted to provide an optimal specific activity ratio of amino acids in isolated proteins (details under *Results*). Media for labeling with one or a few essential amino acids were RPMI 1640 in which the total concentration of the labeling amino acid was typically 10% of normal; all other amino acids were present at their usual levels. For labeling, 20 to 200 $\times$ 10^6 viable lymphoblastoid cells were centrifuged at 300 $\times$ *g* for 10 min, washed in radiolabeling medium made up as usual, but with unlabeled amino acids only, then resuspended at a concentration of 4 $\times$ 10^6 cells per ml in labeling medium with radioactive compounds. The cells were incubated with gentle stirring for 16 hr, harvested by low-speed centrifugation, and washed once in ice-cold phosphate-buffered saline.

Extraction. The washed cells were resuspended gently in 10 ml of ice-cold solubilizing buffer (0.14 M NaCl, 0.5% Triton X-100, 0.01 M Tris·HCl, pH 7.5) with 1% (vol/vol) Trasylol (FBA Pharmaceuticals, Inc., New York, N.Y.). The suspension was kept on ice for 20 min with gentle swirling at 5-min intervals. After extraction, the mixture was centrifuged at 35,000 $\times$ *g* for 15 min and the pellet was discarded.

Immunoadsorption. Purified bovine antibody to human β_2m was prepared and coupled to Sepharose 4B beads as previously described (10). The immunoadsorbent beads (capacity about 1 μg of β_2m per μl) were washed before use once with fetal calf serum (neat), then twice with solubilizing buffer containing 1% fetal calf serum (vol/vol). HLA antigens and β_2m were adsorbed from the detergent extract by several successive treatments with 10 μl of anti-β_2m beads. Adsorption was at 4°, in siliconized centrifuge tubes; after slow stirring for 1 hr, beads were removed by low-speed centrifugation and washed six times with solubilizing buffer containing 1% fetal calf serum (vol/vol), then suspended in an equal volume of two times concentrated sample buffer for electrophoresis (11).

Abbreviations: $NaDodSO_4$, sodium dodecyl sulfate; β_2m, β_2-microglobulin.

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Table 1. Radioactive composition of labeling medium and radioactive output in β_2m isolated from labeled TLCL-19 cells

Amino acid	Specific activity (Ci/mol)	Concentration (mM)	Relative specific activity, Leu = 1	Relative specific activity in isolated β_2m, Leu = 1
Ala	27.5	0.056	0.923	1.458
Arg	28.8	0.047	0.965	1.951
Asn	11.0	0.068	0.369	0.507
Asp	40.9	0.044	1.372	
Cys	0.0	0.088	0.0	—
Gln	17.5	0.096	0.588	0.650
Glu	25.3	0.099	0.848	
Gly	9.6	0.042	0.322	0.455
His	31.0	0.032	1.040	1.108
Ile	12.5	0.040	0.419	0.571
Leu	29.8	0.047	1.000	1.000
Lys	30.6	0.020	1.027	0.654
Met	33.1	0.015	1.11	—
Phe	37.6	0.021	1.262	0.970
Pro	23.9	0.063	0.802	0.881
Ser	9.3	0.313	0.312	0.745
Thr	20.4	0.0245	0.685	0.604
Trp	0.0	0.024	0.000	—
Tyr	45.3	0.0088	1.520	0.282
Val	33.4	0.036	1.121	1.210
Prv	8.86	0.706	0.297	—

Only the amino acid and pyruvate components are specified; all other components are as in RPMI 1640. Input amino acids and pyruvate (Prv) were uniformly labeled with ^{14}C, except for methionine, which was labeled with ^{35}S only.

Electrophoresis. To monitor the progress of adsorption, analytical disc gel electrophoresis was performed on a small portion of each adsorbate. Polyacrylamide gels (15% acrylamide, 0.2% methylenebisacrylamide, 0.6 × 10 cm) containing sodium dodecyl sulfate ($NaDodSO_4$) were prepared and run as described by Laemmli (11). The gels were fractionated into counting vials with a Gilson model B100 GMA-GCB gel fractionator; radioactivity was measured by liquid scintillation counting. The remaining portions of those adsorbates that showed a good yield were pooled and subjected to preparative $NaDodSO_4$ electrophoresis on standard sized gels (0.6 × 10 or 20 cm) that had been dialyzed against lower gel buffer.

These gels were fractionated as described above; crushed gel fractions were rocked overnight in 0.01 M NH_4HCO_3, 0.1% $NaDodSO_4$ containing 100 μg/ml of egg white lysozyme. Gel fragments were removed by low-speed centrifugation; peaks of radioactivity from the supernatants were pooled, dialyzed briefly against 0.01 M NH_4HCO_3, and lyophilized.

Sequencing. The presumptive HLA peak was subjected to automated Edman degradation in an Edman–Begg sequenator (12) (Illinois Tool Works, Illitron Div.) using the procedures for radioactive sequencing described by McKean *et al.* (9). Tritium-labeled ribonuclease T1 was used as an internal standard to monitor sequenator efficiency (13). The thiazolinones were identified by amino acid analysis after back hydrolysis with either HI or NaOH plus sodium dithionite (9, 14), using the Durrum D-500 amino acid analyzer as modified by McKean *et al.* (9). The two procedures together allow identification and quantitation of all amino acids except cysteine. In one experiment, a portion of the thiazolinones was converted to phenylthiohydantoins by heating to 80° for 10 min in 1 M HCl; the phenylthiohydantoin derivatives were analyzed by thin-layer chromatography and autoradiography.

Specific Activity Determination. The radioactive β_2m recovered from $NaDodSO_4$ electrophoresis was hydrolyzed for 24 hr at 127° in constant-boiling HCl. The hydrolysate was analyzed for radioactive amino acids as described for back-hydrolysates (9). The relative specific activities of the amino acids were estimated from the known composition of human β_2m (15).

RESULTS

Labeling. Table 1 presents the amino acid composition of the labeling medium used in a successful experiment, together with the relative specific activities of input amino acids and their output in isolated, radiolabeled β_2m. This mixture represents a compromise between high specific activity in the product, relatively uniform specific activities among the incorporated amino acids, and avoidance of the total depletion of any one amino acid. Some observations which may aid others are: (*a*) Pyruvate aids in the labeling of alanine, aspartic and glutamic acids, and amides. (*b*) Serine labels both glycine and serine. (*c*) The specific activities of arginine and histidine are deliberately set higher than the norm to compensate for analytical problems. (*d*) The starting concentrations of leucine and isoleucine must be at least 5 μg/ml.

In the labeling experiment used to obtain the data shown in Fig. 2, 1.6×10^8 cells were incubated in 40 ml of the labeling medium. Thirty percent of the starting radioactivity was depleted from the medium in 16 hr, while the final viability was greater than 85%. About 2.6×10^8 cpm were incorporated into cellular trichloroacetic-acid-insoluble materials; from this, about 9×10^5 cpm (0.35%) were recovered in purified HLA antigens.

Purification. The use of several sequential adsorptions allows recovery of most of the HLA antigens and β_2m while minimizing a wasteful excess of immunoadsorbent, which would increase the background. This is illustrated by Fig. 1, which shows data from five successive adsorptions with Sepharose-anti-β_2m from a radioactive lymphoblastoid cell lysate (the last two adsorbates were pooled before electrophoresis). The first adsorption (Fig. 1A) reveals only two major radioactive peaks; recovery of these declines in the second and third adsorptions as shown in Fig. 1B and C, until essentially only background is found in the fourth and fifth adsorbates (Fig. 1D). The faster of the two peaks is β_2m, as we have shown by NH_2-terminal sequence analysis (unpublished); its position on the gel corresponds to that expected for a protein having a molecular weight of 12,000. The other peak (HLA heavy chain) is at a position corresponding to a molecular weight between 40,000 and 50,000. Recovery by elution from the crushed gels was greater than 80% of the radioactivity from each of the two major peaks.

Sequencing. Results from one quantitative sequenator run with analysis by back hydrolysis with HI are presented in Fig. 2. Yields have been corrected for background and carryover essentially as detailed by Smithies *et al.* (14). The data for the HLA antigen sequences are shown without (Fig. 2A) and with (Fig. 2B) correction for specific activities of each residue derived from the analysis of concurrently isolated β_2m. These corrections change the apparent yield of some residues (e.g., tyrosine at positions 7 and 27), but the sequence to residue 22 can be easily deduced from the raw data.

Sequencer samples from residue 21 on showed a consistently

Table 2. NH_2-Terminal sequences of TLCL-19 HLA chains

Run	Label	Residue number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
I (HI)	Uniform	G	&	H	&	—	R	Y	F	F/B	T	&	V	&	R	P	G	R	G	Z	P	P/R	F	I	—	V/&	—	Y
II (NaOH)	Uniform	G	—	—	—	M	R	Y	F	F/D	—	—	M/V	—	—	P	G	R										
II (TLC)	Uniform	G	—	—	—	M	—	Y	F	F/D	—	A	M/V	—	—	—	G	—	G	E								
III (HI)	Uniform	G	&	H	&	—	R	Y	F	F/B	T	&	V	&	R													
IV	[^{35}S]M; [^{3}H]F	—	—	—	—	M	—	—	F	F	—	—	—	—	—	—	—	—	—	—	—	—	F	—	—	—	—	—
V	[^{35}S]C; [^{3}H]L,Y,F	—	—	—	—	—	—	Y	F	F	—	—	—	—	—	—	—	—	—	—	—	—	F	—	—	—	—	Y
VI	[^{14}C]H	—	—	H	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
VII	[^{14}C]W	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Deduced sequence		G	S	H	S	M	R	Y	F	F/D	T	A	V/M	&	R	P	G	R	G	E	P	P/R	F	I	—	V/&	—	Y

Several sequencer runs (identified by Roman numerals) were performed on radiolabeled HLA chains as described in the *text*. The sequences deduced from these data are summarized in the last line of the table. Residues in boldface type have been observed by us in at least two runs; residues seen only once are in normal type. Underlined residues have been reported at indicated positions in HLA chains by other workers. & = serine or alanine; TLC = thin-layer chromatography.

higher background than those from the first 20 positions. We suspect that this is a consequence of their having been hydrolyzed in a second batch several days after the first 20 residues. We do not regard our assignments of the residues from 21 to 27 as secure, except where they have been confirmed by subsequent analyses. Radioactivity eluting in the histidine region was particularly high and erratic after residue 21; similar problems have been encountered in the sequence analysis of other ^{14}C-labeled proteins when HI hydrolysis was used (9). For this reason, we deliberately raised the specific activity of histidine well above that of other amino acids.

Several additional sequence runs were performed (Table 2.) Two of these were of uniformly radiolabeled HLA antigens (II and III, Table 2); butyl chloride extracts from one of these runs (II) were analyzed both by thin-layer chromatography of the phenylthiohydantoin derivatives and by amino acid analysis after back hydrolysis with NaOH–dithionite. Several runs were also performed on material labeled with specific amino acids in various combinations; in these cases only amino acids known from our previous work not to label other residues were used, so that data could be obtained directly from double-label counting of the butyl chloride extracts.

Serine was distinguished from alanine by specific activity, by NaOH hydrolysis (14), and by thin-layer chromatography; we cannot exclude serine at position 11. Separate labelings with cysteine and tryptophan documented the absence of these residues. Histidine, methionine, phenylalanine, and tyrosine were all confirmed by individual labeling experiments. In labeling IV (Table 2), methionine was found at positions 5, 12 (½ yield), and 45; phenylalanine occurred at position 8, 9 (½ yield), 22, 33, and 36.

Aspartic acid and phenylalanine appeared in nearly identical

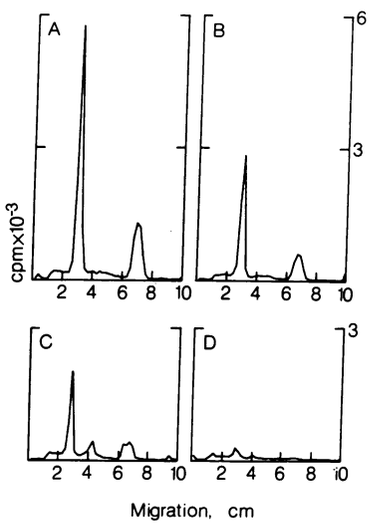

FIG. 1. NaDodSO$_4$-polyacrylamide gel electrophoresis of five successive immunoadsorptions to anti-β_2m. Radiolabeling of TLCL-19 lymphoblastoid cells, extraction, and adsorption were as described in the *text*. Two percent of each adsorbate was loaded onto a 0.6 × 10 cm polyacrylamide gel; electrophoresis was as described (11). Gels were crushed as 2 mm fractions on a Gilson model B-100/GMA-GCB gel fractionator and counted in a liquid scintillation counter. (A) first adsorption; (B) second adsorption; (C) third adsorption; (D) pool of fourth and fifth adsorptions.

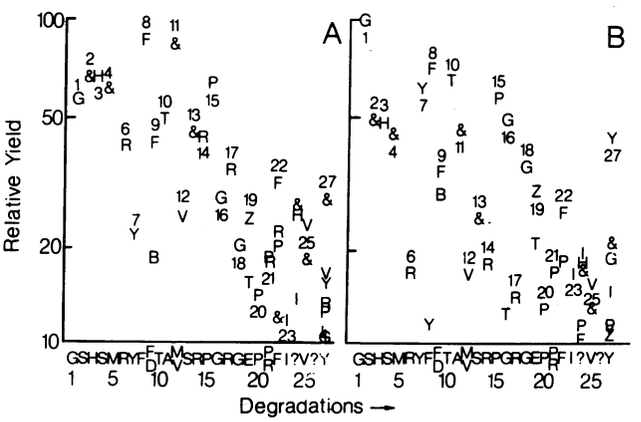

FIG. 2. Relative yield plotted on a logarithmic scale against step number for the NH_2-terminus of mixed HLA-antigens from TLCL-19. Data were plotted essentially as in Smithies *et al.* (14). The standard one-letter symbols (16) for amino acids are used, along with & for serine or alanine. (A) Data corrected for carryover and background only; (B) additional correction for specific activities. The actual yield at residue no. 1 (G) was 619 cpm; total radioactivity loaded was 900,000 cpm.

Table 3. NH_2-Terminal sequences of HLA antigens

Antigen(s)	Ref.	Residue number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
A1 + B8	This paper	Gly	Ser	His	Ser	Met	Arg	Tyr	Phe	Phe/Asp	Thr	Ala	Met/Val	Ser	Arg	Pro	Gly	Arg	Gly	Glu	Pro	Pro/Arg	Phe	Ile	—	Val/Ser	—	Tyr
A2	1	Gly	Ser	—	Ser	Met	Arg	Tyr	Phe	Phe	Thr	Ser	Val	Ser	Arg	Pro	Gly	—	Gly	Glu	—	-	—	Phe	Ile	Ala	Val	—	Tyr*
B7	1	Gly	Ser	—	Ser	Met	Arg	Tyr	Phe	Tyr	Thr	Ser	Val	Ser	Arg	Pro	Gly	—	Gly	Glu	—	-	—	Phe	Ile	—	Val	—	Tyr*
B7 + B12	1	Gly	Ser	—	Ser	Met	Arg/Val	Tyr	Phe	Tyr	Thr	Ala	Val	Ser	Arg	Pro	Gly	—	Gly	Glu	—	-	—	Phe	Ile	Ala	Val	—	Tyr*
A1 + A2 + B8 + B13	2	—	Ser	—	Ser	Met	Arg	Tyr	Phe/Tyr	Phe/Tyr	Thr/Ser	Ser/Ala	Val	Ser/Ala	Arg	Pro	Gly	—	-	—	-	—	-	—	-	—	-.	—

* Personal communication from J. L. Strominger.

yield (½) at position 9; valine was also found in ½ yield at position 12 (Fig. 2B). Together with the results of labeling IV, these data provide positive evidence for heterogeneity at these positions.

DISCUSSION

We have shown that radioactive amino acids can be incorporated with sufficiently uniform specific activities to permit protein sequence determination by radioactivity alone. The data presented in Fig. 2A would have permitted the correct assignment of 20 of the first 21 residues (within the constraints of an HI hydrolysis.) Our unfortunate delay in analyzing samples after residue 20 makes the later data less secure; however, we decided not to repeat the long NH_2-terminal runs with [^{14}C]HLA chains, since the material is much more usefully employed for generating peptides that can be used in further sequence studies.

A comparison of the mixed sequence data deduced from our multiple labeling procedure with published sequences from two other laboratories using different methods demonstrates excellent agreement in the results obtained with the three different techniques (Table 3). A minor difference is that Bridgen *et al.* (2) did not report aspartic acid at position 9 or methionine at position 12, although their mixed antigens included specificities 1 and 8. This result could reflect minor differences between molecules bearing similar HLA specificities, a difference between typing antisera, or technical problems. Bridgen *et al.* also reported at position 3 a residue that did not behave as a normal amino acid in thin-layer chromatography. We twice found histidine by amino acid analysis after HI hydrolysis, and also observed that [^{14}C]His alone labels this position. Others have reported that radioactive histidine labels position 3 in mouse H-2 antigens (6, 7).

With these exceptions, the sequences of several HLA specificities derived from both the A and B loci are remarkably uniform at the amino termini, as has been noted by others (1, 2). We have not investigated whether the proteins which determine C-locus specificities are found in our HLA preparations; if so, they must either be present at low concentrations or else have NH_2-terminal sequences very similar to those of the A and B locus products.

We may summarize the technical advantages of our method as follows: (*a*) A relatively small cell culture is required for the operation (40 ml, 1.6×10^8 cells). (*b*) Since only radiochemical purity is required in the protein, no separation from fragments of the immunoadsorbent or removal of unlabeled proteins is required. This dramatically eases problems of yield and purity during the immunoadsorption step (efforts to obtain adequate purity on this scale without some unlabeled protein present as carrier have proved unsuccessful). (*c*) Only a single sequencer run is required to identify most of the amino-terminal residues; this contrasts sharply with the multiple runs required in procedures that label with only a few amino acids (5–8). (*d*) Positions of heterogeneity can be unambiguously defined by applying quantitative techniques. Overlap and background can be assessed and corrected, permitting more confidence in a given run; and chemical or mechanical difficulties that may occur during sequencer operation are readily detected with the reference ^{3}H-labeled protein (13). (*e*) Finally, the entire molecule is available for sequencing; the relatively uniform radiolabel permits easy detection of all of the peptides produced by chemical or enzymic cleavage.

Note Added in Proof. Appella *et al.* (17) have also published sequences for HLA-A2 and mixed HLA-B7,B14 antigen preparations. These authors report serine, rather than histidine, at position 3.

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1. Terhorst, C., Parham, P., Mann, D. L. & Strominger, J. L. (1976) "Structure of HLA antigens: Amino-acid and carbohydrate composition and NH_2-terminal sequences of four antigen preparations," *Proc. Natl. Acad. Sci. USA* **73**, 910–914.
2. Bridgen, J., Snary, D., Crumpton, M. J., Barnstable, C., Goodfellow, P. & Bodmer, W. F. (1976) "Isolation and N-terminal amino acid sequence of membrane-bound human HLA-A and HLA-B antigens," *Nature* **261**, 200–205.
3. Jacobs, J. W. & Niall, H. D. (1975) "High sensitivity automated sequence determination of polypeptides," *J. Biol. Chem.* **250**, 3629–3636.
4. Jacobs, J. W., Kemper, B., Niall, H. D., Habener, J. F. & Potts, J. T., Jr. (1974) "Structural analysis of human proparathyroid hormone by a new microsequencing approach," *Nature* **249**, 155–157.
5. Silver, J. & Hood, L. (1975) "An approach to amino acid sequence analysis of transplantation antigens," *Nature* **256**, 63–64.
6. Henning, R., Milner, R. J., Reske, K., Cunningham, B. A. & Edelman, G. M. (1976) "Subunit structure, cell surface orientation, and partial amino acid sequences of murine histocompatibility antigens," *Proc. Natl. Acad. Sci. USA* **73**, 118–122.
7. Vitetta, E. S., Capra, J. D., Klapper, D. G., Klein, J. & Uhr, J. W. (1976) "The partial amino acid sequence of an H-2K molecule," *Proc. Natl. Acad. Sci. USA* **73**, 905–909.

8. Ewenstein, B. M., Freed, J. H., Mole, L. E. & Nathenson, S. G. (1976) "Localization of the papain cleavage site of H-2 glycoproteins," *Proc. Natl. Acad. Sci. USA* **73**, 915–918.
9. McKean, D. J., Peters, E. H., Waldby, J. I. & Smithies, O. (1974) "Amino acid sequence determination with radioactive proteins," *Biochemistry* **13**, 3048–3051.
10. Faber, H. E., Kucherlapati, R. S., Poulik, M. D., Ruddle, F. H. & Smithies, O. (1976) "β_2-Microglobulin locus on human chromosome 15," *Somatic Cell Genetics* **2**, 141–153.
11. Laemmli, U. K. (1970) "Cleavage of structural proteins during the assembly of the head of bacteriophage T4," *Nature* **227**, 680–685.
12. Edman, P. & Begg, G. (1967) "A protein sequenator," *Eur. J. Biochem.* **1**, 80–91.
13. McKean, D. J. & Smith, G. P. (1974) "The use of radiolabeled T_1 ribonuclease to monitor the efficiency of Edman degradations," *Biochem. J.* **139**, 779–780.
14. Smithies, O., Gibson, D., Fanning, E. M., Goodfleish, R. M., Gilman, J. G. & Ballantyne, D. L. (1971) "Quantitative procedures for use with the Edman-Begg sequenator. Partial sequences of two unusual immunoglobulin light chains, Rzf and Sac," *Biochemistry* **10**, 4912–4921.
15. Peterson, P. A., Cunningham, B. A., Berggard, I. & Edelman, G. M. (1972) "β_2-Microglobulin—A free immunoglobulin domain," *Proc. Natl. Acad. Sci. USA* **69**, 1697–1701.
16. IUPAC-IUB Commission or Biochemical Nomenclature (1968) "A one-letter notation for amino acid sequences: Tentative rules," *J. Biol. Chem.* **243**, 3557–3559.
17. Appella, E., Tanigaki, N., Fairwell, T. & Pressman, D. (1976) *Biochem. Biophys. Res. Commun.* **71**, 286–292.