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Identification of proteinaceous binding media of easel paintings by gas chromatography of the amino acid derivatives after catalytic hydrolysis by a protonated cation exchanger

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Summary. A procedure for the hydrolysis of proteins, based on catalysis by the protonated form of a strong cation exchanger, was established for proteinaceous binding media (e.g. casein, egg, animal collagene) used for objects of art. The experimental parameters for the hydrolysis (temperature, time) were optimized, as well as the conditions for sorption and desorption of the amino acids on the cation exchanger. The results for the identification of proteins by the gas chromatographic pattern of the amino acid derivatives, after hydrolysis by the ion exchanger and by hydrochloric acid were compared. The former method proved to be more efficient due to the mild conditions, avoiding the formation of humins in the presence of carbohydrates, and reducing the dissolution of pigments. The method was applied to identify the proteinaceous vehicles of samples from priming and paint layers of easel and wall paintings from the 16th and 18th century.

1 Introduction

Whereas the analysis of colouring matter, especially of inorganic pigments in paint layers of easel and wall paintings, is in most cases a straightforward task, the identification of binding media offers considerable difficulties. This is partly due to the small percentage – usually less than about 10% of the total weight – contained in the samples which are commonly available in only minute amounts, and partly because they are complicated mixtures of natural substances as: drying oils, native proteins, resins, carbohydrates (starch products, plant gums) and waxes. By microchemical tests these groups can be roughly distinguished, but to identify different species within a particular group is nearly impossible.

This holds especially for the different native proteins. They play an important role as binders; milk and casein, egg tempera, animal glues are prominent examples. On the other hand, these proteinaceous binding media can be characterized and identified by their different amino acid profiles [1–4]. This direct method of identification seems to be more meaningful than approaches investigating the properties of the native protein, because often the more complex original structures of the proteins are lost, especially in aged samples, where denaturation occurs, or where the samples are affected and deteriorated by chemical reactions, e.g. by the interaction of the disulfide- and sulfhydril-groups of amino acids with free radicals [5], or with heavy metal ions stemming from pigments. The very promising sensitive and specific method of fluorescent protein tracing is, however, impeded by the difficult eluation of aged and pigmented protein layers.

For the purpose of identification of the proteins, patterns of the amino acids are determined by gas or liquid chromatography after hydrolysis of the proteinaceous matter. The best established procedure for hydrolysis is based on the cleavage of the amide bonds with hot, concentrated hydrochloric acid (mostly carried out at an acid concentration of 6 mol/l). This crude procedure may lead, however, to misinterpretable results, especially when other binding media like carbohydrates are present in the samples too [3–6]. Caused by losses of amino acids due to humin formation, patterns are then obtained which deviate from those from pure proteins, and identification is complicated, or is even impossible.

In addition to these limiting effects, the reaction products formed may severely interfere with the chromatographic determination in many cases and therefore must be removed. For this aim the preseparation of the amino acids from the interfering matrix is proposed in the literature by adsorption of the analytes via their ammonium groups on a cation exchanger after the hydrolysis with hydrochloric acid [2, 3, 7]. This pretreatment, however, implements an additional step into the analytical procedure, and leads to an increase of manipulation and time of analysis.

Another source of error is the presence of chemically active metal ions in the hydrolysate, contained in the

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pigments in the paint layer, which are often dissolved under the drastic conditions of the hydrolysis with concentrated acid. Additionally, the presence of these metal ions in the hydrolysate can cause an incomplete derivatization [3, 4, 6], which has to be carried out prior to the gas chromatographic analysis of the amino acids. Obviously, the purification step as mentioned above, does not always eliminate this source of error, because these cations are also adsorbed from the hydrolyzate by the ion exchanger and can be coeluted with the amino acids.

These restrictions of the conventional hydrolyzation procedure make the development of a more appropriate method desirable. A procedure carried out under mild conditions is based on the catalytic cleavage of the amide bond of the amino acid chain of peptides or proteins by H^+-ions, adsorbed on a strongly acidic cation exchanger. This method was introduced several ten years ago, mainly for the hydrolysis of dipeptides [8 – 11], but also for proteins [12 – 14]. However, no approach was carried out to evaluate this method with respect to its applicability for the identification of proteinaceous binding media used for objects of art, although its potential utility in this field was mentioned [3].

In order to evaluate the scope of this method, different proteinaceous media were subjected to the hydrolysis with an ion exchanger and the results were compared with those obtained by the hydrolysis by concentrated hydrochloric acid (followed by the preseparation by ion exchange) as described [3]. Finally, the method developed was applied to identify the proteinaceous binding media of some paintings.

2 Experimental

2.1 Materials

If not otherwise stated, the solvents and chemicals used were of p. a. grade (E. Merck, Darmstadt, F.R.G.). Gaseous hydrogen chloride was obtained from Messer-Griesheim (Düsseldorf, F.R.G.). Tetradecane used as the internal standard in gas chromatography was purchased from Chrompack (Middelsburg, The Netherlands). The proteins were of commercial quality. The strongly acidic ion exchanger Dowex 50 WX8 (50 – 100 mesh) was obtained from Fluka (Heidelberg, F.R.G.). Water used was doubly distilled in a quartz apparatus. The pigments were taken from the historic collection of the Academy of Fine Arts, Vienna.

2.2 Apparatus

The gas chromatographic determinations were performed with an instrument (Mega Series HRGC 5300, Carlo Erba, Milano, Italy) equipped with a split injector (inlet split ratio 1:15) and a flame ionization detector. The retention times and the peak areas were determined with an integrator (Mega Series). The capillary column used was made from fused silica (25 m length, 0.2 mm i. d.) and was coated with 5% phenyl-, 95% methyl-polysiloxane (DB-5, Hewlett-Packard, Palo Alto, USA; film thickness 0.33 µm).

The chromatographic conditions were: linear temperature gradient from 100 to 280° C with a rate of 10° C/min.

Temperature of injector and detector was 300° C.

Carrier gas was nitrogen (99.999% v/v purity, Messer-Griesheim).

2.3 Procedures

2.3.1 Hydrolysis of the proteins

2.3.1.1 Hydrolysis by ion-exchanger catalysis. The samples, containing about 10 – 100 µg of proteinaceous media, were placed into a vial, formed from a Pasteur pipet, which was closed on one side by melting off the tight end. 600 µl of an ethanol-water mixture (80/20% v/v) and 600 µl of moist ion-exchange resin were added to the sample. [The commercial resin was previously transferred into the H^+-form by shaking with hydrochloric acid (6 mol/l); then the resin was washed with water to pH 6 – 7, and stored in 80% ethanol.] The vial was then cooled on dry ice to prevent bumping in the following evacuation step. Whilst water jet vacuum was applied, the vial was sealed by melting off its open end. The vial modified in this way was then placed into an oven for 24 h at 110° C. Then the vial was opened after cooling to ambient temperature and its content was transferred into another Pasteur pipet, which was stoppered with a small quantity of glass wool, and was used as an ion-exchange column in this way. The aqueous-ethanolic solvent was discarded and the resin was washed with 6 ml of water. The amino acids were then eluted from the exchanger by 6 ml of aqueous ammonia (7 mol/l). A precipitate, occasionally formed from some pigments in the column, was retained by the glass wool, and was therefore not transported into the effluent. The remaining, clear eluate, containing the amino acids of the hydrolyzed proteins, was pretreated prior to derivatization of the acids as described below.

2.3.1.2 Conventional hydrolysis by hydrochloric acid [3]. The samples, containing about 10 – 100 µg of proteinaceous matter were hydrolyzed by hydrochloric acid (6 mol/l) for 24 h at 110° C under vacuum in a vial, prepared as in section 2.3.1.1. The subsequent derivatization was carried out in the same manner as for the eluate from the ion exchanger.

2.3.2 Derivatization of the amino acids

2.3.2.1 Pretreatment of the hydrolysate. Prior to the derivatization of the acids which was carried out by methods described in the literature [15 – 26], a pretreatment of the solution obtained as the effluent of the ion exchanger was necessary in order not only to remove the solvent, but also to eliminate traces of water, which would interfere with the subsequent chemical reactions. Therefore, the effluent of the ion exchanger was transferred into a conical bulb (with a volume of about 30 ml), and the volume of the solution was reduced to about 500 µl, using a rotary evaporator under vacuum at about 50 – 70° C. The remaining solution was then transferred into a reaction vial (with a volume of about 1.5 ml). The vial was placed into a bulb and the solvent was totally removed with the rotary evaporator. Finally, 100 – 200 µl dichloromethane were added and the sample was evaporated to dryness to eliminate traces of water. The residue was subjected to the derivatization procedure as described below.

2.3.2.2 Derivatization of the carboxylic group. Transformation of the carboxylic functions into the butyl esters was carried out in the usual way [15 – 17] by adding to the residue 300 µl of butanol, in which hydrogen chloride was dissolved

at a concentration of 3 mol/l[1]. The reaction vial was capped under a stream of nitrogen and the mixture was heated for 35 min at 100° C. After cooling to ambient temperature, the solvent and excess reagent were removed with the rotary evaporator, and again 100–200 µl dichloromethane were added and evaporated. Acylation of the dry sample was then carried out.

2.3.2.3 Derivatization of the amino group. 150 µl trifluoroacetanhydride and 150 µl dichloromethane were added to the dry residue [15–17], the vial was then capped under a stream of nitrogen and the solution was kept for 20 min at 100° C. After cooling to ambient temperature, the solvent and the excess reagent were removed with a rotary evaporator. The residue was taken up in 100 µl of ethylacetate, in which the internal standard for the gas-chromatographic determination was dissolved. Aliquots of these solutions were directly injected into the gas chromatograph.

3 Results and discussion

3.1 Optimization of the hydrolysis method

The optimization of the entire method has to include two different steps:

– the optimization of the experimental conditions to achieve maximum yield of cleavage of the amide bonds of the proteins, namely temperature and time of hydrolysis, and

– the adjustment of the appropriate conditions for the adsorption of the amino acids, which are catalytically abstracted from the proteins (and converted into the actually adsorbed ammonium ions under the given conditions), and of the respective conditions for the quantitative elution from the ion exchanger.

3.1.1 Conditions for adsorption and elution of the amino acids

In order to investigate the latter problem, namely the elution profile of the amino acids from the cation exchanger under different experimental conditions, casein as model protein was hydrolyzed by the catalytic method with the exchanger as described in the Experimental Section. The resulting aqueous-ethanolic suspension of the resin (with the adsorbed hydrolysate) was transferred into a Pasteur pipet used as ion-exchange column. Since it was found that the adsorption of the ammonium cations formed from the amino acids is partially inhibited in acidic solutions by the concurrence of H_3O^+-ions, the absence of excess acid is important for an efficient procedure. Therefore the sample should not be applied in an acidic solution.

The first effluent from the column obtained in this way (300 µl of the solvent, in which the hydrolysis took place) was collected. One washing step was carried out with 6 ml of water, followed by 4 elution steps with aqueous ammonia (3 ml each). All effluents were collected separately and their

[1] This solution was prepared by passing gaseous hydrogen chloride first through a washing flask containing concentrated sulfuric acid and then dissolving the gas in butanol, which was placed into a second washing flask on line. After the saturation of the alcohol with HCl, the concentration of the acid was determined by titration, and was finally adjusted to 3 mol/l by dilution with butanol

Table 1. Distribution of the total amino acid content in the different effluents obtained by the sample handling after the hydrolysis of the protein with the ion exchanger. Casein was used as model protein

Type of effluent	Volume [ml]	% Amino acids found in the particular effluent	
Ethanolic hydrolysis solution	0.6	4	3
Aqueous washing solution	6.0	3	2
1st Ammonia displacement solution	3.0	67[a]	90[b]
2nd Ammonia displacement solution	3.0	20[a]	3[b]
3rd Ammonia displacement solution	3.0	4[a]	2[b]
4th Ammonia displacement solution	3.0	2[a]	0[b]
Total		100	100

The concentration of the ammonia solution used to desorb the amino acids from the ion exchanger was [a] 2 and [b] 7 mol/l

amino acid content was quantified by gas chromatography as described. The results are given in Table 1 for two different ammonia concentrations. One can see that a negligible amount of the amino acids are found in the first effluent and in the washing water, respectively. The main part of the amino acids is found in the first ammonia fraction, where 90% are recovered using 7 mol/l ammonia as displacer. However, such high yields were also observed when the first two fractions from 2 mol/l ammonia applied are connected (87% of the total). Only a few percents are found in the third and fourth basic fractions. Thus, 6 ml of 7 mol/l ammonia were used to elute the amino acids quantitatively from the ion exchanger, on which hydrolysis was performed.

3.1.2 Experimental variables of the hydrolysis

Two main variables can be expected to influence the yield of the hydrolytic procedure: the temperature, at which hydrolysis is carried out, and the reaction time. The dependence of the extent of the hydrolysis on both variables was investigated, using gelatine as model protein. Two different temperatures were applied (80° C and 110° C), and the variation of the amount of the amino acids abstracted from the protein with time was determined. The results are shown in Fig. 1. An almost linear increase of the extent of the hydrolysis with increasing reaction time can be observed for the first hours for both temperatures, but the curve obtained at the higher temperature has a steeper slope. For both temperatures the curves reach the same plateau, but this constant value is reached after different times: after about 15 h at 110° C, and after about 40 h at 80° C. In practice, the hydrolysis of the samples was therefore carried out at 110° C for 24 h.

3.2 Hydrolysis by the ion exchanger compared with hydrolysis with hydrochloric acid

3.2.1 Hydrolysis of "pure" proteinaceous binding media

In a first step, the performance of the identification of proteins by the aid of the ion-exchanger hydrolysis was compared with the usual HCl method, applied for the type of samples under discussion. The latter method consists

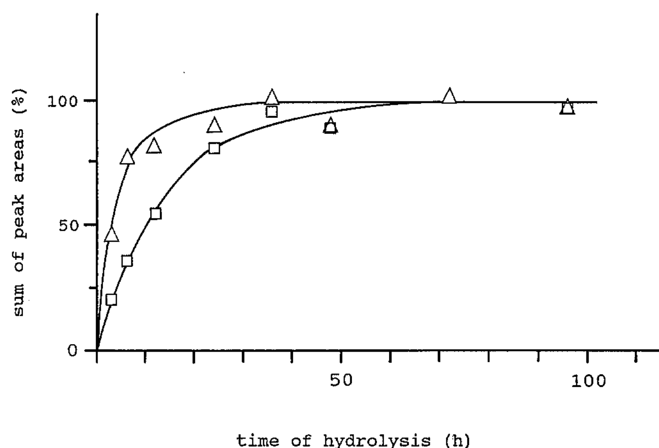

Fig. 1. Dependence of the yield of the protein hydrolysis, catalysed by the ion exchanger, on time and temperature. The yield was determined from the sum of the gas-chromatographic peak areas of the amino acid derivatives. Gelatine was used as model protein. □ 80°C; △ 110°C

in a hydrolytic step carried out with hydrochloric acid (6 mol/l), followed by the purification of the hydrolyzate with an ion exchanger [2, 3, 7].

For the purpose of comparison, different proteinaceous media, which are commonly used for paintings (commercial gelatine, rabbit skin glue, casein and egg) were investigated. At this step, no other type of binding media or pigments were added to the samples. The proteinaceous materials were hydrolyzed by both methods, and the amino acid profiles were determined by gas chromatography after derivatization.

The resulting chromatograms are shown in Figs. 2–4. It can be seen that both procedures yield nearly identical patterns for the particular proteins. Fluctuations of the absolute amounts of the amino acid (reflected by the variations of the absolute peak heights) are caused by variations of the sample amounts and can be neglected for the characterization of the proteins, where only the relative amount of the amino acids is decisive. The statistical variation of these relative peak areas is obviously greater after the hydrolysis procedure compared to the error originated by the derivatization and the gas chromatographic determination only. For the two latter procedures, the relative standard deviation, derived by analysis of variance from pure amino acids, is about 5%, whereas that for the overall procedure could even reach several ten percents, independent of the type of hydrolysis. Based on these relative amounts, the identification of the different proteins is, however, easily possible.

The measured values are presented in Table 2. They are in good agreement with those given in the literature [1–5, 27]. Even assuming the same performance of both methods in characterizing proteins, the procedure based on the catalysis with the ion exchanger offers greater simplicity of the experimental handling.

3.2.2 Hydrolysis in the presence of other binding media

In order to prove the applicability of the method also for the case, that the protein is present in the sample together with other types of binding media, 1:1, 1:10 and 1:100

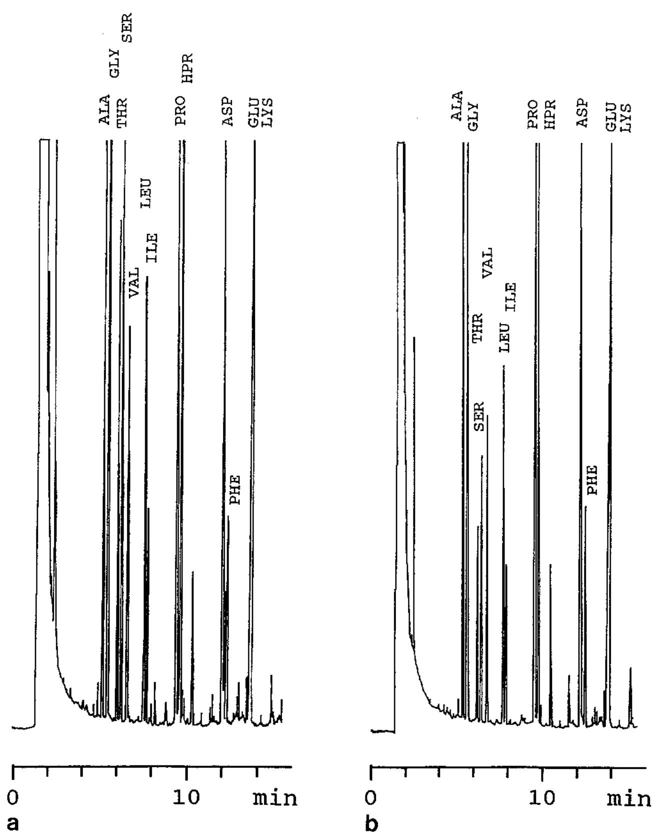

Fig. 2. Gas-chromatograms of the amino acid derivatives obtained after hydrolysis of gelatine, catalysed by the ion exchanger (**a**) and with concentrated HCl (**b**). Abbreviations of the amino acids see Table 2. Gas-chromatographic conditions are described in the Experimental Section

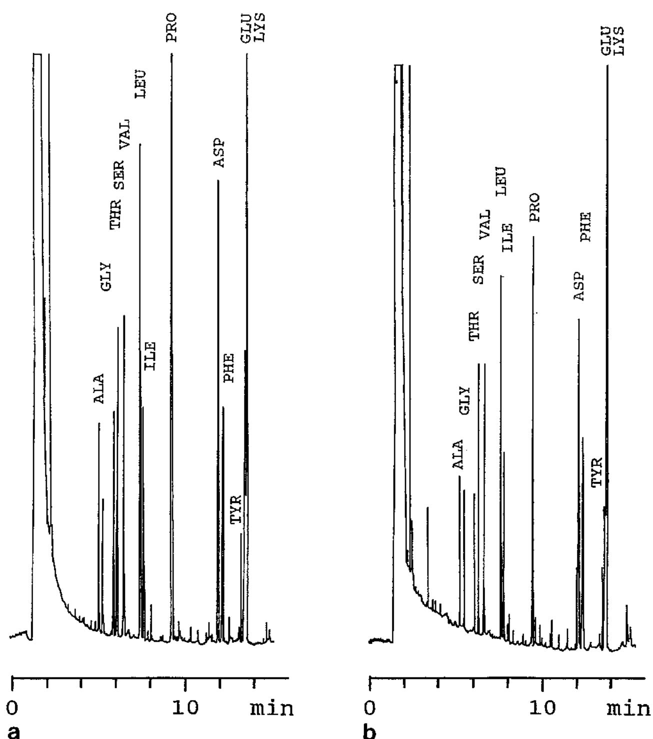

Fig. 3. Gas-chromatograms of the amino acid derivatives obtained after hydrolysis of casein catalysed by the ion exchanger (**a**) and with concentrated HCl (**b**). See also Fig. 2

mixtures of the different proteinaceous materials with lineseed oil and carbohydrate (starch), respectively, were prepared. The amino acid profiles were determined in these mixtures after hydrolysis by both methods. It was found, that the hydrolysate obtained after the catalysis by the ion exchanger always remained perfectly clear and colourless. In contrast, the formation of a dark, solid residue in addition to a brownish discolouring of the solution was observed in the case of hydrolysis with HCl in the presence of carbohydrate. This effect is well-known and described in literature [3–7]; it seems to be caused by the formation of humins. In the presence of oil, also a discolouration took place, which was localized mainly in the oily matter. It was found, however, that the HCl-method did not influence the amino acid profiles in most cases to such an extent that the characterization of the proteins would be complicated seriously.

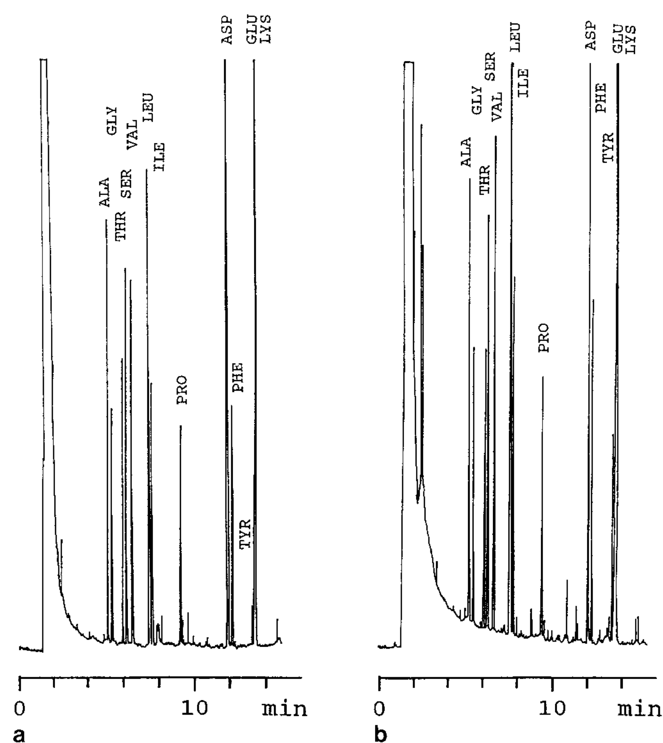

Fig. 4. Gas-chromatograms of the amino acid derivatives obtained after hydrolysis of egg catalysed by the ion exchanger (**a**) and with concentrated HCl (**b**). See also Fig. 2

3.2.3 Hydrolysis in the presence of pigments

The influence of a variety of pigments on the characterization of proteinaceous binding media was investigated by adding the following pigments (in a five- to tenfold excess) to egg yolk, casein and gelatine: zinc white (ZnO), chalk ($CaCO_3$), yellow ocre ($Fe_2O_3 \cdot H_2O$), chrome yellow ($PbCrO_4 \cdot 2PbSO_4$), vermillion (HgS) and verdigris ($Cu(CH_3COO)_2 \cdot H_2O$).

The mixtures of the proteins with the pigments were subjected to both hydrolysis procedures. It was observed that all pigments were quantitatively dissolved under the conditions of the hydrolysis with HCl (Pb and Hg form a precipitate of their chlorides as the products of a consecutive reaction). In contrast to this finding, the pigments were not or only to a small extent dissolved in the ethanolic medium of the hydrolysis with the ion exchanger: reduced interferences in the subsequent derivatization step of the amino acids can be expected.

After the derivatization procedure the proteinaceous media in the pigment/binder mixtures were characterized by the different amino acid profiles as discussed. In accordance with the literature [1–3, 27] it was found, that the quantitation of the amino acids seems to be sensitive to the presence of ions in the sample – especially for Ca or Fe containing pigments – when the HCl-procedure was applied for hydrolysis. Especially serine and threonine – acids used as markers for the characterization of the binding media [1–4, 27] – show large fluctuations of their relative concentrations up to 100% or even more. It should be kept in mind that a significant variation of their concentrations lead to

Table 2. Relative peak areas of the derivatized amino acids from the different proteinaceous binding media and the samples of objects of art. The samples are described in Table 3

Amino acid	IUPAC abbr.	Relative peak area (normalized to 100%)										
		Casein	Egg	Gelatine	Sample of the object of art							
					A	B	C	D	E	F	G	H
Alanine	ALA	3– 6	7–10	10–16	16	18	15	8	6	10	9	9
Glycine	GLY	2– 6	4– 5	20–30	22	8	26	16	17	27	17	17
Threonine	THR	3– 6	5– 8	1– 4	5	4	3	5	2	2	6	5
Serine	SER	6–12	7–11	2– 6	12	16	4	11	6	7	12	8
Valine	VAL	5– 7	5–10	2– 4	7	3	2	3	3	2	4	4
Leucine	LEU	8–12	8–12	2– 5	7	<1	4	3	2	2	4	4
iso-Leucine	ILE	3– 8	5– 7	1– 3	1	<1	1	3	1	1	2	3
Proline	PRO	10–15	5– 6	9–15	5	16	10	4	11	13	2	3
Hydroxy-proline	HPR	<0.5	<0.5	9–13	<0.5	<0.5	9	2	5	12	2	<0.5
Aspartic acid	ASP	7–12	12–18	6– 8	8	7	15	3	15	9	13	15
Phenylalanine	PHE	2– 6	4– 6	1– 3	<1	4	<0.5	4	4	2	6	4
Tyrosine	TYR	1– 3	3– 4	<0.5	<1	<1	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Lysine	LYS	2– 6	4– 6	1– 3	2	7	3	8	8	3	7	8
Glutamic acid	GLU	17–24	12–16	6–12	5	18	7	20	21	9	17	20

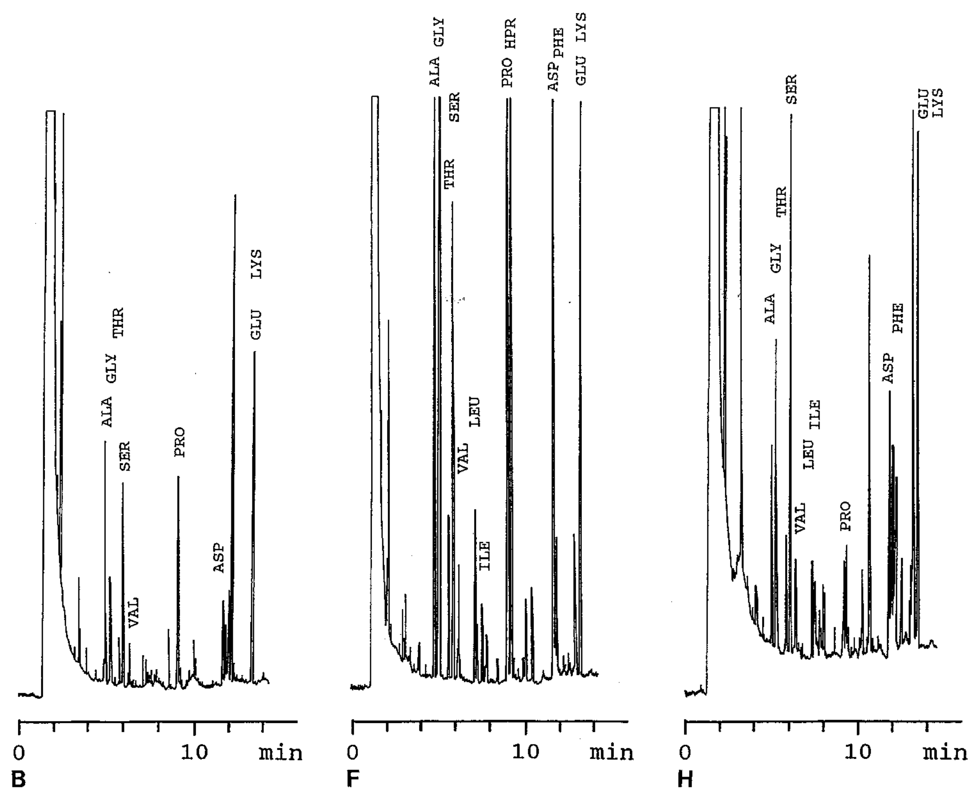

Fig. 5 B, F, H. Typical gas-chromatograms of the amino acid derivatives obtained after the cation exchanger catalized hydrolysis of the proteinaceous binding media of historic samples. The chromatograms are from the glue sample from the 16th century (**B** from Table 3), the ground layer of the painting from B. Zanganelli (ca. 1500, sample **F**) and the pigment layer of the painting from Michelangelo followers (ca. 1500, sample **H**). Gas-chromatographic conditions see Experimental Section

Table 3. Examined objects. List of samples taken

Artist	Object	Date	Sample	Sign
–	Paint film, egg tempera pigmented with yellow ochre	1970	Paint layer	A
Vredeman de Vries	"Interior of a gothic church"	16. c.	Glue from original craddling	B
Anonym.	Polychromated timber ceiling	18. c	Paint layer	C
Anonym., Piemont	"Sacra conversatione" (after Mantegna); Vienna, Academy of Fine Arts Inv. No. 1084	16. c.	Gesso ground (with traces of paint layer)	D
Pannini, F.	"Landscape with roman monuments"; Vienna, Academy of Fine Arts Inv. No. 534	18. c.	Dark red ground layer	E
Zanganelli, B. (?)	"Circumcision of Christ"; Vienna, Academy of Fine Arts Inv. No. A 18	ca. 1500	White ground layer	F
Anonym., Italian	"Madonna with child and St. John" (Michelangelo followers)	ca. 1500	White ground layer	G
Anonym., Italian	See object G		Paint layer	H

systematic errors in the determination of the corresponding concentrations of other amino acids. This fact can impede the proper identification of the proteinaceous binder.

In contrast to the results found after the hydrolysis with HCl, no interferences were observed in the quantitative analysis of the amino acids after hydrolysis with the ion exchanger. Only in the presence of Fe-, Pb- and Cu-containing pigments an artefact was found in some cases. This compound elutes together with iso-leucine, but this amino acid is not used as a characteristic constituent of the proteins of interest. Furthermore, the deviation from the true concentration of iso-leucine can be controlled by the peak area ratios given e.g. for leucine and iso-leucine. It was found at least for the marker acids, that in all pigment containing samples the relative concentrations are in the range given in the literature.

3.3 Applicability of the hydrolysis procedure with ion exchanger to samples of objects of art and identification of the binder based on the relative content of amino acids

With a series of samples, given in Table 3, the reliability of the hydrolysis procedure described in the present paper was tested. Typical chromatograms obtained from samples of objects of art are shown in Fig. 5.

The results of the relative quantitation of the amino acids are given in Table 2. An attempt to identify the binder in the

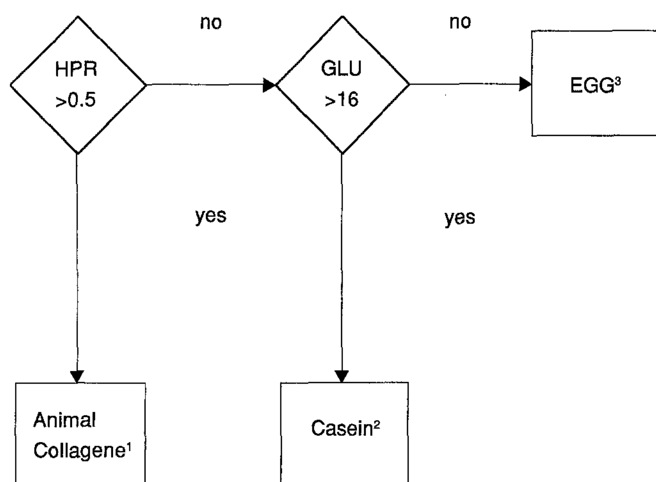

Fig. 6. Scheme for the identification of proteinaceous binding media based on the relative peak areas of marker amino acids (normalized to 100%). The abbreviations of the amino acids are according to IUPAC (see Table 3). The relative peak areas are given as percentages of the summarized areas. Additional criteria for confirmation: *1* GLY 15–30, PRO, ALA 6–12, VAL, LEU <5; *2* ASP <12, PRO >9, ALA <6, VAL, LEU >5; *3* ASP >12, PRO <9, ALA >5, VAL, LEU >5, THR >5, SER >7

samples can be made by comparison with the values derived for the proteinaceous binders given also in Table 2. By the aid of the relative peak areas, a scheme for the identification of the particular proteins can be derived, which is similar to that given by Keck [1] (for the amino acid derivatives determined by the detection of the reaction products with ninhydrin after liquid chromatographic separation of the free acids on an ion exchanger) and White et al. [3, 4] (for the amino acid derivatives determined in a similar way as in the present paper by gas chromatography on a packed column, but using some different criteria to characterize the protein). Using this scheme, which is shown in Fig. 6, the binding media of the samples of the objects of art can be identified from the peak area values given, leading to the following results.

It can be concluded that sample A contains egg protein as binder, sample B contains casein, the proteinaceous medium of the panel painting (sample C) contains animal collagene. Samples D, E, F and G, which are ground layers from panel paintings consist of animal collagene, too. Sample H, which is a pigment layer of the same object as sample G seems to be egg or casein. The binder of this sample is difficult to be clearly identified as egg protein, which is the more probable result from the technical point of view: the content of glutamic acid seems to be too high. This case demonstrates again the fact that only a combination of different methods like microchemical group tests, inspection of cross sections and staining techniques together with a thorough knowledge of painting techniques yields meaningful results in the identification of vehicles.

References

1. Keck S, Peters T (1969) Stud Conserv 14:75–82
2. Birstein VJ (1975) Stud Conserv 20:8–19
3. White R (1984) Techn Bull 8:5–14 (National Gallery, London)
4. Mills JS, White R (1987) The organic chemistry of museum objects. Butterworths, London
5. Karpowicz A (1981) Stud Conserv 26:153–160
6. Mairinger F (1986) Fresenius Z Anal Chem 324:147–153
7. Adams RF (1974) J Chromatogr 95:189–212
8. Lawrence L, Moore WJ (1951) J Am Chem Soc 73:3973–3977
9. Niemann A (1960) Naturwissenschaften 47:514
10. Niemann A (1961) Fresenius Z Anal Chem 181:543–544
11. Pöhm M (1961) Naturwiss 48:551
12. Paulson JC, Beatherage FE, Emory FA (1953) J Am Chem Soc 75:2039–2041
13. Whitaker JR, Beatherage FE (1955) J Am Chem Soc 77: 3360–3365
14. Kuznetsova NP, Samsonov GV (1969) Biokhimiya 31:51–54
15. Roach D, Gehrke CW (1969) J Chromatogr 44:269–278
16. Cancalon P, Klingham JD (1974) J Chrom Sci 12:349–355
17. Blau K, King G (1978) Handbook of derivatives for chromatography. Heyden, London
18. James LB (1987) J Chromatogr 408:291–295
19. Pettitt PC, Stouffer JE (1970) J Chrom Sci 8:735–737
20. Drozd J (1975) J Chromatogr 113:303–356
21. James LB (1987) J Chromatogr 403:271–274
22. MacKenzie SL, Tenaschuk D (1974) J Chromatogr 97:19–24
23. Moodie IM, Burger J (1981) JHRC CC 4:218–223
24. Frank H (1984) Gaschromatographie von Aminosäuren. Analytiker Taschenbuch, vol 4. Springer, Berlin Heidelberg New York, pp 337–373
25. Bruckner H, Bosch J (1987) J Chromatogr 395:569–590
26. Husek P, Macek K (1975) J Chromatogr 113:139–230
27. Schramm HP, Hering B (1989) Historische Malmaterialien und ihre Identifizierung. VEB Deutscher Verlag der Wissenschaften, Berlin