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CHROMIUM-AMINO ACID CONJUGATES AS ELICITORS IN CHROMIUM-SENSITIZED GUINEA PIGS*

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ABSTRACT

Chromium-amino acid conjugates were isolated from systems initially containing either potassium dichromate and amino acid or chromic nitrate and amino acid. The elicitating properties of these chromium-amino acid conjugates were evaluated by intradermal injection of guinea pigs sensitized to chromium. Positive results were obtained with several conjugates. The conjugates prepared from chromic nitrate and amino acid were stronger elicitors than those prepared from potassium dichromate and amino acid.

Landsteiner [1, 2] proposed the concept that allergy to simple chemicals involved conjugation of the simple chemical with a protein carrier, forming the complete antigen. This was confirmed by later workers. Eisen and co-workers [3-5] reported on the chemical reactions of the conjugation of dinitrobenzene residues to protein and the elicitation of allergic reactions with the resulting conjugates. Benacerraf and Gell [6, 7] reported on reactions to picryl conjugates in guinea pigs. Gell and Silverstein [8] demonstrated a carrier specificity in the formation of antigens. Cohen [9] reported that human serum albumin acts as a carrier, while human gamma globulin participates to a much lesser extent (if at all) in chromium sensitivity. In a subsequent paper, Cohen [10] proposed that mucopolysaccharides also function as carriers of chromium in the manifestation of the allergic response mechanism. Based on studies in our laboratories‡, we would agree that serum albumin-chromium conjugates may be elicitors, but we cannot confirm the role attributed to the mucopolysaccharides.

Berrens [11] proposed that amino acid-hapten conjugates have an important function as intermediate messengers in the process of sensitization. Jansen and co-workers [12, 13] demonstrated that a nickel-alanine complex can elicit reactions in sensitized animals. There are, however, no reports of the eliciting properties of chromium-amino acid conjugates in chromium-sensitive subjects. Consequently, we undertook the preparation of chromium-amino acid conjugates and an evaluation of their eliciting properties in guinea pigs sensitized to chromium.

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MATERIALS AND METHODS

Chromium-amino acid conjugates were prepared as follows: Approximately 1 millimole of the desired amino acid was dissolved in 3 ml of 0.1 N hydrochloric acid. One-ml aliquots of the amino acid solutions were treated with either 1 ml of 1.0×10^{-3} M chromic nitrate solution to produce complexes with trivalent chromium or 1 ml of 1.0×10^{-3} M potassium dichromate solution to produce complexes with hexavalent chromium. Control solutions were prepared by mixing 1 ml of the amino acid solutions with an equal volume of distilled water and carrying them through the remainder of the procedures. In addition 1-ml samples of 0.1 N hydrochloric acid were treated with 1 ml of either 1.0×10^{-3} M chromic nitrate or 1.0×10^{-3} M potassium dichromate and carried through the procedure as controls. A distilled-water control was treated similarly.

The amino acid-chromium mixtures and the controls were allowed to stand at room temperature for five days. After that time, each solution was neutralized to litmus with 0.1 M TRIS (trishydroxymethylaminomethane) and batch extracted with two 1-gm portions of Dowex-50 to remove uncomplexed cationic trivalent chromium. The solutions were each treated with 1 ml of dilute hydrochloric acid and batch extracted with two 1-ml portions of MIBK (4-methylpentanone, 2) to remove excess hexavalent chromium. Each solution was evaporated to dryness under vacuum. The solid residues were taken up in 1 ml of physiologic saline solution, filtered, and sterilized by passage through Millipore filters.

Conjugates of chromium with alanine (ALA), arginine (ARG), aspartic acid (ASP), cystine (CYS), glycine (GLY), histidine (HIS), leucine (LEU), lysine (LYS), methionine (MET), norvaline (NOR), ornithine (ORN), phenylalanine (PHE), proline (PRO), serine (SER), tryptophan (TRY), and tryosine (TYR) were prepared in the above manner. The chemical properties of these conjugates will be the subject of a future report.

Two sets of guinea pigs were used. One set was sensitized to potassium dichromate by the technique of Gross et al [14]. (Albino guinea pigs weighing 300 to 500 gm were sensitized to hexavalent chromium by three subcutaneous injections in the nape one week apart. The following emulsion was injected: 0.5 ml Freund's complete adjuvant [Difco] with 0.5 ml of 3.4×10^{-3} M $K_2Cr_2O_7$). The second set was not sensitized; it served as a control group in the detection of the irritant effects of the materials used. The eliciting properties of the chromium conjugates were evaluated by intradermal

injections of 0.1 ml of each conjugate and each control in the two sets of guinea pigs. The eliciting doses contained 1×10^{-4} millimole of chromium–amino acid conjugate (corresponding to 0.025 to 0.045 mg depending on the amino acid) or 3×10^{-2} millimoles of uncomplexed amino acid (corresponding to 3 to 7 mg of amino acid again depending on the amino acid).

RESULTS

Results of skin tests are summarized in the Table.

The following chromium–amino acid conjugates were elicitors in chromium-sensitized guinea pigs: alanine-Cr (III), alanine-Cr (VI), arginine-Cr

TABLE I

Results of skin tests with hexavalent and trivalent chromium-amino acid conjugates in sensitized and control guinea pigs

Challenge material	Reactions in sensitized animals							Reactions in control animals				
	A	B	C	D	E	F	AVG.	G	H	I	J	AVG.
ALA	0	0	0	0	0	0	0.0	0	0	0	0	0.0
ALA-Cr(III)	+3	+2	+2	+2	+1	+2	2.0	0	0	0	0	0.0
ALA-Cr(VI)	+2	±	+2	+2	+1	+2	1.6	+1	0	0	0	0.3
ARG	0	0	0	0	0	0	0.0	0	0	0	0	0.0
ARG-Cr(III)	+1	+1	+1	+2	±	+2	1.2	0	0	0	0	0.0
ARG-Cr(VI)	±	0	0	+1	±	+1	0.5	0	0	0	0	0.0
ASP	+1P	+1P	+1P	+1P	+2P	+1P	1.1P	±	±	+1	0	0.4
ASP-Cr(III)	+1P	+2P	+2P	±P	+2P	+2P	1.6P	±	+1	+1	0	0.6
ASP-Cr(VI)	±	+2	+1P	±P	+1P	+2P	1.1P	+1	+1	+2	0	1.0
CYS	+1	±	±	±	±	+1	0.7	0	0	±	0	0.1
CYS-Cr(III)	+2	+1	+1	+1	±	+2	1.3	0	±	±	0	0.3
CYS-Cr(VI)	+2	+1	+2	+1	±	+3	1.6	±	0	0	0	0.1
GLY	0	**	0	±	0	0	0.1	0	0	**	**	0.0
GLY-Cr(III)	0	**	0	+1	+2	0	0.6	0	0	**	**	0.0
GLY-Cr(VI)	±	**	±	+2	+1	+1	1.0	0	+1	**	**	0.5
HIS	+1	**	+1	±	+2	+1	1.1	±	±	**	**	0.5
HIS-Cr(III)	+3	**	+2	+2	+2	+1	2.0	+1	+1	**	**	1.0
HIS-Cr(VI)	+2	**	+1	+2	+2	+1	1.6	±	+1	**	**	0.8
LEU	0	0	0	0	0	0	0.0	0	0	0	0	0.0
LEU-Cr(III)	+1	+1	+2	0	0	+1	0.8	0	0	+1	0	0.3
LEU-Cr(VI)	±	0	+2	0	0	+1	0.6	0	0	±	0	0.1
LYS	±	**	±	0	±	±	0.4	0	0	**	**	0.0
LYS-Cr(III)	+2	**	±	0	+2	+2	1.3	0	+1	**	**	0.5
LYS-Cr(VI)	+1	**	+1	+1	0	+1	0.8	0	0	**	**	0.0
MET	0	0	0	0	0	0	0.0	0	0	0	0	0.0
MET-Cr(III)	+1	+1	+2	0	+1	+1	1.0	0	0	0	0	0.0
MET-Cr(VI)	+1	±	+1	0	±	+1	0.7	0	0	0	0	0.0
NOR	+1	**	+1	0	0	+1	0.6	0	0	**	**	0.0
NOR-Cr(III)	+2	**	+1	+1	+2	+2	1.6	0	+1	**	**	0.5
NOR-Cr(VI)	+1	**	+1	0	0	+1	0.6	+1	+1	**	**	1.0
ORN	+1P	**	+1	0	+1P	**	0.8P	+1P	0	**	**	0.5P
ORN-Cr(III)	+2	**	+2	+2	+3	**	2.3	+1	+2	**	**	1.5
ORN-Cr(VI)	+1	**	+2	+1	+1	**	1.2	+1	+1	**	**	1.0
PHE	±	±	±	0	0	0	0.3	0	0	0	0	0.0
PHE-Cr(III)	+1	+1	+2	+1	+1	+1	1.1	0	0	+1	0	0.3
PHE-Cr(VI)	+1	+1	+1	+1	0	+1	0.9	0	0	±	0	0.1

TABLE I—*Continued*

Challenge material	Reactions in sensitized animals							Reactions in control animals				
	A	B	C	D	E	F	AVG.	G	H	I	J	AVG.
PRO	0	**	0	±	0	±	0.2	0	0	**	**	0.0
PRO-Cr(III)	+4	**	+4	+3	+4	+4	3.8	+1	+3	**	**	2.0
PRO-Cr(VI)	+3	**	+2	+1	+2	+3	2.2	±	+1	**	**	0.8
SER	0	0	0	0	0	0	0.0	0	0	+1	0	0.3
SER-Cr(III)	0	+1	0	0	0	0	0.2	0	0	0	0	0.0
SER-Cr(VI)	+1	+2	+1	0	0	0	0.7	+1	+1	+1	0	0.8
TRY	±	**	±	0	±	0	0.3	0	0	**	**	0.0
TRY-Cr(III)	+3	**	+3	+3	+4	+3	3.2	+2	±	**	**	1.3
TRY-Cr(VI)	+2	**	+2	+2	+2	+2	2.0	0	±	**	**	0.3
TYR	0	0	0	0	0	0	0.0	0	0	0	0	0.0
TYR-Cr(III)	+1	+1	+1	0	0	0	0.5	0	0	0	0	0.0
TYR-Cr(VI)	+2	+1	+2	0	0	0	0.8	+1	0	+1	0	0.5
4.2×10^{-4} M $K_2Cr_2O_7$	+3	**	+4	+3	+4	+3	3.4	0	0	**	**	0.0
4.2×10^{-4} M $CrCl_3$	+1	**	0	+1	+1	+2	1.0	0	0	**	**	0.0
Distilled Water	0	**	0	0	0	0	0.0	0	0	**	**	0.0

P = Pustule
** = animal died
Skin tests were evaluated as follows:
0 no response
± equivocal results
+1 response of 10 mm diameter
+2 response of 15 mm diameter
+3 response of 20 mm diameter
+4 response of 20 mm diameter with central necrosis

(III), cystine–Cr (III), cystine–Cr (VI), methionine–Cr (III), phenylalanine–Cr (III), proline–Cr (III), proline–Cr (VI), tryptophan–Cr (III), tryptophan–Cr (VI). The remaining conjugates tested produced either low reactions (less than 1+) or were irritating in the control animals.

DISCUSSION

Although the guinea pigs were sensitized with hexavalent chromium and responded more strongly to challenge with unconjugated hexavalent chromium than with unconjugated trivalent chromium, the amino acid–chromium (III) conjugates consistently produced more pronounced reactions than the amino acid–chromium (VI) conjugates. We interpret this as a further indication that chemical reduction of hexavalent chromium precedes conjugation. At this time, we are unable to define the mechanism of this reduction, nor can we define the exact role played by the amino acids themselves. We have previously demonstrated [15] that the sulfur-containing amino acids are able to chemically reduce hexavalent chromium and that trivalent chromiun forms complexes with several amino acids. Conjugation of chromium (III) with these amino acids apparently yields a product of three to five times greater antigenicity than unconjugated chromium (III). By the same token, conjugation with hexavalent chromium decreased antigenicity.

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