provide an exceptionally important model for the study of the biology and biochemistry of chemotactic peptide receptor-mediated functions in mammalian cells. Although the occupancy of *N*-formylated peptide receptors induces one receptor-coupled process, it fails to induce another that is thought to be initiated by the same mechanism. The reason for this discrepancy is unknown. It is possible that the coupling processes that link this receptor with the effector mechanisms for the two different biological functions are discrete, with the transducer for secretion intact while the one for chemotaxis is nonfunctional. Alternatively, the number of receptors available may be insufficient for chemotaxis, but sufficient for secretion. In studies of chemotactic factor receptors on chemotactically responsive cells, the estimates have ranged from 2000 to 250,000 receptors per cell. Equine PMN's contain < 1000 receptors per cell. Although these cells clearly have enough *N*-formylated peptide receptors to initiate secretion, they may not have a sufficient number to sense a gradient across their surfaces. Chemotaxis, unlike secretion, may require an asymmetric distribution of chemoattractant binding sites on the cell surface for directional movement. It is also possible that secretion and chemotaxis induced by the *N*-formylated peptides are initiated by similar but independent receptors.

In any case, the transduction signals resulting from the binding of the *N*-formylated oligopeptides to their receptors differ for chemotaxis and secretion in equine PMN's—the former signal being ineffective while the latter signal is operative. It should now be possible, by comparing equine and human PMN's, to separate the biochemical events following *N*-formylated oligopeptide receptor occupancy that result in secretion alone and those that result in both secretion and chemotaxis.

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9. Horse plasma was incubated with zymosan (5 mg/ml) for 60 minutes at 37°C, then for 30 minutes at 56°C. After the zymosan was removed by centrifugation, 4 ml of activated plasma was applied to a Sephadex G-100 column, and the fractions were eluted with 0.01*M* sodium phosphate-buffered isotonic saline, *p*H 7.2. Active fractions were pooled and stored at −20°C (*5*).
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14. Horse PMN's were suspended in phosphate-buffered isotonic saline, *p*H 7.4, at a concentration of 2.5×10^6 to 3.0×10^6 cells per milliliter in the presence or absence of 10^{-5}*M* cytochalasin B. After incubation for 15 minutes at 37°C, buffer or chemotactic factors were added, and incubation was continued for an additional 15 minutes at 37°C. Enzyme release was terminated by immersing the tubes in an ice bath, followed by centrifugation. Lysozyme was assayed in the supernatant by measuring turbidometrically the lysis of *Micrococcus lysodeikticus* at *p*H 7.0 after 15 minutes at 37°C [D. Shugar, *Biochim. Biophys. Acta* **8**, 302 (1952)]. β-Glucuronidase was assayed by measuring the release of phenolphthalein from its β-glucuronate after an 18-hour incubation at *p*H 4.5 [W. H. Fishman, B. Springer, R. Brunetti, *J. Biol. Chem.* **173**, 449 (1948)].
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5 February 1980

Ion Pairing Techniques: Compatibility with On-Line Liquid Chromatography–Mass Spectrometry

Abstract. *The mass spectrometric properties of a series of model ion pairs were examined. In the cases studied it was possible to vaporize the ion pair consituents and to produce spectra corresponding to those of the unpaired materials. These findings offer a convenient means for derivatizing certain ionic compounds and demonstrate the feasibility of analyzing ionic species by on-line liquid chromatography–mass spectrometry.*

The direct analysis of ionic species by conventional mass spectrometric (MS) techniques [that is, electron impact ionization (EI) or chemical ionization (CI)] is difficult if not impossible, and chemical derivatization is usually required for these types of compounds. This requirement complicates the analysis of large and important classes of compounds, particularly those of biological and pharmaceutical origin that are ionic in character.

With high-performance liquid chromatography (HPLC), ionic compounds are being successfully analyzed by ion pairing techniques (*1*), but there is limited information on the MS characteristics of the resultant neutral derivatives. The direct combination of reversed phase HPLC and MS has recently been accomplished (*2*), and we report here our findings regarding the MS properties of model ion pairs that demonstrate both the compatibility of on-line LC-MS with ion pairing techniques and the potential for using ion pair derivatives for the direct MS analysis of ionic compounds.

As a model system for this study, we chose ion pairs formed from *n*-alkyl sulfates and sulfonates (counterions) and primary amines (solutes). This system is relatively general and is routinely applied to the HPLC analysis of biological and pharmaceutical materials. To be ideally compatible with MS analysis, both constituents of the ion pair should be volatilized under normal MS operating conditions and should provide spectra indicative of or consistent with the structure of the unpaired materials.

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The data presented here indicate that these requirements are met.

In the first part of this study we examined the MS properties of a series of *n*-alkyl sodium sulfate and sulfonate salts (counterion salts) by EI-MS (70 eV) with the direct inlet probe. The sulfates (C_6, C_7, C_8, C_{10}, C_{12}, and C_{14}) produced spectra at probe temperatures $\leq$ 200°C corresponding to *n*-alkenes arising from the elimination of $NaHSO_4$. The spectrum obtained from *n*-octyl sodium sulfate (Fig. 1A) is typical. No molecular ion of the salt was observed in any case, and the $[M\text{-}HSO_4Na]^+$ ion was invariably the highest mass detected. In contrast to these results, no spectra were obtained from a series of sodium sulfonates (C_6, C_7, C_8, and C_{10}) at probe temperatures up to 300°C.

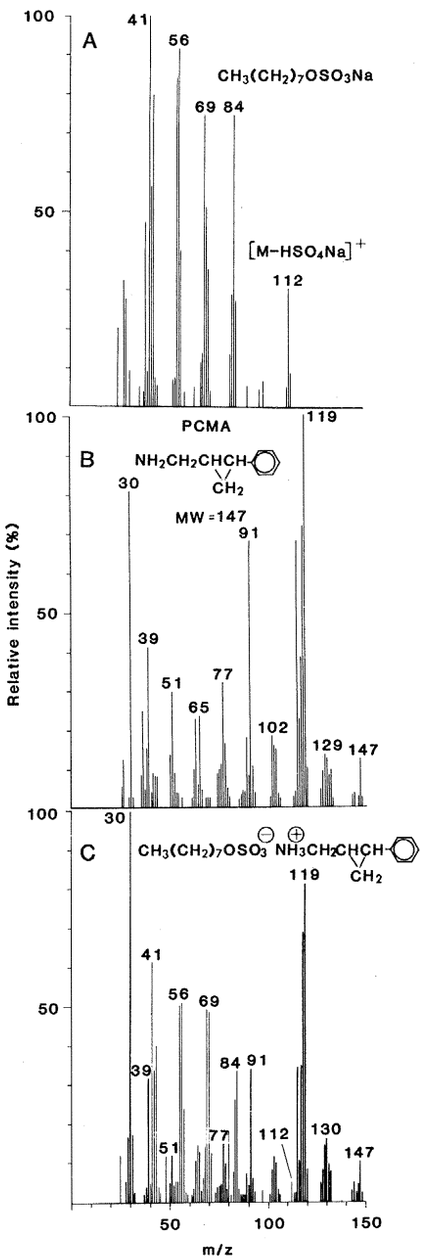

Fig. 1. The EI-MS (70 eV) spectra obtained from (A) neat *n*-octyl sodium sulfate, (B) neat PCMA, and (C) the ion pair formed from *n*-octylsulfate and PCMA (PCMA related ions are marked in bold lines); MW, molecular weight.

Solute spectra were obtained under similar conditions. The spectrum of PCMA (1-phenylcyclopropanemethylamine) (Fig. 1B) is typical.

Next we examined by EI-MS a series of ion pairs formed between the solutes and counterions. Ion pairs were extracted from an acidic phosphate buffer into methylene chloride and were admitted to the MS by the direct inlet probe. Introduction of the sulfate ion pairs into the MS at probe temperatures $\lesssim$ 200°C resulted in spectra that contained ions indicative of both the solute and counterion. Each spectrum contained all the major ions associated with the respective anions and cations, and these were present in approximately the same relative intensities as in the spectra of the unpaired materials. The spectrum obtained from the *n*-octylsulfate-PCMA ion pair (Fig. 1C) is typical. Ions at a mass-to-charge ratio (*m/z*) of 41, 56, 69, 84, and 112 are indicative of the counterion, whereas those at *m/z* 30, 51, 65, 77, 91, 103, 119, 130, and 147 are indicative of the solute. Thus, given the spectrum or identity of the counterion, one can readily discern the spectrum of the solute.

In a similar manner spectra were obtained from sulfonate ion pairs at probe temperatures $\leq$ 100°C. These spectra also contained ions characteristic of both the anionic and cationic components and were very similar to the spectra from the sulfate ion pairs. This result is significant in that without the utilization of the ion pairing technique we had been unable to observe any ions from the sulfonate sodium salts, and it demonstrates that ion pair derivatization can be used as a means of increasing the volatility of certain ionic compounds. This form of derivatization may be applicable to a wide variety of analytical problems. No higher mass ions indicative of an intact ion pair were observed with either the sulfates or the sulfonates.

In the final part of this study we examined the model ion pairs under CI-MS (reagent gas, isobutane) conditions. As expected, the CI spectra were much simpler than the corresponding EI spectra. Typical results are shown in Figs. 2 and 3. Introduction of either the sulfate or sulfonate ion pairs into the MS yielded $[M + H]^+$ and $[M + H\text{-}NH_3]^+$ ions from each amine solute (*m/z* 148 and 131, respectively, for the examples in Figs. 2 and 3). The spectra obtained from the counterion portions of the sulfate ion pairs contained the protonated alkene ion and one or more less intense hydrocarbon fragments (*m/z* 113 and 71, respectively, for the example in Fig. 2). In contrast, however, the spectra obtained from the counterion portions of the sulfonate ion pairs contained only a single ion that corresponded to the protonated sulfonic acid of the salt (*m/z* 195 for the example in Fig. 3). In neither case did we observe higher mass ions indicative of an intact ion pair, even under milder ionization conditions with NH_3 used as the CI reagent gas.

The observation of recognizable fragment ions originating from both the anion and cation, including the sulfate (*m/z* 48, 64, and 80, Fig. 1C) (*3*) and sulfonate portions (*m/z* 195, Fig. 3) suggests that both moieties of the ion pair are volatilized. It seems doubtful that the ion pair is volatilized intact. It is more likely that thermal decomposition of the ion pair precedes ionization in a manner analogous to that of alkyl ammonium halides (*4*), ionic dyes (*5*), or salts of or-

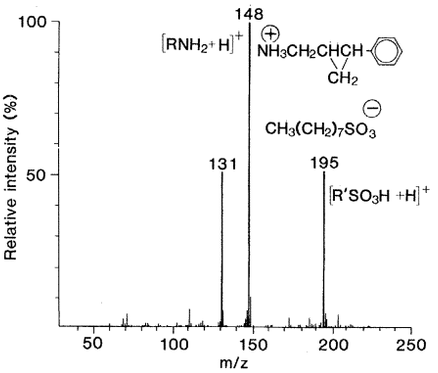

Fig. 2. The CI-MS (isobutane) spectra obtained from the ion pair formed from *n*-octylsulfate and PCMA.

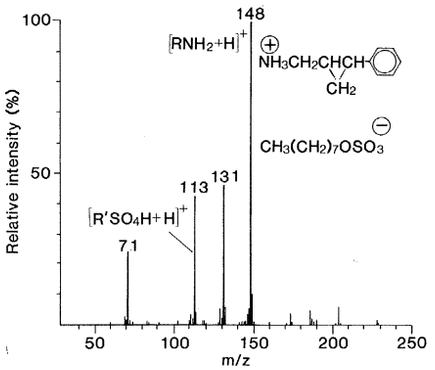

Fig. 3. The CI-MS (isobutane) spectra obtained from the ion pair formed from *n*-octylsulfonate and PCMA.

ganic acids (*6*), where a proton transfer from a counterion or a reagent mixed with the salt leads to the formation of a neutral compound which can then be vaporized. However, although the exact mechanistic details need further study, this technique can be directly applied to specific analytical problems.

Our results demonstrate the compatibility of a model ion pairing system with MS analysis and its compatibility with on-line LCMS. This technique provides a convenient method for both the separation and MS analysis of ionic compounds. Moreover, the data demonstrate the ability to convert ionic compounds into a volatile form by ion pair derivatization. Significantly, the resulting spectra correspond to those of the unpaired material and contain no mass increment as is usually the case with ordinary derivatization schemes. The ion pairing technique should be readily adaptable to the analysis of many sulfate and sulfonate surfactants and eventually should be extendable to other classes of ionic compounds.

Since the completion of these experiments, we have evaluated the on-line LCMS analysis of ion pairs. A system consisting of a continuous extraction interface and moving belt inlet to the MS, as described in (*2*), was used. The resulting spectra (both EI and CI) were identical to those shown here, and no detrimental effects on the operation of the MS were observed (*7*).

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28 January 1980; revised 9 April 1980

Human Hepatocellular Carcinoma Cell Lines Secrete the Major Plasma Proteins and Hepatitis B Surface Antigen

Abstract. *Analysis of the cell culture fluid from two new human hepatoma-derived cell lines reveals that 17 of the major human plasma proteins are synthesized and secreted by these cells. One of these cell lines, Hep 3B, also produces the two major polypeptides of the hepatitis B virus surface antigen. When Hep 3B is injected into athymic mice, metastatic hepatocellular carcinomas appear. These cell lines provide experimental models for investigation of plasma protein biosynthesis and the relation of the hepatitis B virus genome to tumorigenicity.*

Plasma protein synthesis, a specialized function of liver parenchymal cells, has been studied in vitro in liver slices from experimental animals (*1*), in suspension and in primary cultures of isolated hepatocytes (*2–6*), and in hepatoma-derived cell lines (*7–9*). From these investigations, general principles for the control of some of these liver-specific functions in rodents have been derived. However, no experimental system for the investigation of plasma protein synthesis in the human has been described.

We have isolated two human cell lines (Hep G2 and Hep 3B) from liver biopsies of two children with primary hepatoblastoma and hepatocellular carcinoma (*10*). We found that 17 of the major plasma proteins are secreted into cell culture medium. A previously described (*11*) hepatoma cell line (PLC/PRF/5) produces seven of the same human plasma proteins. The Hep 3B and PLC/PRF/5 cell lines synthesize hepatitis B virus surface antigen (HBsAg) (*10, 11*); the Hep G2 line does not synthesize HBsAg. We found that the two major polypeptides of HBsAg are synthesized by Hep 3B and PLC/PRF/5. Hepatitis B virus (HBV) has been epidemiologically implicated as a probable causative agent of the majority of hepatocellular carcinomas (*12*). We now report that Hep 3B and PLC/PRF/5 form tumors when injected into nude mice. These cell lines thus provide an excellent model for the investigation of human plasma protein synthesis and the hepatitis B virus–host cell relationship.

Table 1. Identification of plasma proteins in tissue culture medium of human hepatoma cell lines by the Ouchterlony double-diffusion technique.

Human protein	Reaction with antiserum*		
	Hep G2	Hep 3B	PLC/ PRF/5
α-Fetoprotein	+	+	−
Albumin	+	+	−
α_2-Macroglobulin	+	+	+
α_1-Antitrypsin	+	+	+
α_1-Antichymotrypsin	+	+	−
Transferrin	+	+	+
Haptoglobin	+	+	−
Ceruloplasmin	+	+	+
Plasminogen	+	+	+
Gc-globulin	−	+	−
Complement C3	+	+	+
Complement C4	+	−	−
C'3 activator	+	+	−
α_1-Acid glycoprotein	+	+	+
Fibrinogen	+	+	−
α_2-HS-glycoprotein	+	+	−
β-Lipoprotein	+	+	−
Retinol-binding protein	+	+	−

*Indicates line of identity with either normal human plasma or human placental cord serum. Negative controls include culture fluid from human fibroblasts and mouse cells. Samples taken from confluent flasks, which contained the same culture fluid throughout the growth cycle of the culture (7 to 14 days), were concentrated tenfold by use of Minicon-B15 concentrators (Amicon, Lexington, Mass.). No reactivity was noted with antibody to prealbumin and hemopexin. All antibodies were purchased from Cappel Laboratories (Cochranville, Pa.) or Calbiochem-Behring (San Diego, Calif.). The amounts of albumin and α-fetoprotein produced by cultures initiated with 10^6 Hep 3B cells and grown for 2 weeks without medium change (1.1×10^7, final cell number) are 300 and 500 μg, respectively (*10*).

The Hep G2 and Hep 3B cell lines were initially derived from tissue minces of hepatoma biopsies placed on feeder layers of the irradiated mouse cell line STO (*13*) in William's E medium (Gibco) supplemented with 10 percent fetal bovine serum. This method of isolation promotes the growth of cells that have fastidious requirements while preventing fibroblastic overgrowth. After an initial period of apparent cell proliferation, growth was restricted to single colonies that were maintained in flasks for several months. Cells from flasks containing single large colonies were dissociated by trypsinization (0.25 percent trypsin, 0.1 percent EDTA in phosphate-buffered saline) and transferred to new feeder layers, eventually producing proliferating cell lines that have now been serially passaged more than 50 times. Sublines of both Hep G2 and Hep 3B have been selected for feeder independence. These cell lines resemble liver parenchymal cells morphologically and are chromosomally abnormal: Hep G2, $\bar{X} = 55$ (50 to 56); Hep 3B, $\bar{X} = 60$, with a subtetraploid population, $\bar{X} = 82$. Both cell lines contain distinctive rearrangements of chromosome 1.

When Hep 3B was injected under the

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